"DNA Intercalating Compounds with a Tetra- or Pentacyclic Nucleus: Design and Biological Evaluation"

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INTRODUCTION

1.1 Epidemiology of Cancer

The World Health Organisation (WHO) describes cancer as "a generic term for a large group of diseases that can affect any part of the body." One feature of cancer is the rapid creation of abnormal cells that grow beyond their boundaries, their invasion to adjoining parts of the body and spreading to other organs, a process which is called metastasising. Metastases are the main cause of death from cancer. Benign tumours do not spread into other organs and are therefore not called cancer.

With approximately 14 million new cases and over 8.2 million cancer related deaths worldwide, it is one of the leading causes of mortality and morbidity in 2012. It is expected that the number of new cases will rise by about 70 % in the next 20 years. The most five diagnosed types of cancer in men (2012) were lung, prostate, colorectal, stomach and liver cancer, whereas in women breast, colorectal, lung, cervix and stomach cancer are dominant.

About one third of all deaths of cancer can be attributed to five dietary and behavioural risks. These are smoking, alcohol abuse, low intake of fruit and vegetables, low physical activity and a high body mass index (BMI). Among these, the most important risk factor is the use of tobacco, which causes 20 % of global cancer deaths and about 70 % of global lung cancer deaths.

In low- and middle developed countries viral infections such as HBV/HCV and HPV are responsible for up to 20 % of cancer deaths. In Africa, Asia and Central and South America occur more than 60 % of the annual new cases of cancer and over 70 % of the world’s cancer deaths.

It is estimated that annual cancer cases will rise from 14 million in 2012 to 22 in the next 20 years.

1.2 The Classification of Cancer

There are over 100 types of cancer known in humans affecting virtually every organ or tissue. They are described by their primary site or the location (topographic) where they have first developed (lung, brain, etc.), and based on the histological type (morphologic), because all organs or body parts are composed of different types of cells. The international standard for the classification and nomenclature of histologies is the International Classification of Diseases for Oncology, Third Edition (ICD-O-3). These types include

- carcinoma, which is derived from epithelial cells; the most common cancers are carcinoma, for example many breast, lung, prostate, colon and lung cancers
- sarcoma, if a cancer originates from connective tissue, for example bone, cartilage, fat; each of which develops from cells originating from mesenchymal cells;
- lymphoma and leukemia are derived from blood forming cells;
- germ cell tumours which are derived from pluripotent cells, for example seminoma originating from the testicle or dysgerminoma from the ovary;
- blastoma which are cancers from embryonic tissue or immature precursor cells and are more common in children than in adults.

In cancer nomenclature the suffix -carcinoma, -sarcoma or -blastoma is used with the Greek or the Latin word for the source as the root, for example "hepatocarcinoma" as cancer originating from ma-
lignant epithelial cells of the liver parenchym.
Benign tumours are usually nomenclated with organ name as the root and the suffix -oma. Confusingly, some types of malignant cancer use the -oma suffix, such as melanoma or seminoma. Some types of cancer are named after their morphology under a microscope, such as giant cell carcinoma or small-cell carcinoma.

1. 3 Causes of Cancer

90 to 95% of the cancer cases are due to environmental factors, only 5 to 10% can be attributed to inherited gene mutations.\(^4\) In this context, the term environmental is by cancer researchers not used to merely describe pollution of air or water but also behavioural, economic and lifestyle factors.\(^5\)

The environmental factors contributing to cancer deaths include tobacco (25 to 30%), physical inactivity, diet and obesity (30 to 35%), radiation (up to 10%), infection diseases (15 to 20%), stress and environmental pollutants.\(^4\)

1. 3. 1 Chemicals

Chemicals which have been linked to specific types of cancers are called carcinogens, Tobacco smoke contains over fifty known carcinogens, including polycyclic aromatic hydrocarbon and nitrosamines.\(^6\) Smoking tobacco causes 90% of lung cancers\(^7\) and is responsible for one third of all cancer deaths in the developed world\(^8\) and for about 20% worldwide.\(^6\) Cancer in the larynx, neck, head, esophagus, stomach, kidney, bladder, and pancreas are also related to tobacco use.\(^6\)

The International Agency for Research on Cancer (IARC) classifies alcoholic beverages as a group 1 carcinogen (carcinogenic to humans) and as a cause of female breast cancer, as well as larynx, pharynx, esophagus, liver (hepatocellular carcinoma), oral cavity and colorectal cancer. It is also a probable cause of pancreatic cancer.\(^9\)

Alcohol intake is responsible for 3.6% of all cancer cases and 3.5% of global cancer deaths\(^10\) and for 10% of cancers in males and 3% in females in Western Europe, especially cancer of the liver and the digestive tract.\(^11\)

Chemical exposure at work is believed to cause 2 to 20% of all cancer cases. In 1996, the Harvard Center for Cancer Prevention (HCCP) classified 32 substances or industries as carcinogenic in humans. Recently, 28 agents have been considered as definite occupational carcinogens in human, 27 as probable occupational carcinogens and 113 as possible occupational carcinogens."\(^12\)

1. 3. 2 Diet and Exercise

The risk of cancer is significantly influenced by dietary factors, which can have both an increasing or reducing effect. 30 to 35% of cancer deaths may be related to diet, lack of physical activity and obesity.\(^4\) The most important dietary cause of cancer is overnutrition; obesity and consumption of alcohol are accepted causes of cancer.\(^13\) Consequently, many dietary recommendations have been proposed
but only few are supported by scientific evidence. Some specific foods are associated with specific cancers. Studies have demonstrated that the consumption of red or processed meat is linked to an increased risk of breast cancer, colon cancer, prostate cancer and pancreatic cancer. At least partially responsible are carcinogens which are formed at high temperatures during the cooking process. The food contaminant Aflatoxin B1 is known to cause liver cancer, whereas drinking coffee reduces the risk. Dietary habits in different countries partially explain the differences in cancer incidence. The fundamental link between cancer and diet is suggested by the observation that immigrant communities often develop the cancer risk of their new country, often within one generation.

1.3.3 Infection

Infectious diseases are responsible for 16.1% of cancers worldwide, varying in different regions with 32.7% in Sub-Saharan Africa to only 3.3% in Australia and New Zealand. The main infectious agents causing cancer are viruses, called oncoviruses, but *Mycobacterium* and other bacteria or parasites are also responsible.

These oncoviruses include human papillomavirus which causes cervical carcinoma, Epstein-Barr virus, responsible for B-cell lymphoproliferative disease and nasopharyngeal carcinoma, Kaposi’s sarcoma herpesvirus causing Kaposi's Sarcoma and primary effusion lymphomas, hepatitis B and C viruses causing hepatocellular carcinoma, and Human T-cell leukemia virus-1.

The most prominent example for bacterial infection that may increase the risk of cancer is *Helicobacter pylori*, responsible for inducing gastric carcinoma.

One example for a cancer-related parasite is *Schistosoma haematobium*, causing bladder cancer, probably due to chronic inflammation triggered by the worm's eggs.

1.3.4 Radiation

Ionising and non-ionising UV radiation exposure are responsible for up to 10% of invasive cancers. From the non-invasive cancers the majority are non-melanoma skin cancers which are caused by ultraviolet radiation, mostly from UVB waves of sunlight. Long-term exposure to sunlight can lead to melanoma. Radon gas and medical imaging are the main sources for ionising radiation, especially computed tomography is a widespread source of ionising radiation in the modern world and the carcinogenic effects are unclear. Although ionising radiation is not particularly strong it can be a potent source of cancer in combination with other cancerogenic agents such as smoking tobacco. Ionising radiation is also used to treat cancers, but can also form secondary neoplasms as a consequence. The world health organisation has described non-ionising radio frequency radiation from power lines, mobile phones and similar sources as possible risk for the development of cancer.
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1. 3. 5 Heredity

The majority of all cancers are sporadic, only 3 to 10% of annual diagnosed cancers are triggered by an inherited genetic mutation. For example, mutations in the genes BRCA1 and BRCA2 are associated with a risk of more than 75% to develop breast and ovarian cancer.29

1. 3. 6 Hormones

The development of certain cancers can be triggered by hormones that are promoting cell proliferation such as insulin-like growth factors.30 Especially sex-related cancers such as prostate, ovary, testis and breast cancer, but also thyroid or bone cancer can be attributed to hormones.30 Elevated levels of estrogen and progesterone for example in daughters of women with breast cancer may explain their higher risk of developing breast cancer, even without breast-cancer genes.

For example, the daughters of mothers who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters without breast cancer. These higher hormone levels may explain why these women have higher risk of breast cancer, even in the absence of a breast-cancer gene.30 Similarly, men with African ancestors have a higher rate of prostate cancer due to significantly higher testosterone level compared to men with European ancestors whereas the low levels of testosterone-activating androstanediol glucuronide in men with asian ancestors reflect the low rates of prostate cancer.30

1. 4 Carcinogenesis - A Multistep Model

The development of cancer, called carcinogenesis is a multistage process that transforms a normal cell to a malignant cell. This is characterised by changes at the genetic and epigenetic level altering the cellular program to undergo uncontrolled cell division. Under physiological conditions of healthy cells and tissues proliferation and programmed cell death (apoptosis) are in balance. This process is sustained by a series of a complex regulation machinery which is eventually damaged by mutations and epimutations in the DNA, in particular in DNA repair genes and genes involved in cell growth, cell division or apoptosis. Genes that govern cell growth are called proto-oncogenes, which become to oncogenes when their function is disabled by mutation, whereas tumour suppressor genes and related proteins suppress mitosis and cell growth. Mutations in these genes cause a deregulation of signalling pathways and eventually tumour cells start to divide uncontrollably.

The development of cancer can be divided into four conceptual stages: initiation, promotion, malignant conversion, and tumour progression. Malignant conversion of benign hyperplastic cells to a malignant state is a prerequisite for carcinogenesis, invasion and metastasis are the result of further genetic and epigenetic changes.30, 31, 32 The study of this process in humans relies merely on information from occupational exposures to chemical carcinogens and lifestyle. Age-dependent cancer cases have demonstrated that the rate of tumour development is proportional to the sixth power of time, suggesting that at least four to six independent steps are necessary.34
1. 4. 1 Tumour Initiation

The early concept of tumour initiation indicated an irreversible genetic damage as first step in chemical carcinogenesis, but recent data from molecular studies also indicate that epigenetic changes as another early event are involved. These are caused by DNA methylation of promoter regions of genes that are capable of silencing tumour-suppressor genes. The accumulation of mutations can only occur in cells that proliferate and survive the lifetime of the affected organism. According to Knudson's hypothesis, mutations in both tumour suppressor genes and proto-oncogenes are required to transform a cell into a cancer cell. Only when enough tumour suppressor genes are deactivated or damaged and proto-oncogenes have mutated into oncogenes cell proliferation gets out of control.

1. 4. 2 Tumour Promotion

The term tumour promotion describes the selective clonal expansion of initiated cells. The high accumulation rate of mutations is proportional to rate of cell divisions or at least the rate of stem cell replacement and promotes the clonal expansion of initiated cells, thus producing a great number of cells which are prone to further genetic mutations and malignant conversion. Tumour promoters are not carcinogenic alone, nonmutagenic and often capable of mediating their effect without metabolic activation. After exposure to an initiator, tumour promoters are capable of reducing the latency period for tumour formation, or increasing the number of tumours formed in that tissue. In addition, in conjunction with a dose of an initiator, that is too low to be carcinogenic alone the promoter can induce tumour formation. Agents capable of triggering initiation and promotion are called complete carcinogens, such as benzo[a]pyrene.

1. 4. 3 Malignant Conversion

The transformation of a preneoplastic cell into a cell with malignant phenotype is called malignant conversion, a process that requires further genetic changes, again in connection with tumour suppressor genes and activation of proto-oncogenes. These further genetic changes are to some extent the result from errors in DNA synthesis. The relatively low probability of malignant conversion can be increased substantially by the exposure of preneoplastic cells to DNA-damaging agents. The total dose of a tumour promoter is not as significant as frequently repeated administrations and in case it is discontinued before the malignant transformation has occurred the benign lesion may regress.

1. 4. 4 Tumour Progression

Tumour progression is characterised by the expression of malignancy and a tendency to develop more aggressive properties over time. A prominent example is the tendency for genomic instability on either the nucleotide or the chromosomal level and uncontrolled growth. This results in further genetic and epigenetic changes, again including activation of proto-oncogenes and inactivation of tumour suppressor genes. Also, a tumour can develop the ability to secrete proteases that allow invasion beyond its primary location, a process referred to as metastasis. Although a certain scheduling of these mutational incidents is suggested, merely the accumulation of genetic changes appears to be the determining factor, not the stage of carcinogenesis in which they occur.
1. 5  The Hallmarks of Cancer

In 2000, cancer researchers Douglas Hanahan and Robert Weinberg have published an article in the Journal "Cell" in which the complex mechanism of multistep carcinogenesis is simplified to six common traits ("hallmarks") that govern the transformation of normal cells to malignant cells. 11 years later two further hallmarks ("deregulated metabolism" and "avoiding immune destruction") were added, which are labelled "emerging hallmarks", since they are not yet fully validated. In addition, two capabilities of neoplasia are described which facilitate the acquisition of both core and emerging hallmarks. These are "genomic instability" and thus mutability which endows cancer cells with genetic alterations that drive tumour progression and "inflammation" by innate immune cells can instead result in their inadvertent support of multiple hallmark capabilities, thereby manifesting the now widely appreciated tumour-promoting consequences of inflammatory responses.

1. 5. 1  Self-Sufficiency In Growth Signals

In order to grow and divide, normal cells need external stimuli via growth factors which are transmitted through receptors in the cell membrane. If these signals are missing they don't grow. In contrast, cancer cells don't need that stimulation, they can generate their own growth signals, possess overexpressed receptors or have mutated receptors that transmit signals without growth factors. Glioblastoma cells, for example, can produce their own platelet-derived growth factor (PDGF) whereas the epidermal growth factor receptor (EGF-R/erbB) is overexpressed in brain, stomach, and breast cancers; in stomach and breast cancers HER2/neu receptor is overexpressed.

1. 5. 2  Insensitivity To Anti-Growth Signals

In the same manner normal cells receive growth signals to divide and grow they are kept under control by growth inhibitors of the surrounding environment, on the surface of neighbouring cells and in the extracellular matrix. These inhibitors influence the cell cycle clock by interrupting mitosis. Cancer cells are generally resistant to growth-preventing signals from their neighbours. Eventually, the growth inhibitor signals are transmitted via the retinoblastoma protein (pRB), which prevents the G1 to S phase transition. If pRB is damaged by a mutation or by interference from human papilloma virus, cells can divide uncontrollably, leading to cervical cancer.
1. 5. 3  Evading Apoptosis

Apoptosis is the term of programmed cell death (cell suicide) in the event they become irreparably damaged. It is characteristic for cancer cells to be able to overcome this mechanism.

The apoptotic machinery can be divided into sensors, which monitor the cell for abnormal behavior, and effectors, which cause apoptosis. Sensors include IGF-1/IGF-2 and their receptors and IL-3 and its receptor. The effectors include FAS ligand and its receptor, and TNF-α and its receptor.

The p53 tumour suppressor protein is an important trigger of apoptosis and acts on DNA damage. If p53 is mutated, which is the case in over 50% of cancers, this regulation mechanism fails.

1. 5. 4  Limitless Replicative Potential

Normal cells have a limited number of divisions before they die. This number is called the Hayflick limit, which is about 60 to 70 doublings before the cell reaches the state of senescence. Cancer cells, on the other hand, have to overcome this limit and acquire immortality, that means the capability of limitless growth and cell division. However, these immortalised cells have damaged chromosomes which may become cancerous. The counter for cell doublings is the telomere, a sequence of repetitive nucleotides at the tips of each chromatide, of which some are lost during each cell cycle. Many cancers involve the upregulation of telomerase, the enzyme that replenishes nucleotides of the telomere.

1. 5. 5  Sustained Angiogenesis

Angiogenesis is the term used for the formation of new blood vessels. Cancer cells are able to start this process, ensuring to receive a sufficient amount of oxygen and nutrients. Initially cancer cells lack that capability, limiting their further growth (usually about 1 to 2 square mm). This process, which is governed by inducers and inhibitors is vital for cancer cells to expand (for example cervix, breast and melanoma tumours). Among the inducers are vascular endothelial growth factor (VEGF) and acidic and basic fibroblast growth factor (FGF-1 and FGF-2), which bind to transmembrane tyrosine kinase receptors expressed on endothelial cells. An inhibitor is thrombospondin-1 which is regulated by p53, thus angiogenesis can be triggered by loss of p53. Drugs targeting the inhibition of angiogenesis in tumours are a promising research field, under investigation and is tightly connected with the capability of metastasis.

1. 5. 6  Tissue Invasion and Metastasis

The probably most dangerous ability a cancer cell acquires is to spread from its place of origin to invade the surrounding tissue and into distant organs or body parts. This capability is referred to as metastasis and is a complex process comprised of a series of steps which involve the mimicking of normal cell-cell interactions through cell adhesion molecules (CAMs) and integrins. Matrixdegrading proteases are also vital to facilitate the invasion into the new tissue. Moreover, the body’s natural defense mechanisms, such as the expression of E-cadherin which transmits antigrowth signals must be overcome by cancer cells to grow. The probably most critical event required for successful metastasis is angiogenesis, the process of forming new blood vessels which is why this process is under investigation.
1.5.7  Deregulated Metabolism

Cancer cells can reprogram their energy metabolism to use primarily glycolysis for energy production instead of oxidative phosphorylation in the mitochondria. This is seemingly counterintuitive as the cancer cell has to compensate for the 18-fold lower efficiency of ATP production. This is achieved in part by upregulating glucose transporters to increase the glucose import into the cytoplasm. On the other hand, increased glycolysis allows diversion of glycolytic intermediates into diverse biosynthetic pathways, including those producing amino acids and nucleosides. This facilitates the biosynthesis of organelles and other macromolecules required for new cells. Since an altered energy metabolism is as common as other cancer-associated hallmarks of cancer the designation as emerging hallmark seems appropriate.

1.5.8  Evading the Immune System

A still unresolved issue related tumour formation involves the role of the immune system. Cancer cells seem to be able to avoid detection of the immune system or evade eradication by disabling components of the immune system which were designed to eliminate them. This might be modulated by secretion of TGF-β or other immunosuppressive factors. In studies, genetically engineered immunodeficient mice were assessed for the development of tumours. Indeed, tumours grew more frequently and/or faster than in the immunocompetent control group. In addition, human patients with colon and ovarian tumours that are infiltrated with NK cells and CTLs have a better prognosis than patients lacking those cells. On the other hand, epidemiological data does not show an increased incidence of cancer in immunosuppressed people, which might be attributed to a residual defense capability mediated through NK and other innate immune cells. Yet, the scientific demonstrations of antitumour immunity as barrier against tumourigenesis is still rudimentary and needs further confirmation.
1.6 Therapeutic Concepts - Mechanism-based Targeting

With the introduction of mechanism-based targeting myriads of therapies to treat cancer have evolved reflecting the description of the hallmark principles. The rapidly growing armamentarium of cancer therapeutics can be categorised according to their effects on a hallmark capability and their efficacy demonstrates the validity of that concept (Figure 1).

Most of the hallmark-targeting cancer drugs developed to date have been directed toward specific molecular targets which in theory should provide inhibitory activity while having fewer off-target effects, leading to reduced toxicity. This is not the case, a circumstance that can be interpreted by the fact that each hallmark capability has redundant signalling pathways which results in only a partial inhibition of a capability instead of a complete shutdown, leaving behind some cells with residual function. They or their progeny can eventually adapt by mutation of epigenetic reprogramming or remodelling of their microenvironment which causes relapses in clinical therapy.

The emerging of adaptive resistance should be prevented by therapeutically adressing all of the supporting pathways of a certain hallmark. Instead, recent discoveries suggest that cancer cells might be able to shift their dependence from one particular capability to another, which can be regarded as completely different form of acquired drug resistance. This was found in antiangiogenic therapies where tumours adapted from a dependence on angiogenesis to increased invasiveness and metastasis which resulted in invasion to surrounding tissue where preestablished blood vessels could restore the oxygen supply of the tumour.46, 47, 48

Such observations suggest that future anti-cancer drugs and treatment will benefit from incorporating the concept of hallmark capabilities and of the supporting biochemical pathways in each of them. In particular, "we can envisage that selective cotargeting of multiple core and emerging hallmark capabilities and enabling characteristics in mechanism-guided combinations will result in more effective and durable therapies for human cancer."42
1.7 Therapeutic Concepts - Intercalation and Topoisomerase Inhibition

Despite the latest advances in targeting hallmark capabilities as anticancer agents with varying success and the first steps in immunotherapy there are still many small molecules with cytotoxic properties in clinical use and there is still need of compounds with an optimised side effect profile. The small molecules comprise several classes of cytotoxic compounds: antimetabolites, alkylating agents, microtubule inhibitors, intercalating agents and topoisomerase inhibitors.

1.7.1 Intercalation

The concept of intercalation was first described by Leonard Lerman in 1961 for the acridine Proflavin. Intercalation is the term for the insertion of virtually any polyaromatic structure between the base pairs of the DNA with the right steric properties. Based on his research a number of structural requirements for molecules were proposed by Moore et al. to optimise intercalation. A system should be polyaromatic with 3 to 4 rings and coplanar, with a width of 3-4 Å and a length of 6-8 Å. The surface area should have approximately 28 Å². Intercalative binding is primarily driven by charge-transfer-complexes, dipole-dipole- and electrostatic interactions. The redox activity of the para-conjugated quinone ring is also beneficial for the radical cleavage of DNA, as shown in doxorubicin, daunorubicin or mitoxantrone. A positive charge is generally needed for activity, which is usually provided by a protonable or quaternised aromatic nitrogen of the chromophore. A very large number of chemically heterogenous compounds has been screened and positively identified as DNA intercalating compounds, of which many show cytotoxic activity. It could be demonstrated that the cytotoxic effects were primarily caused by inhibiting the topoisomerase enzymes by formation of ternary complexes with DNA, called the “ternary cleavable complex”. Many DNA intercalators are now recognised as a class of topoisomerase inhibitors.

1.7.2 Topoisomerase Inhibitors - Modes of Action and Cardiomyopathy

Topoisomerase enzymes are necessary during replication and transcription where they regulate the overwinding and underwinding of the DNA. This is achieved by forming a DNA-topoisomerase-complex, which is referred to as “cleavable complex” which temporarily introduces DNA single strand (topoisomerase I, TOP1) or double strand breaks (topoisomerase II, TOP2). After successful replication or transcription the strands are reannealed and the enzyme dissociates from the DNA.

Human DNA topoisomerase I (TOP1) is an essential enzyme that relaxes DNA supercoiling during replication and transcription by generating DNA single-strand breaks and religates the cleaved strand to reestablish intact duplex DNA. TOP1 is target for the treatment of human cancers. Camptothecin derivative topotecan and irinotecan belong to the most effective anticancer agents in clinical practice. Topotecan is approved for the treatment of ovarian and lung cancer, irinotecan for the treatment of colon cancer.

Clinical limitations of the camptothecin derivatives include spontaneous inactivation to a lactone form in blood, overexpressing membrane transporters resulting in resistance of cancer cells, rapid
reversal of the trapped cleavable complex after drug removal, requiring prolonged infusions, and dose-limiting side effects of neutropenia and diarrhea.

To overcome these limitations, non-camptothecin indenoisoquinoline derivatives as inhibitors of TOP1 were developed which are chemically stable in blood, more effective as anti-cancer compounds in animal models, inhibitors of TOP1 cleavable complexes at distinct sites and not substrates of membrane transporters. Based on these characteristics, the indenoisoquinoline derivatives indotecan and indimitecan are currently under evaluation in a Phase I clinical trial for patients with relapsed solid tumours and lymphomas.

Topoisomerase II has two closely related isoforms, designated α and β, in vertebrate species. TOP2α and TOP2β share 70 % amino acid sequence identity, but are encoded by separate genes and have different molecular masses (170 kDa and 180 kDa, respectively). The α isoform is the type II topoisomerase originally described in mammalian species and mainly represents the enzyme that was characterised as topoisomerase II in early studies.

Drugs targeting TOP2 can be divided into TOP2 poisons and TOP2 catalytic inhibitors. TOP2 poisons can be further subdivided into intercalating and non-intercalating poisons. The intercalators include doxorubicin and other anthracyclines, mitoxantrone and other compounds that are not currently in clinical use, such as ellipticine and amonafide. They share only the ability to intercalate into the DNA, but there is no apparent chemical similarity that would explain their ability to trap TOP2. Non-intercalating TOP2 poisons include teniposide and etoposide, as well as fluoroquinolones, a class of widely used oral broad-spectrum antibiotics, which are mainly affecting prokaryotic topoisomerases. Non-intercalating TOP2 poisons don't interact tightly with the DNA, hence it has been suggested that protein–drug interactions are responsible for trapping TOP2 covalent complexes. The biochemical mechanism by which TOP2 poisons exert their cytotoxic effects seem to be multifaceted, however their role as a topoisomerase II poison has been well established.

Unlike catalytic inhibitors of topoisomerase II, which block the catalytic function of the TOP2 enzyme, the drugs poison the enzyme by increasing the steady-state concentration of its cleavage complexes. This converts TOP2 into a physiological toxin inducing double-stranded DNA breaks triggering numerous mutagenic and cytotoxic events, such as deletions, insertions, and illegitimate recombination. "Due to their mechanism of action, these drugs are considerably more lethal to cells that contain high levels of topoisomerase II and are undergoing high rates of DNA replication. Since the concentration of the enzyme is usually elevated in rapidly proliferating or transformed cells, clinically aggressive cancers appear to be most responsive to these drugs. Although both isoforms of topoisomerase II probably interact with anticancer drugs in human cells, several lines of evidence suggest that the α-isoform of the enzyme is the primary cytotoxic target of these agents." Associated with this mode of action seems to be the cardiotoxicity which is inherent to the anthracyclins such as doxorubicin and the anthracenedione derivative mitoxantrone. This side effect is generally attributed to the generation of free radicals by the anthracyclines. This is usually explained with the quinone moiety in ring B, which can undergo a reduction to its hydroquinone form via an intermediate semiquinone which subsequently reduces oxygen into reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radicals. ROS can exert cellular damage via DNA, cell membrane, and other intracellular macromolecules. The relevance of ROS regarding antitumour effects and cardiomyopathy is still heavily discussed, as it
INTRODUCTION

is uncertain if the generated amounts of free radicals at clinically relevant drug concentrations could be responsible for anthracycline toxicity to the tumour and to cardiac tissue. The application of antioxidants such as α-tocopherol, probucol, melatonin, N-acetylcysteine to counteract cardiotoxicity was promising in preclinical models, but the outcome in clinical studies was not convincing.57, 58 In this context, lipid peroxidation of cell membranes through free-radical production and their antitumour effects is doubtful.

Instead, an alternative molecular mechanism was described recently by Zhang and co-workers,59 which could - if confirmed in future investigations - broaden our molecular understanding about TOP2-inhibitors and their associated therapeutic disadvantages.

Of both isoforms TOP2α and TOP2β the latter is the only isoform expressed in mammalian cardiomyocytes, whereas proliferating cells, such as cancer cells preferentially express TOP2α. The authors demonstrated in a mouse model with cardiomyocyte specific deletion of TOP2β that "in the presence of TOP2β, doxorubicin activates the DNA response and apoptosis pathways and triggers a marked alteration in the transcriptome that selectively affects oxidative phosphorylation and mitochondrial biogenesis in cardiomyocytes."59 These results suggest that redox cycling of doxorubicin is not solely responsible for doxorubicin-induced cardiotoxicity, but implicate that TOP2β is the essential driver of doxorubicin-induced cardiotoxicity.59 Once confirmed, these findings could have the potential to incorporate new aspects in cancer therapy. Novel selective TOP2α inhibitors without cardiotoxic properties or selective TOP2β protecting molecules could be designed.

1.7.3 Mitoxantrone

Bearing the cardiotoxic properties of doxorubicin in mind hundreds of anthracyclines have been synthesised in hope to overcome these side effects but only daunorubicin, doxorubicin, epirubicin, idarubicin, aclarubicin, pirarubicin and valrubicin earned clinical approval.60 These second generation anthracyclins have altered pharmacokinetic and pharmacodynamic profiles as well as improved efficacy, but the cardiomyopathy is still an unresolved issue. Another disadvantage is the complex chemical structure which makes synthesis difficult.

Originally designed as stable dye, mitoxantrone can be regarded a simplified analogue of anthracyclines with a planar anthracenedione nucleus. The non-planar ring was removed to form a tricylic system with two identical side chains (Figure 2). Structure-activity-relationship (SAR) studies revealed the importance of one hydroxyl and one carbonyl group. The nitrogen-containing side chains is also important for the activity as it stabilises the ring in between base pairs by intercalating with the negatively charged phosphate backbone of the DNA.63

![Figure 2. Simplification of Doxorubicin](image)
Mitoxantrone is used in the treatment of metastatic breast cancer, acute myeloid leukemia, and non-Hodgkin's lymphoma. In combination with prednisone it is approved as a second-line treatment for metastatic hormone-refractory prostate cancer. Mitoxantrone also inhibits T-cell, B-cell, and macrophage proliferation, and is indicated for reducing neurologic disability and relapse frequency in patients with secondary progressive multiple sclerosis (SPMS), progressive relapsing MS, or worsening relapsing-remitting MS (RRMS).\(^\text{62}\)

In addition to the topoisomerase-based mechanism there is one which was discovered first in doxorubicin as well as daunorubicin, finding stable covalent DNA adducts in the presence of formaldehyde as illustrated in Figure 3.

Later, this mechanism was also discovered in mitoxantrone and pixantrone. Formaldehyde activates mitoxantrone to produce the adducts which stabilise DNA by forming interstrand crosslinks (Figure 4).\(^\text{64, 65}\)

The drug is attached covalently to only one strand with sufficient local stabilisation deriving from the intercalated drug plus hydrogen bonding (the localisation of the presumed hydrogen bonds has not yet been clarified) to the second DNA strand (non-covalent), similar to that of doxorubicin-DNA adducts.\(^\text{64}\)
This may be biologically relevant since cells of myeloid origin have elevated levels of formaldehyde and mitoxantrone is effective against myeloid tumours.\textsuperscript{65} Inflammation sites of solid tumours attract neutrophils releasing hydrogen peroxide which oxidises the biologically available polyamines, such as spermine to generate formaldehyde upon respiratory burst.\textsuperscript{65, 66} This appears to be a Fenton reaction and is preceded by the Cu(II)/EDTA-catalysed oxidation of Tris to formaldehyde.\textsuperscript{67, 68} This provides an increased level of formaldehyde at the site of these solid tumours and may also result in activation of mitoxantrone. It could also be demonstrated that the crosslink formation is concentration dependent from both formaldehyde and mitoxantrone. The same correlation could be shown for pixantrone.\textsuperscript{69} Similarly, synthetic doxorubicin-formaldehyde-derivatives showed increased cytotoxic activity, presumably because they do not require drug-induced intracellular production of formaldehyde.

\subsection*{1.7.4 Pixantrone\textsuperscript{69}}

Pixantrone was prepared as second-generation group of anthracenediones with the aim to develop compounds with better therapeutic efficacy and reduced side-effects. The anthraquinone nucleus of mitoxantrone was retained, and the 5,8-dihydroxy phenylene ring implicated in its cardiotoxicity was substituted by a pyridine moiety. Krapcho et al. rationalised that these nitrogen atoms may provide basic sites or improved hydrogen bonding, therefore providing the analogues with a potentially greater affinity for DNA and topoisomerase II. Moreover, the side chain was truncated to an aminoethyl group as a primary amino function (\textit{Figure 5 on page 15}).

Pixantrone emerged as the most promising drug candidate and demonstrated superior anti-leukemic activity in mice over a wide, well tolerated range of doses compared with mitoxantrone. Further studies showed that pixantrone has a wide spectrum of anti-tumour activity, and a marked efficacy against lymphomas and leukemias. Histopathological evaluation of the heart tissue in these studies revealed that pixantrone induced no detectable delayed cardiotoxicity. These key findings prompted the entry of pixantrone into clinical trials for further development. In 2012, pixantrone was approved by the EMA in the treatment of relapsed or refractory aggressive NHL.

The mechanism of action is like in mitoxantrone probably multimodal. Pixantrone interacts with DNA via intercalation and as topoisomerase II poison. Mitoxantrone and pixantrone share close structural similarity, nevertheless the primary amino group of pixantrone in each of its side chains is more susceptible to formaldehyde activation and consequently formaldehyde-activated pixantrone reacts far more efficiently with DNA in vitro to generate covalent drug-DNA adducts than mitoxantrone and also exhibits greater stability than the corresponding mitoxantrone species. This may also be important since an extended pixantrone–DNA adduct half-life may maximise cytotoxicity and enable an enhanced disruption of critical cellular processes such as DNA transcription and replication.
2 AIMS OF THE THESIS

The aim of this research was to modify and expand the core structure which was previously synthesised at the Department of Drug and Natural Product Synthesis to further enhance its cytotoxic potential.

The rationale for the development of novel cytotoxic compounds was based on the works of Krapcho and Menta who have synthesised a number of aza-bioisosteric chemotypes related to mitoxantrone that lack the 5,8-dihydroxy substitution pattern and have shown that in these chemotypes the positioning of the nitrogen atom exerts a profound effect on the expressed antitumour activity. This modification led to the development of the topoisomerase II inhibitor BBR 2778, which showed antitumour activity, but lacks cardiotoxicity. It is now known by its international non-proprietary name pixantrone dimaleate, and in 2012 it received conditional marketing authorisation in the European Union (Pixuvri) as monotherapy to treat adult patients with relapsed or refractory aggressive Non-Hodgkin B-Cell lymphomas. However, pixantrone lacks the wide range of therapeutic use compared to doxorubicin and mitoxantrone. The goal to develop a potent anticancer agent lacking the cardiotoxicity while retaining the broad therapeutic spectrum of mitoxantrone could not be achieved yet.

![Figure 5. Bioisosteric Modification of Mitoxantrone](image)

In this context, in cooperation with Aeterna-Zentaris our department subjected the tricyclic skeleton of pixantrone to bioisosteric replacement of the diamino substituted benzene ring with a pyrrole unit. Extensive variations on pyrrole, as well as pyridine, pyrazine and pyrimidine annulation were performed. Furthermore, different basic amino and DNA alkylating side chains were linked to the pyrrole nitrogen (Figure 6 on page 16).
In the following, the tricyclic nucleus was expanded by one benzene ring into a pyrido-annulated carbozole dione of the ellipticine quinone type which is known to possess cytotoxic properties (Figure 7). A regioisomer of ellipticine quinone endowed with the same side chains as its tricyclic precursors was prepared.

The synthesised compound library was tested by Aeterna-Zentaris against a panel of standard cancer cell lines using a XTT antiproliferative assay revealing that the ellipticine quinone isomer endowed with a \( N,N \)-dimethylaminoethyl side chain (16) was the most cytotoxic compound so far (Table 1).

<table>
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</tbody>
</table>

*Table 1. Biological Evaluation of 16*
The aim of this thesis was to use compound 16 as a scaffold for further investigations. We were interested in modifying the heterocyclic system as well as expanding the core to pentacyclic isomers of the known anticarcinogenic natural compounds calothrixin A and B\(^8\) linked to the same basic side chain which has demonstrated to be the most promising so far (Figure 8).

![Figure 8. Structural Modification of 16](image)

Thus, we were interested in expanding our library by the following target compounds and evaluating their cytotoxic potential (Figure 9). In the following, the most active candidates from the antiproliferation assays should serve as scaffolds for further side chain modifications.

![Figure 9. Target Compounds](image)
3 RESULTS AND DISCUSSION

3.1 Synthesis of 6-[2-(dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (7)

The construction of the 5H-pyrido[3,2-b]carbazole-5,11(6H)-dione ring system 6 was achieved by the known procedure of Ashcroft et al.\textsuperscript{84,85} in three steps. The synthesis of 7 required in total seven steps which is outlined in Scheme 1.

\textbf{Scheme 1. Synthesis of 7}
3. 1. 1 Synthesis of Furo[3,4-\textit{b}]pyridin-5,7-dione (1)

The synthesis of commercially available 1 was achieved by a simple condensation reaction of pyridine-2,3-dicarboxylic acid in the presence of acetic anhydride which was carried out according to the procedure by Vitry et al.\textsuperscript{82} by refluxing pyridine-2,3-dicarboxylic acid in acetic anhydride for 3 h and purification by sublimation to give 1 as white crystals in 75 \% yield. The identity was confirmed by NMR spectroscopy and was in accordance with literature (compare 6. 2. 1 on page 83).

3. 1. 2 One-Pot-Synthesis of Furo[3,4-\textit{b}]pyridin-5(7)-one (2)\textsuperscript{81}

Key intermediate 2 was prepared by a more recent procedure described by Inoue et al.\textsuperscript{81} which comprises two steps from pyridine-2,3-dicarboxylic acid or one step from commercially available furo[3,4-\textit{b}]pyridin-5,7-dione (1), respectively, and is thus favourable to the 4-step synthesis by Ashcroft et al.\textsuperscript{79} which results in lower overall yields and also employs a reduction step using highly inflammable LiAlH\textsubscript{4}.

The reaction involves the regioselective reduction of anhydride 1 with NaBH\textsubscript{4} in the presence of acetic acid in dry THF. The authors suggest that in the presence of the additive acetic acid the pyridine nucleus of 1 might be partially protonated or chelated and the 7-carbonyl group would be more activated for an attack by the hydride ion in comparison with the 5-carbonyl group, which would preferentially produce intermediate 2-(hydroxymethyl)nicotinic acid. The lactonisation thereof was executed without isolation by treatment with acetic anhydride. After the aqueous workup and extraction with dichloromethane, the pure product could be obtained by recrystallisation from isopropanol, affording long, pale brown needles. Some discolouration and little of the undesired isomer could be removed by purification using column chromatography (silica gel, ethyl acetate) affording a colourless crystalline product. The identity of the product was confirmed by NMR spectroscopy which is in accordance with literature\textsuperscript{81} (compare 6. 2. 2 on page 84).
3. 1. 3 Synthesis of 1-(phenylsulfonyl)-1H-indole (3)

Building block 3 was prepared according to the procedure described by Conway et al.\textsuperscript{83} Indole was added to an ice-cold mixture of powdered NaOH and tetra-n-butylammonium hydrogen sulfate in dry dichloromethane under argon in one portion, followed by a solution of benzenesulfonyl chloride in dry dichloromethane. After 2 h of vigorous stirring the obtained thick orange oil was recrystallised twice from hot methanol to afford white needles in 75 % yield. The identity of the product was confirmed by NMR spectroscopy (\textit{compare 6. 2. 3 on page 85}).

3. 1. 4 Synthesis of [2-(hydroxymethyl)-3-pyridinyl][1-(phenylsulfonyl)-1H-indol-2-yl]methanone (4)\textsuperscript{84}

The condensation of 2-lithiated synthon 3 and lactone 2 gave the desired ketone 4. The lithiation was conveniently effected with commercial n-BuLi initially at -78 °C and then at room temperature. The condensation yielded 21.8 % of the desired intermediate (after purification) which could be used without purification in the following cyclisation step. Yet, the product was purified by column chromatography for NMR spectroscopic purposes and melting point determination. The successful reaction was indicated in the $^1$H spectra by the absence of the H-2 proton of the indole, and the emerging of the methylene singlet counting two protons at 5.12 ppm and a broad singlet at 4.93 ppm, which can be attributed to the hydroxy group showing no correlation to a carbon atom in the HSQC spectrum. Moreover, the $^{13}$C NMR clearly indicates the introduction of the carbonyl-C at 193.1 ppm. HRMS analysis is in agreement with the calculated mass (\textit{compare 6. 2. 4 on page 86}).

Ketone 4 was transformed into the corresponding pyrido-oxepino-indole 5 by intramolecular indole-β-nucleophilic substitution. This reaction was performed in refluxing aqueous methanolic NaOH. The reaction time of not exceeding 2-3 minutes is essential for good yields, longer reaction time deteriorates the results, as the initial product disappears. The successful ring-formation was indicated in the $^1H$ spectrum by the absence of the indole H-3 proton an the OH-proton of 4, also the aromatic signals of the protecting group have disappeared and the broad singlet at 11.39 ppm can be assigned to the indole NH proton. The $^{13}$C spectrum is also consistent with these results as are the 2D-NMR experiments. These findings are also in agreement with HRMS analysis (compare 6. 2. 5 on page 87).

3. 1. 6  **Synthesis of 5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (6)**

Pyrido-oxepino-indole 5 was subsequently transformed into quinone 6 under refluxing aqueous methanolic NaOH with the passage of air. Literature suggests that “the quinone-forming process to work best/at all when the methylene hydrogens in the oxepino-indole are acidified (either by a fused pyridine or quinoline ring or by an aromatic nitro-group).” The formed anion seems to be in equilibrium with the benzene oxide which is trapped by oxygen giving the product 6 at the oxidation level of the quinone (Figure 10).

*Figure 10. Suggested Quinone-forming Process*
The mixture was poured into water and the reddish-brown precipitate was filtered off, dried and purified by sublimation to afford 95% of 6 as yellow crystals. The identity of the desired compound was elucidated by NMR spectroscopy and HRMS analysis (compare 6.2.6 on page 88). In particular, the carbazole NH proton gives a broad singlet at around 12 ppm in the 1H spectrum which was found to be characteristic for all nuclei of these series, as are the two quinone carbons at about 178 ppm to 180 ppm.

3.1.7 Synthesis of 6-[2-(dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (7)

The alkylation of compound 6 is a S$_N$2 reaction which was carried out by a standard procedure described in countless publications by forming the pyrido-carbazole anion using sodium hydride followed by substitution of 2-chloro-$N,N$-dimethylethanamine hydrochloride in dry DMF. The product was extracted with ethyl acetate and purified using column chromatography to yield 26% of 7 as orange-brown crystals. Structure elucidation via NMR spectroscopy showed two N-methyl groups giving a singlet at 2.41 ppm and two pseudo-triplets at 4.84 ppm for the methylene group attached to the carbazole N and at 2.78 ppm for the methylene group adjacent to the dimethylamino group. The absence of the carbazole NH singlet and the spatial proximity of the methylene signals to H-7 demonstrated via NOE experiment are indicative for the desired substitution pattern. HRMS analysis supported the results of the NMR experiments (compare 6.2.7 on page 89).

3.2 Synthesis of 10-[2-(dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (12)

The synthesis of 12 in analogy to the methodology for 16 described by Ashcroft et al. would have required the preparation of the corresponding isomeric lactone as necessary building block in 3 steps employing the double esterification of pyridine-2,3-dicarboxylic acid followed by reduction to the diol with LiAlH$_4$ and finally oxidation with MnO$_2$ in chloroform (Scheme 2 on page 23).
Additional four steps for the construction of the final compound in analogy to compound 7 makes this synthetic pathway unattractive and was dismissed in favour of a method developed by Ketcha and Gribble\cite{86}, which was successfully used for the construction of 16 by Leber\cite{70}.

This strategy should employ a Friedel-Crafts C-3 acylation of 1-(phenylsulfonyl)-1\textsubscript{H}-indole with carboxylic acid anhydrides or acid chlorides in the presence of aluminium chloride to give 3-acyl-1-(phenylsulfonyl)indoles in good yields. The ketoester would then be prepared by Fischer esterification of the carbocyclic acid with ethanol in presence of p-TsOH over 5 days followed by the ring-formation step with LHMDS with in situ N-deprotection. The synthesis of compound 12 should be achieved in analogy to this methodology as depicted in Scheme 3.

1-(phenylsulfonyl)-1\textsubscript{H}-indole (3) was stirred with furo[3,4-\textit{b}]pyridine-5,7-dione (1) in the presence of aluminium chloride in dry dichloromethane at room temperature for two hours. After the workup procedure NMR spectroscopy revealed that the desired product was not formed, but anhydride 1
was hydrolysed presumably during the aqueous workup and only pyridine-2,3-dicarboxylic acid and 1-(phenylsulfonyl)-1H-indole (3) could be recovered. Several attempts to modify the reaction conditions with varying amounts of aluminium chloride and/or reaction times did not succeed either.

Following a literature survey of similar compounds we abandoned the Friedel-Crafts-acylation strategy in favour of an ortho metallation strategy applied for the construction of the calothrixins by Watanabe and Snieckus\(^8\) and further developed by Kelly et al.\(^9\)

The well-established directed ortho metallation\(^9\) strategy to construct the tetracyclic nucleus should be applied, starting from key compounds 1-(methoxymethyl)-1H-indole-3-carbaldehyde (8) and \(N,N\)-diethylpyridine-2-carboxamide (9). Although this method is unattractive in terms of overall yield compared to other strategies, it is highly convergent and the building blocks 8 and 9 are easily prepared in good yields.

Since the reaction conditions reported by Watanabe and Snieckus\(^8\) failed in the construction of the calothrixins, the optimised reaction conditions of Kelly et al.\(^9\) were applied. The same methodology was later applied for the preparation of calothrixin analogues. After the construction of the MOM-protected nucleus the protecting group would be removed and the side chain should be linked to the carbazole nitrogen (Scheme 4).

\[\text{Scheme 4. Directed Ortho Metallation}\]
3. 2. 1  Synthesis of 1-(methoxymethyl)-1H-indole-3-carbaldehyde (8)

The method of Comins et al.\textsuperscript{91} was used. A solution of 1H-indole-3-carbaldehyde in dry THF was added to a cooled suspension of sodium hydride in dry THF. The indole proton is abstracted by sodium hydride giving the indole anion and the N-methoxymethyl protecting group (N-MOM) was introduced in a nucleophilic substitution (S\textsubscript{N}2) reaction by slowly adding chloro(methoxy)methane into the reaction vessel. After warming to room temperature and stirring for 30 min the reaction mixture was poured into cold aqueous 5 % NaHCO\textsubscript{3} solution, extracted with diethyl ether, and evaporated in vacuo. The resulting crude product was purified by balltube distillation or recrystallisation from ethyl acetate/light petroleum (1:1, v/v) to afford beige crystals in good yields. Structure elucidation via NMR spectroscopy confirmed the identity of the product (compare 6. 2. 8 on page 90).

3. 2. 2  Synthesis of N,N-Diethylpyridine-2-carboxamide (9)

The method of Swain et al.\textsuperscript{92} was used to prepare the amide. It employs a simple condensation reaction of a carboxylic acid and an amine with the strong dehydrating agent phosphorous pentoxide. Pyridine-2-carboxylic acid and diethylamine were refluxed in dry toluene in presence of excess phosphorous pentoxide for 4 hours. The product was extracted with toluene/diethyl ether, dried, and the solvent was removed in vacuo. The brown residue was purified by balltube distillation or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to give the title compound as yellow oil in good yields. A benefit of this method is the simple reaction setup and the proceeding of the reaction requires little attention. Therefore, this method was extended to the following diethylcarboxamides which were prepared accordingly from the corresponding carboxylic acids to give yellow to orange oils in 64 to 84 % yields.

The identity of all compounds was verified via NMR spectroscopy (compare 6. 2. 9 on page 91, 6. 2. 13 on page 95, 6. 2. 18 on page 100 and 6. 2. 24 on page 106).
3. 2. 3 **Synthesis of 10-(methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (10)**

The building blocks 8 and 9 were coupled in a one-pot operation to give 10 directly. None of the putative intermediates were isolated, but the choice of LiTMP as base was crucial. Although Snieckus and Watanabe successfully constructed 6-(methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (18) using sec-BuLi as base, Kelly et al. failed in the construction of the calothrixin system. Tert-BuLi and hexamethyldisilazide were equally useless resulting in no product. The more basic LDA produced only small amounts. The even more basic LiTMP proved to be most effective.

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine to a cold (−70 °C) solution of n-BuLi in dry THF, under argon, and then stirring the mixture at −5 to 0 °C for 1 hour. The temperature was then reduced to −78 °C and a solution of the diethylamide 9 and aldehyde 8 in dry THF was added dropwise in such a rate, that the temperature did not exceed −60 °C. The reaction solution turned orange. The flask was then taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h). During this time the solution turned dark. The time allowed to reach room temperature was crucial for acceptable product yield. Prolonged stirring in the cold as well as stirring overnight generated undesired side products.

The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried over Na₂SO₄ and the solvent was evaporated to furnish an orange residue which was purified by column chromatography, eluting with ethyl acetate/light petroleum (4:6, v/v) giving the desired product in 33 % yield as an amorphous orange solid which could be further purified by recrystallisation from ethyl acetate.

After our first attempt of reacting 8 with 9 by adding the electrophile 30 min after lithiating the amide, NMR structure elucidation revealed that the amide had undergone a dimerisation process to give N,N-diethyl-3-(2-pyridinylcarbonyl)-2-pyridinecarboxamide (**Figure 11 on page 27**). This is a recurring problem in lithiations with -CONEt₂, which was overcome by an in situ trapping technique by adding substrate and electrophile simultaneously to the LiTMP solution.
We could demonstrate that this procedure could be extended to the synthesis of the intermediary MOM-protected isomeric pyridocarbazole-diones 14, 18 and the pyrazinocarbazole-dione 24, from which we could access the desired basic substituted derivatives in two steps. Nevertheless, the construction of compound 24 was more difficult, yields were comparably low (approximately 10%) generating unwanted tarry side products which made the purification process challenging. These findings were not surprising and are usually explained with the preferential reactivity of the electrophiles with the nitrogen atoms and the very low reactivity of these heterocycles towards electrophilic substitution on the carbon atoms. The electron-withdrawing effect of the additional nitrogen ring atom decreases the energy level of the LUMO of the pyrazines making them more reactive towards nucleophiles than pyridines. Although the lithiated species is stabilised by the electron-withdrawing effect of the ring nitrogen atom, it is, however, destabilised by electronic repulsion between the carbanion and the lone pair of the adjacent nitrogen.\(^\text{24}\)

\begin{align*}
1^\text{H NMR (500 MHz, CDCl}_3\text{)} & \delta 8.65 (dd, J = 4.9, 1.7 Hz, H-6, 1H), 8.54 (ddd, J = 4.8, 1.7, 0.9 Hz, H-6', 1H), 8.18 (ddd, J = 7.9, 1.2 Hz, 0.9 Hz, H-3', 1H), 8.08 (ddd, J = 7.8, 1.7 Hz, H-4, 1H), 7.88 (ddd, J = 7.9, 7.5, 1.7 Hz, H-4', 1H), 7.44 (dd, J = 7.7, 4.9 Hz, H-5, 1H), 7.43 (ddd, J = 7.5, 4.8, 1.2 Hz, H-5', 1H), 3.52 (q, J = 7.0 Hz, H-10', 2H), 3.25 (q, J = 7.1 Hz, H-10, 2H), 1.33 (t, J = 7.1 Hz, H-11', 3H), 0.80 (t, J = 7.1 Hz, H-11, 3H).
\end{align*}

\begin{align*}
1^3\text{C NMR (125 MHz, CDCl}_3\text{)} & \delta 193.9 (C-7), 167.8 (C-8), 155.6 (C-2), 153.8 (C-2'), 149.1 (C-6), 148.0 (C-6'), 137.8 (C-4), 137.1 (C-4'), 134.3 (C-3), 126.6 (C-3'), 123.6 (C-5), 123.5 (C-3'), 43.3 (C-10'), 39.6 (C-10), 13.7 (C-11'), 11.6 (C-11).
\end{align*}

\begin{align*}
1^5\text{N NMR (50 MHz, CDCl}_3\text{)} & \delta -68.6 (N-1'), -74.3 (N-1), -252.6 (N-9).
\end{align*}

The identity of all MOM-pyridocarbazole-diones was confirmed by NMR structure elucidations studies and HRMS (compare 6.2.10 on page 92, 6.2.14 on page 96, 6.2.19 on page 101 and 6.2.25 on page 107). In particular, in all instances the MOM protection group can be identified in the \(^1\)H NMR spectrum by the methyl singlet at approximately 3.40 ppm and its methylene singlet at approxi-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure11.png}
\caption{Dimerisation Product of 9}
\end{figure}
In the $^{13}$C spectrum the two carbonyl groups can be observed at approximately 180 ppm. In case of MOM-protected pyrazine derivative 24, an unambiguous NMR spectroscopic signal assignment of the fused pyrazine unit was not possible via HMBC experiment, which is also valid for all other pyrazine derivatives.

3.2.4 General Procedure for the Synthesis of 11, 15, 19 and 35 by BBr$_3$ Effected MOM Cleavage

The cleavage of the MOM protecting group was accomplished according to literature procedure employed by Bernardo et al. by reacting the educts with 1.2 eq. of BBr$_3$ in dry dichloromethane at 0 °C and stirring at room temperature for 1 h (Scheme 5). The reaction mixture was quenched with saturated NaHCO$_3$ solution and stirred for 1 h at 60 °C. The product was collected by suck filtration. The filter cake was washed with water and remaining starting material could be removed by washing with dichloromethane which is an excellent solvent for the starting material, but not for the product. The filter cake was recrystallised from ethyl acetate to afford the unprotected nucleus in 75 to 95 % yield. The successful deprotection step was verified by NMR investigation and HRMS. The $^1$H spectra show the absence of the methyl and the methylene protons of the MOM group, and a broad singlet of the newly formed carbazole NH proton at approximately 13.20 ppm can be observed. Moreover, in case of 11 (the first compound derived from this approach) a NOESY experiment was performed, showing the spatial proximity of the carbazole NH and the adjacent H-9 proton (compare 6.2.11 on page 93, 6.2.15 on page 97, 6.2.20 on page 102 and 6.2.26 on page 108).

Scheme 5. BBr$_3$ Effected MOM Cleavage
3. 2. 5 General Procedure of Sodium Hydride Effected Alkylation of 11, 15, 19 and 25

The functionalisation of the nuclei to give the title compounds was carried out in analogy to compound 7 (compare 3. 1. 7 on page 22) as outlined in Scheme 6. In all cases except for compound 26 the purification step of the desired compounds involved column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v), followed by ethyl acetate/methanol (8:2, v/v) and recrystallisation from ethyl acetate to give orange or brown crystals. Compound 26 was purified eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) and recrystallisation from ethyl acetate. NMR experiments showed a consistent set of signals for the side chains in the $^1$H spectra with very similar shifts and comparable peak patterns relating to compound 7. HRMS analyses are in agreement with the NMR spectroscopic identification.

Scheme 6. Sodium Hydride Effected Alkylation
We were also interested in the synthesis of isomeric calothrixin analogues functionalised with basic amino side chains and the evaluation of their cytotoxic potential in vitro. The tetracyclic pyridocarbazole system should be expanded by benzo-annulation into pentacyclic quinoline-annulated carbazoledione systems to give calothrixin isomers. Then different basic side chains should be linked to the skeleton. This step is consistent with previous structure modification steps and in-vitro evaluation. The decision was supported by the known cytotoxic properties of the calothrixin skeleton against certain cancer cell lines as well as their inhibitory effect on the growth of plasmodium falciparum.

Some of the isomeric calothrixin analogues were constructed by directed ortho metallation following the method of Kelly et al. (Scheme 7), which was also successfully adapted for the synthesis of some tetracyclic compounds, as described in the previous chapters.

Scheme 7. Synthesis of Calothrixin B Derivative 65
3.3.1 Synthesis of $N,N$-diethylquinoline-4-carboxamide (61)

![Chemical structure of 61]

The known method of Gilman and Spatz was modified. Oxalyl chloride was used instead of phosphoryl chloride for the formation of the intermediate acid chloride which resulted in shorter reaction time and better product yield. Quinoline-4-carboxylic acid was treated under cooling with oxalyl chloride in presence of catalytic amounts of DMF to form an intermediate acid chloride which reacts in an $S_{N}2t$ type reaction with $N,N$-diethylamine to give the title compound (61) which was obtained in good yield as yellow-orange oil after extraction and purification by column chromatography eluting with ethyl acetate/light petroleum (4:1, v/v). The identity of the product was confirmed by NMR spectroscopy (compare 6.2.63 on page 145).

3.3.2 Synthesis of 12-(methoxymethyl)-7$H$-indolo[3,2-j]phenanthridine-7,13(12$H$)-dione (62)

![Chemical structures of 61, 8, and 62]

The construction of compound 62 was performed in analogy to 10, 14, 18 and 24 except for the trapping technique applied during the reaction. A dimerisation process occurring during the ring formation process of the tetracyclic nuclei required an in situ trapping of substrate and electrophile to give the desired compounds. Following the publication for constructing the calothrixin B skeleton this was unnecessary.

The DoM reaction was accomplished by adding a solution of the diethylamide 61 and after 15 minutes of stirring a solution of aldehyde 8 to a prepared solution of LiTMP at -78 °C. After allowing to reach room temperature over 2 h, the reaction mixture was quenched with water and after extraction with ethyl acetate and purification by column chromatography eluting with ethyl acetate/light petroleum 4:6 (v/v) the title compound was obtained as an amorphous orange-red solid. The structure of the title compound was confirmed via 2D-NMR technique and HRMS and is in agreement with literature.
3. 3. 3 Synthesis of 7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (63)

The deprotection of 62 was realised by the same procedure as described for compounds 11, 15, 19, and 25 (compare 3. 2. 4 on page 28). The detailed procedure as well as NMR spectroscopic assignment is described in chapter 6. 2. 65 on page 147.

3. 3. 4 Synthesis of 12-[2-(dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (65)

The alkylation of 63 was performed in analogy to 6 by treating the calothrixin B framework with sodium hydride in dry DMF and subsequent reaction with 2-chloro-N,N-dimethylethanamine hydrochloride in a S_N2 type reaction to give 65 as red-orange crystals in 39.5 % yield. The detailed procedure as well as NMR spectroscopic assignment is referenced in chapter 6. 2. 67 on page 149.

3. 4 Synthesis of 12-[2-(dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (66)

The synthesis of the title compound was achieved by following the procedure of Kelly et al.\(^8\) which involved N-oxidation of calothrixin B (63) with 3-chloro perbenzoic acid in dichloromethane and alkylation of the resulting product. As would be expected upon switching from 65 to the corresponding calothrixin-N-oxide 66 a shift of δ to lower frequency of N-5 from -53.7 ppm to -88.3 ppm was
RESULTS AND DISCUSSION

apparent, although higher than in the transition from pyridine to pyridine-N-oxide (approximately 23 ppm). This shift can be explained by resonance structures which describe both π-electron donor- and acceptor functions of the N-oxide moiety.\textsuperscript{139}

In the $^{13}$C NMR spectra of 64 and 66, the expected shifts to lower frequencies for the carbons in ortho and para positions to N-5 (C-6: -15.7/-15.5 ppm, C-4a: -8.2/-8.0 ppm, C-13a: -10.7/-10.4 ppm) can be observed (relative to the corresponding shifts in the parent compounds 63 and 65). This can be explained by resonance structures which indicate an increase in electron density in these positions.\textsuperscript{140}

A detailed NMR spectroscopic characterisation of 64 and 66 is in chapter 6. 2. 66 on page 148 and 6. 2. 68 on page 150, respectively.

3. 5 Synthesis of 8-[2-(dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (80)\textsuperscript{142}

The synthesis of the isomeric calothrixin derivative 80 should be achieved in analogy to 65 as outlined in Scheme 8.\textsuperscript{142} It was uncertain which position of the quinoline system would be attacked preferentially by the electrophile. The successful cyclisation step would depend on a lithiation at position 4. A literature review by Snieckus\textsuperscript{90} suggested that the electrophile would indeed preferentially attack position 4 over position 2. In the following, Quinoline-3-carboxylic acid was converted into its corresponding ortho directing diethylamide derivative via acid chloride by treatment with oxalyl chloride in dry dichloromethane and a catalytic amount of DMF followed by reacting with diethylamine. The resulting diethylamide was subjected to the ortho lithiation strategy with 8 to give the corresponding MOM-protected pentacyclic product.

After the BBr$_3$ effected MOM cleavage the indole nitrogen was alkylated with 2-chloro-$N,N$-dimethyllethanamine hydrochloride to give 80 as orange-red crystals. The identity of the title compound was confirmed by NMR spectroscopy and HRMS in chapter 6. 2. 82 on page 164.

![Scheme 8. Synthesis of Isomeric Calothrixin B Derivative 80](image-url)
3. 6 Synthesis of 7-[2-(dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (76)

The synthesis of 76 was the third of four pentacyclic isomers based on a quinoline system. In this case, the lithiation of the 73 would clearly occur at position 3. The resulting system was the linear annulated indolo[3,2-b]acridine system bearing the quinoline nitrogen at position 5. The procedure was performed in analogy to compounds 65 and 80 as depicted in Scheme 9. The detailed spectroscopic characterisation of the title compound can be found in chapter 6. 2. 78 on page 160.
3.7 Synthesis of 11-[2-(dimethylamino)ethyl]-6\textit{H}-indolo[2,3-\textit{b}]acridine-6,12(11\textit{H})-dione (60)

Compound 60 could not be realised by employing an \textit{ortho} lithiation strategy but should be accessible \textit{via} the same route as compound 7 (\textit{compare 3.1 on page 18}). Lactone 57 was accessible in 71\% yield by reacting commercially available 2-aminobenzaldehyde with tetronic acid in ethanol,\textsuperscript{96} and was then reacted with 3 to give a keto-alcohol which was subjected to an intramolecular \textbeta\-nucleophilic substitution without isolation.\textsuperscript{85} Refluxing the resulting intermediate 58 for 6 days in methanolic NaOH converted the oxepino-indole into its quinone 59 which was finally connected to the side chain using the usual procedure (\textit{Scheme 10}).

\begin{equation}
\begin{array}{c}
\begin{array}{c}
\text{NH}_2 \\
\text{O} \\
\text{O} \\
\text{EtOH} \\
\end{array} \\
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{N} \\
\end{array} \\
\begin{array}{c}
\text{N} \\
\text{O} \\
\end{array} \\
\end{array}
\end{equation}

\textit{Scheme 10. Synthesis of Isomeric Calothrixin B Derivative 60}
3.8 Biological Evaluation - Viability Assay of the First Set of Compounds

Since the synthesis of the pyrimidine and pyridazine derivatives could not be accomplished in time, viability assays of the first series of 9 compounds were performed using both EZ4U® XTT assay and a Neutral Red assay for comparison (a detailed protocol of the assays is referenced in chapter 5.4 on page 80).

Two different viability assays targeting different cell organelles were performed to assess the antiproliferative activity of these compounds. Neutral Red measures lysosomal activity to assess viability, whereas XTT targets mitochondrial enzymes. Judged by structural properties an intercalating effect on the DNA and the topoisomerase enzymes was expected, which should result in a quantifiable antiproliferative effect. The rationale for using two different assays was to minimise the chance of flawed test results caused by unknown effects of the test compounds on the targeted organelle. Influences on two different cellular targets by compounds of similar structure on the other hand is unlikely.

The first set of compounds consisted of 9 structures sharing a basic $N,N$-dimethylaminoethyl side chain linked to the carbazole nitrogen of 4 ellipticine quinone and 5 calothrixin analogues, respectively. In previously synthesised compounds of our research group this side chain had exerted the highest cytotoxic effect. The previously synthesised and biologically evaluated compound 16 was used for comparison with the new compounds, mitoxantrone dihydrochloride served as reference compound.

The cells were cultivated on 96-well plates and exposed to increasing concentrations of each compound for 24 h. The results of at least 3 independent experiments with Neutral Red in at least quadruplicate was used to calculate IC$_{50}$ values. For comparison, at least 1 experiment in quadruplicate using the XTT assay was performed. (compare 3.19 on page 68).

IC$_{50}$ values for all compounds were established in SW480 cells (colon carcinoma). All IC$_{50}$ values were calculated with GraphPad Prism® 6 using a non-linear regression model (variable slope, 4 parameters). The expressed values are µM.

As would be expected, a comparable outcome of both assays could be demonstrated on all cell lines with all tested compounds. Exemplarily, comparative dose-response-curves of the most active compounds 20 and 26 against SW480 cells are depicted in Figure 12 on page 38 and Figure 13 on page 38. Since the XTT assay is easier to handle and more sensitive, the second set of compounds, consisting of 17 compounds was performed using XTT only.

Highly cytotoxic tetracyclic derivatives 20 and 26 and one also highly active representative of the pentacyclic series (calothrixin A derivative 66) were also tested against A549 (lung carcinoma), Hep3B (liver cell carcinoma), HTB65 (melanoma) and U373 (glioblastoma) cells (Table 2 on page 37). The skeletons of 20 and 26 were used as lead structures for further side chain modifications.

Our efforts to open a synthetic pathway to the pyrimidine and the pyridazine nuclei were continued.
### Table 2. Antiproliferative XTT (EZ4U®) and Neutral Red Assays.
*IC₅₀* values are expressed in µM. Values of Neutral Red assay are expressed in Red Italic. Numbers in square brackets represent *IC₅₀* values in the 95% confidence interval.

<table>
<thead>
<tr>
<th>Compound</th>
<th>SW480 (Colon) [95%-CI]</th>
<th>A549 (Lung) [95%-CI]</th>
<th>HEP3B (Liver) [95%-CI]</th>
<th>U373 (Brain) [95%-CI]</th>
<th>HTB65 (Skin) [95%-CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>2.69 [1.13 - 6.41]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3.68 [2.10 - 6.43]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.07 [1.16 - 3.69]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.76 [1.49 - 5.11]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.796 [0.696 - 0.911]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.41 [1.00 - 1.97]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.519 [0.499 - 0.539]</td>
<td>0.514 [0.485 - 0.544]</td>
<td>0.627 [0.562 - 0.700]</td>
<td>0.961 [0.804 - 1.15]</td>
<td>0.582 [0.543 - 0.623]</td>
</tr>
<tr>
<td></td>
<td>0.924 [0.708 - 1.21]</td>
<td>0.194 [0.153 - 0.246]</td>
<td>0.584 [0.506 - 0.673]</td>
<td>1.12 [0.643 - 1.94]</td>
<td>0.452 [0.382 - 0.536]</td>
</tr>
<tr>
<td>26</td>
<td>0.492 [0.351 - 0.688]</td>
<td>1.06 [0.971 - 1.16]</td>
<td>0.371 [0.327 - 0.419]</td>
<td>0.645 [0.465 - 0.894]</td>
<td>0.225 [0.204 - 0.248]</td>
</tr>
<tr>
<td></td>
<td>0.389 [0.278 - 0.544]</td>
<td>0.436 [0.378 - 0.503]</td>
<td>0.452 [0.409 - 0.499]</td>
<td>0.717 [0.592 - 0.869]</td>
<td>0.336 [0.318 - 0.355]</td>
</tr>
<tr>
<td>60</td>
<td>&gt;10</td>
<td>1.34 [1.11 to 1.62]</td>
<td>0.772 [0.704 - 0.846]</td>
<td>1.64 [1.00 - 1.16]</td>
<td>0.569 [0.541 - 0.598]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.01 [0.789 - 5.10]</td>
<td>0.907 [0.819 - 1.01]</td>
<td>0.902 [0.831 - 0.980]</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td></td>
<td></td>
<td>1.04 [0.927 - 1.16]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td></td>
<td></td>
<td>0.861 [0.765 - 0.969]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.629 [0.556 - 0.711]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>&gt;10</td>
<td>1.02 [0.941 - 1.11]</td>
<td>1.05 [0.819 - 1.01]</td>
<td>0.902 [0.831 - 0.980]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.64 [1.30 - 2.09]</td>
<td>0.907 [0.819 - 1.01]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Figure 12. Dose-Response Curve of Compound 26 with Neutral Red and XTT Assay. Dotted lines indicate the respective IC_{50} value. Error bars represent ± standard error mean of at least 3 independent experiments in quadruplicate (NR) or at least 2 experiments in quadruplicate (XTT).

Figure 13. Dose-Response Curve of Compound 20 with Neutral Red and XTT Assay. Dotted lines indicate the respective IC_{50} value. Error bars represent ± standard error mean (SEM) of at least 2 independent experiments in quadruplicate (NR) or at least 1 experiment in quadruplicate (XTT).
3. 9 General Alkylation Procedure of 1H-Indole-3-carbaldehyde

After the biological evaluation of the first series of compounds had been completed one aim was to modify the substitution pattern of the most active compounds to further enhance their activity. In this context we were able to optimise the synthetic strategy by avoiding the MOM-protection/deprotection step. This was achieved by linking the desired side chain to the indole unit prior to the cyclisation step. The substituted aldehydes could be prepared by the standard alkylation procedure using sodium hydride and the respective side chain in DMF affording the desired products in good yields as yellow or orange oils or as beige low melting solids (Scheme 11). The detailed procedure and NMR spectroscopic assignments are referenced in the experimental part. Compared with the MOM-protected compounds yields were a bit lower, nevertheless we favoured this method saving one step.

Scheme 11. General Alkylation Procedure
3.10 Synthesis of 5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (19) and 5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (25) with Alternate Substitution Patterns

Having prepared the respective substituted 1H-indole-3-carbaldehyde the cyclisation step was performed using the usual procedure (Scheme 12) to afford the desired compounds which were purified using column chromatography to afford orange to brown crystals. The prepared compounds are depicted in the scheme below. The detailed procedure, yield and spectroscopic characterisation for each compound is referenced in the experimental part. Employing this methodology also enabled us to shorten the route for the already prepared compounds 20 and 26.
3. 11 Synthesis of 5-[2-(dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (72)

The viability assay of the first set of compounds showed high activity of the tetracylic pyrazine derivative 26 against SW480 cells (IC\textsubscript{50} = 0.492 µM), but low activity of the pentacyclic derivatives 60 and 76 (IC\textsubscript{50} > 10 µM). Hence we were interested in the activity of the pentacyclic pyrazine derivative 72. The synthetic pathway is depicted in Scheme 13.

The synthesis was accomplished by the preparation of amide 69 in three steps and cyclisation with functionalised aldehyde 27. The pentacyclic framework was prepared in the same way by cyclisation with MOM-protected aldehyde 8 and subsequent BBr\textsubscript{3} effected MOM cleavage of 70 to afford 71.

Scheme 13. Synthesis of 71
Building block 69 was prepared from D-fructose in three steps. D-Fructose was subjected to condensation with 1,2-benzenediamine under acidic conditions, followed by oxidation with $\text{H}_2\text{O}_2/\text{NaOH}$. The amidation of the carboxylic acid function was achieved following the routine procedure via intermediary acid chloride and reacting with diethylamine. The identity of all intermediates and the target compound was confirmed via NMR spectroscopy and HRMS and are referenced in the experimental section.

3. 12 Synthesis of 6-[2-(dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (22)

The biological evaluation revealed the significantly higher activity of calothrixin A derivative 66 endowed with N-oxide function than calothrixin B derivative 65 lacking thereof. This prompted us to investigate if its corresponding tetracyclic counterpart would also benefit from a pyridine N-oxide moiety. Thus, we synthesised 22 in analogy to 66 by reacting derivative 19 with $m$-CPBA to afford N-oxide 21 in over 90 % yield, followed by alkylation with 2-chloro-$N,N$-dimethylethanamine hydrochloride to give the target compound as orange crystals (Scheme 14). As expected a shift of $\delta$ to lower frequency of N-2 from -51.0 ppm to -76.9 ppm (for compound 21) and from 55.0 ppm to 77.7 ppm (for compound 22) was apparent, running completely parallel to the pyridine $\rightarrow$ pyridine N-oxide transition (approximately 23 ppm).139 As in the $^{13}$C NMR spectra of N-oxides 64 and 66, the expected shift to lower frequency for the carbons in ortho and para positions to N-2 (C-1: -11.5/-11.5 ppm, C-3: -12.8/-12.6 ppm, C-4a: -11.0/-10.7 ppm) could be observed (relative to the corresponding shifts in the parent compounds 19 and 20). A full NMR and HRMS spectroscopic analysis of 21 and 22 is referenced in chapters 6. 2. 22 on page 104 and 6. 2. 23 on page 105.


Among the basic substitution pattern we were particularly interested in evaluating the effect of the 2-(2-hydroxyethylamino)-ethyl group which is an essential motif of our lead compound mitoxantrone. The free amino and hydroxy group of the side chain prohibits a simple alkylation preceding the metallation step. Instead, we envisaged the introduction of a chloroethyl group and subsequent $S_2N_2$ reaction in an alkaline milieu with 2-aminoethanol (Scheme 15 on page 43).
RESULTS AND DISCUSSION

This should be achieved by employing the routine procedure in DMF with sodium hydride and 1-bromo-2-chloroethane under moderate heating. The intermediary compound 99 was obtained in good yield as orange crystals, m.p. 235-237 °C. The identity was confirmed by NMR spectroscopy showing a consistent signal set for the ring system and two triplets indicating the successful introduction of the chloroethyl group. This finding was also confirmed by HRMS.

Intermediate 99 was then reacted with 2-aminoethanol and sodium hydride in DMF to substitute the chlorine of the side chain. However, the isolated product was not the desired target compound but was elucidated as 6-vinyl-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (Figure 14 on page 44). Evidently, the basic reaction conditions led to HCl elimination affording olefine 100 as orange crystals, m.p. 210-212 °C. The same phenomenon occurred on treating 99 with the less basic K₂CO₃ and KI as catalyst in DMF. This reaction might be favoured due to a conjugational effect of the vinyl group to the aromatic system.

\[ \text{Scheme 15. Synthesis of Olefine } 100 \]

\[ \text{H NMR (400 MHz, DMSO-d₆) } \delta 9.25 \text{ (d, } J = 0.7 \text{ Hz, H-1, } 1\text{H}), 9.08 \text{ (d, } J = 5.0 \text{ Hz, H-3, } 1\text{H}), 8.29 \text{ (m, H-10, } 1\text{H}), 7.95 \text{ (dd, } J = 5.0 \text{ Hz, } 0.7 \text{ Hz, } 1\text{H}), 7.91 \text{ (m, H-7, } 1\text{H}), 7.56 \text{ (m, H-8, } 1\text{H}), 7.45 \text{ (m, H-9, } 1\text{H}), 5.08 \text{ (t, } J = 6.0 \text{ Hz, H-12, } 2\text{H}), 4.09 \text{ (t, } J = 6.0 \text{ Hz, H-13, } 2\text{H}). \]

\[ \text{C NMR (100 MHz, DMSO-d₆) } \delta 180.3 \text{ (C-11), 177.2 \text{ (C-5), 155.3 \text{ (C-3), 147.2 \text{ (C-1), 139.7 \text{ (C-6a), 138.9 \text{ (C-4a), 134.8 \text{ (C-5a), 127.8 \text{ (C-8), 125.8 \text{ (C-11a), 125.0 \text{ (C-9), 122.7 \text{ (C-10a), 122.6 \text{ (C-10), 118.6 \text{ (C-4), 118.2 \text{ (C-10b), 112.8 \text{ (C-7), 46.1 \text{ (C-12), 43.3 \text{ (C-13).}}} \]

\[ \text{N NMR (40 MHz, DMSO-d₆) } \delta -237.9 \text{ (N-6), not found (N-2).} \]
3. 13. 1 Synthesis of 3-(2-chloroethyl)-1,3-oxazolidin-2-one (41)

Finally we introduced the side chain masked as carbamate with subsequent alkaline hydrolysis. This procedure was performed according to the procedure by Leepasert. For that intention building block 3-(2-hydroxyethyl)-1,3-oxazolidin-2-one (40) had to be prepared which was achieved by reacting 2,2'-iminodiethanol with diethyl carbonate and sodium methoxide to afford intermediate 40 in almost quantitative yield. Subsequent treatment with thionyl chloride in dry dichloromethane and a small amount of absolute DMF converted the alcohol into desired halide 41. NMR spectroscopic information of 40 and 41 is according to literature and referenced in the experimental section in chapters 6. 2. 41 on page 123 and 6. 2. 42 on page 124.
3. 13. 2  Synthesis of 6-[2-(2-oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (42)

In the following compound 19 was treated with sodium hydride in dry DMF in the presence of 41. Surprisingly, heating at 70 °C for 6 d was necessary to obtain the target compound in good yields as bright red needles.

$^1$H NMR spectrum of 42 shows pseudo-triplets at 3.32 ppm and 4.09 ppm which are assigned to the methylene protons of the oxazolidine ring whereas the triplets at 4.92 ppm ($J = 6.3$ Hz, 2H) and 3.75 ppm ($J = 6.3$ Hz, 2 H) are attributed to the side chain (compare 6. 2. 43 on page 125). Since the carbamate moiety is susceptible to enzymatic hydrolysis compound 42 should act as precursor thus releasing active compound 43. In order to test this hypothesis both compounds should be evaluated in the viability assays to compare their potency. However, it turned out, that precursor 42 was considerably weaker after 24 h treatment on SW480 cells. Prolonged treatment for 48 h and 72 h showed no significant improvement of cytotoxic activity.

3. 13. 3  Alkaline Carbamate Hydrolysis of 42

The carbamate hydrolysis was performed under harsh conditions by refluxing 42 in methanolic 2M NaOH furnishing only 11 % of 43. Employing only 0.5 M NaOH required longer reaction times to convert the starting material, thus having the same detrimental effect in terms of yield. Moreover, the purification process of 43 was tedious due to the high polarity of the attached side chain which forced us to apply RP column chromatography to purify the product. The identity of 43 was confirmed by NMR and HRMS analysis (compare 6. 2. 44 on page 126).
3. 14 Synthesis of 6-[2-(2-oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (44)

The synthesis of the carbamate bearing pyrazine derivative 44 was accomplished in analogy to 42. The NMR spectroscopic characterisation is referenced in chapter 6. 2. 45 on page 127.

![Chemical structure of 44]

Due to the poor yields of 43 and the associated difficulties in the purification process we refrained from the carbamate hydrolysis in the case of 44. Instead, we postponed our efforts awaiting the results from the biological tests.

The successful introduction of a mesyl group and the substitution with aziridine in acceptable yields during our ongoing research prompted us to explore this methodology further with the prospect of opening a more practical approach to the 2-(2-hydroxyethylamino)-ethyl substituted pyrazine derivative. This needs to be investigated in future works.

3. 15 Attempted Synthesis of the Isomeric Pyrimidocarbazolediones

For the construction of the first of two possible pyrimidine-annulated carbazoledion derivatives we envisaged the construction via the proven ortho lithiation strategy. Already available MOM-protected indole-3-carbaldehyde 8 should be cyclised with amide 87 to give the MOM-protected framework which should then be converted into the desired compound in two steps as depicted in Scheme 16 on page 47.
3. 15. 1 Attempted Synthesis via Directed Ortho Lithiation

Amide 87 was prepared starting from commercially available ethyl 5-pyrimidinecarboxylate which was subjected to alkaline ester hydrolysis using 4M NaOH following the procedure of Maier and co-workers. The free carboxylic acid could be obtained in over 80% yield and was used in the next step without purification. The modified procedure by Gilman and Spatz was used to convert 86 in its corresponding amide 87 via $S_N^2$ reaction by treatment with oxalyl chloride and diethylamine in dichloromethane yielding 67% as a yellow oil. The identity of the product was confirmed via NMR spectroscopy.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.24 (d after Gauss multiplication, $J = 0.7$ Hz, H-2, 1H), 8.78 (d, $J = 0.7$ Hz, H-4/6, 2H), 3.56 (br q, H-9', 2H), 3.28 (br q, H-9, 2H), 1.26 (br t, H-10', 3H), 1.17 (br t, H-10, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 165.8 (C-7), 159.0 (C-2), 154.6 (C-4/6), 131.0 (C-5), 43.5 (C-9), 39.8 (C-9'), 14.4 (C-10), 12.8 (C-10').

$^{15}$N NMR (40 MHz, CDCl$_3$) $\delta$ -88.6 (N-1/3), -248.3 (N-8).
Amide 87 should then be reacted with MOM-protected aldehyde 8 in a \textit{ortho} directed metallation reaction. Unfortunately, after the usual workup procedure and purification by column chromatography only the starting materials could be recovered along with many tarry side products. Adding an additional eq. LiTMP didn't furnish the desired product. Reacting the educts at -100 °C in a bath of liquid nitrogen/ethanol was also not successful. Subsequently, this route was abandoned in favour of the following 7-step synthesis as depicted in Scheme 17.

3.15.2 Attempted Synthesis \textit{via} Palladium-mediated Biaryl Coupling Reaction\textsuperscript{141, 143}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Scheme_17.png}
\caption{Palladium-mediated Biaryl Coupling}
\end{figure}

In contrast to our previous strategy, the requisite quinone system should be constructed first which should allow us to effect the coupling of aniline; the subsequent ring-closure step should be realised in a palladium(II) acetate mediated biaryl C-C coupling reaction to afford the desired nucleus.

The methods of Shen et al.\textsuperscript{98} and Groszek et al.\textsuperscript{99} were adapted to prepare the 2-nitrobenzaldehyde derivative 88 by treating a solution of 2,5-dimethoxybenzaldehyde in glacial acetic acid at 0 °C with nitric acid (1 to 1.5 mol-eq.). After the workup procedure and rerystallisation from dichloromethane the desired 2-nitro isomer could be obtained as yellow needles, m.p. 163-165 °C. The mother liquor containing additional product could be separated from the undesired 4-nitro isomer by column chromatography eluting with ethyl acetate/hexane (1:1, v/v). Both isomers could be discriminated \textit{via} NMR spectroscopy. In particular, for the 2-nitro isomer the aromatic protons H-4 and H-5 can be observed as two doublets at 7.28 ppm and 7.11 ppm ($J=9.3$ Hz), in contrast to the protons H-2 and H-5 of the 4-nitro derivative which give two singlets. Moreover, the spatial proximity of the methoxy protons (H-8 and H-9) to the adjacent aromatic protons H-4 and H-5 is apparent in the NOE spectrum.
RESULTS AND DISCUSSION

Following the procedure of Shaikh et al.\textsuperscript{100}, 3,6-dimethoxy-2-nitrobenzaldehyde was converted into its respective aminal 89 under stirring in formamide in the presence of dry HCl gas.

\begin{align*}
{^1}H \text{ NMR (400 MHz, CDCl}_3) & \delta 10.35 (s, H-7, 1H), 7.28 (d, J = 9.3 \text{ Hz, H-5, 1H}), 7.11 (d, J = 9.3 \text{ Hz, H-4, 1H}), 3.94 (s, H-9, 3H), 3.86 (s, H-8, 3H).
\end{align*}

\begin{align*}
{^{13}}C \text{ NMR (100 MHz, CDCl}_3) & \delta 186.0 (C-7), 155.3 (C-3), 144.5 (C-6), 138.6 (C-2), 120.0 (C-5), 116.1 (C-1), 114.1 (C-4), 57.2 (C-8), 56.7 (C-9).
\end{align*}

Subsequently, crude intermediate 89 was subjected to reductive cyclisation with Zn metal in glacial acetic acid to give 5,8-dimethoxyquinazoline (90) as pale yellow crystals, m.p. 116-117 °C. \textsuperscript{1}H NMR data is in agreement with literature\textsuperscript{100} and was amended by \textsuperscript{13}C and \textsuperscript{15}N NMR spectra.

\begin{align*}
{^1}H \text{ NMR (400 MHz, DMSO-}d_6) & \delta 8.67 (d, J = 8.1 \text{ Hz, H-10/10}', 2H), 7.91 (dd, J = 1.4, 0.9 \text{ Hz, H-11/11}', 2H), 7.26 (s, H-4/5, 2H), 6.77 (t, J = 8.1 \text{ Hz, H-9, 1H), 3.86 (s, H-8, 3H), 3.80 (s, H-7, 3H).
\end{align*}

\begin{align*}
{^{13}}C \text{ NMR (100 MHz, DMSO-}d_6) & \delta 160.2 (C-11/11'), 150.8 (C-6), 143.6 (C-3), 139.6 (C-2), 119.6 (C-1), 114.3 (C-5), 113.9 (C-4), 57.0 (C-7), 56.7 (C-8), 48.5 (C-9).
\end{align*}

90 should be converted into its respective quinone by oxidative demethylation with ceric ammonium nitrate (CAN) in a mixture of acetonitril/water, described by Potts et al.\textsuperscript{101} in 1986. However, following this procedure we couldn't reproduce their results. The product could only be obtained in 3 % yield in unsatisfying grade of purity. Adjusting the reaction parameters by using different eq. of CAN, varying reaction times and/or temperature did not have any impact on the results. Most interestingly, in two later publications\textsuperscript{102, 103} a different research group has prepared 90 via oxidation of the intermediate 5,8-quinazolinediol employing Fremy’s Salt (disodium nitrosodisulfonate) or K$_2$Cr$_2$O$_7$ as oxidising agents. This implicates the insertion of an additional reaction step, because 5,8-quinazolinediol in turn had to be prepared from 90 by heating with AlCl$_3$. 

\begin{align*}
\text{55} & \quad \text{44} \\
\text{33} & \quad \text{22} \\
\text{11} & \quad \text{66} \\
\text{7.28 (d)} & \quad \text{120.0} \\
\text{7.11 (d)} & \quad \text{114.1} \\
\text{3.94 (s)} & \quad \text{56.7} \\
\text{1H NMR (400 MHz, CDCl}_3) & \delta 10.35 (s, H-7, 1H), 7.28 (d, J = 9.3 \text{ Hz, H-5, 1H}), 7.11 (d, J = 9.3 \text{ Hz, H-4, 1H}), 3.94 (s, H-9, 3H), 3.86 (s, H-8, 3H).
\end{align*}

\begin{align*}
\text{13C NMR (100 MHz, CDCl}_3) & \delta 186.0 (C-7), 155.3 (C-3), 144.5 (C-6), 138.6 (C-2), 120.0 (C-5), 116.1 (C-1), 114.1 (C-4), 57.2 (C-8), 56.7 (C-9).
\end{align*}

\begin{align*}
\text{55} & \quad \text{44} \\
\text{33} & \quad \text{22} \\
\text{11} & \quad \text{66} \\
\text{7.28 (d)} & \quad \text{120.0} \\
\text{7.11 (d)} & \quad \text{114.1} \\
\text{3.94 (s)} & \quad \text{56.7} \\
\text{1H NMR (400 MHz, DMSO-}d_6) & \delta 8.67 (d, J = 8.1 \text{ Hz, H-10/10}', 2H), 7.91 (dd, J = 1.4, 0.9 \text{ Hz, H-11/11}', 2H), 7.26 (s, H-4/5, 2H), 6.77 (t, J = 8.1 \text{ Hz, H-9, 1H), 3.86 (s, H-8, 3H), 3.80 (s, H-7, 3H).
\end{align*}

\begin{align*}
\text{13C NMR (100 MHz, DMSO-}d_6) & \delta 160.2 (C-11/11'), 150.8 (C-6), 143.6 (C-3), 139.6 (C-2), 119.6 (C-1), 114.3 (C-5), 113.9 (C-4), 57.0 (C-7), 56.7 (C-8), 48.5 (C-9).
\end{align*}
RESULTS AND DISCUSSION

This step could be circumvented by preparing 91 following a modified procedure\textsuperscript{104} by Ubeda et al.\textsuperscript{105} which involved oxidative demethylation of 90 with CAN in a biphasic mixture of chloroform/water (1:2, v/v). Chloroform was substituted with dichloromethane due to its lower carcinogenic potential. After stirring 90 in a mixture of dichloromethane/water (1:2, v/v) for 1 h at room temperature in the presence of three eq. CAN the organic layer was collected and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water and the solvent was evaporated to afford 91 without further purification as brown crystals of high purity in 80 to 90 % yield, m.p. 113-115 °C.

A regioselective addition\textsuperscript{102, 104} of aniline to the prepared quinone at position 6 was achieved by stirring 91 in ethanol in presence of the lewis acid cerium(III)chloride heptahydrate at room temperature for 24 h. The reaction was quenched by pouring onto ice-cold water that was acidified with 3 % acetic acid. The product was collected by suck-filtration and purified by column chromatography eluting with dichloromethane followed by column chromatography eluting with ethyl acetate/light petroleum (8:2, v/v) to give 34 % of 6-anilino-5,8-quinazolinedione (92) as purple crystals, m.p. 209-211 °C, which should be subjected to a palladium catalysed biaryl coupling reaction.\textsuperscript{143}

The palladium acetate promoted cyclodehydrogenation procedure was based on the works of Sridharan et al.,\textsuperscript{107} Knoelker et al.,\textsuperscript{110} Choi et al.,\textsuperscript{109} and Liegault et al.\textsuperscript{108} using catalytic amounts of palladium for the synthesis of various carbazole-based compounds.

\textsuperscript{1H} NMR (400 MHz, DMSO-d$_6$) $\delta$ 9.55 (s, H-4, 1H), 9.24 (s, H-2, 1H), 7.30 (d, $J = 8.7$ Hz, H-7, 1H), 7.00 (d, $J = 8.7$ Hz, H-6, 1H), 3.92 (s, H-10, 3H), 3.90 (s, H-9, 3H).

\textsuperscript{13}C NMR (100 MHz, DMSO-d$_6$) $\delta$ 154.8 (C-4), 154.4 (C-2), 148.2 (C-5), 147.9 (C-8), 141.5 (C-8a), 117.1 (C-4a), 113.4 (C-7), 106.0 (C-6), 56.03 (C-9), 55.96 (C-10).

\textsuperscript{15}N NMR (40 MHz, DMSO-d$_6$) $\delta$ -89.1 (N-3), -106.5 (N-1).

\textsuperscript{1H} NMR (400 MHz, DMSO-d$_6$) $\delta$ 9.66 (s, H-2, 1H), 9.41 (s, H-4, 1H), 7.27 (d, $J = 10.5$ Hz, H-7, 1H), 7.17 (d, $J = 10.5$ Hz, H-6, 1H).

\textsuperscript{13}C NMR (100 MHz, DMSO-d$_6$) $\delta$ 184.1 (C-5), 182.9 (C-8), 162.1 (C-2), 156.3 (C-4), 152.6 (C-8a), 139.5 (C-7), 137.8 (C-6), 124.6 (C-4a).

\textsuperscript{15}N NMR (40 MHz, DMSO-d$_6$) $\delta$ -73.6 (N-3), -94.4 (N-1).
RESULTS AND DISCUSSION

First, a microwave-assisted approach was attempted by treating with Pd(OAc)$_2$ (0.4 eq.) and Cu(OAc)$_2$ (2 eq.) as co-oxidant in DMF in a microwave reactor (100 W, 130 °C, 60 min). Unfortunately, the reaction was unsuccessful and the starting material was decomposed. Shorter reaction times or prolonged reaction times at reduced microwave power had no influence on the result. In any case either the starting material was destroyed or no reaction at all could be observed.

Next we chose a conventional reaction setup following a procedure by Norcott and co-workers. 92 was refluxed with Pd(OAc)$_2$ (1 eq.) and Cu(OAc)$_2$ (1 eq.) in glacial acetic acid. The reaction process was monitored by TLC. However, the reaction was unsuccessful, leading to the decomposition of the educt. By lowering the temperature no reaction was observed.

Liegault et al. reported the intramolecular Pd(II)-catalysed oxidative biaryl synthesis under air with substituting acetic acid with 2,2-dimethylpropionic acid (pivalic acid, PivOH), which gives the prospect of not only increasing the reactivity but also improving the selectivity for the formation of the carbazole product. Previous work in their group has also demonstrated that PivOH can play an important role as a co-catalyst in Pd(0)-catalysed benzene arylation. Schultz et al. have also demonstrated the efficiency of the pivaloyl moiety for palladium-catalysed aerobic oxidation of alcohols. In both cases, the pivalate ligand is proposed to play a key role during the C-H bond cleavage.

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.59 (s, H-2, 1H), 9.55 (s, H-9, 1H), 9.39 (s, H-4, 1H), 7.46 (m, H-3'/5', 2H), 7.38 (m, H-2', 1H), 7.25 (m, H-4', 1H), 6.24 (s, H-7, 1H).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 180.5 (C-5), 180.0 (C-8), 162.6 (C-2), 155.6 (C-4), 153.7 (C-8a), 146.2 (C-6), 137.5 (C-1'), 129.4 (C-3'/5'), 125.8 (C-4'), 124.1 (C-4'), 124.0 (C-2'/6'), 103.5 (C-7).

$^{15}$N NMR (40 MHz, DMSO-$d_6$) $\delta$ -78.3 (N-3), -94.1 (N-1), -274.9 (N-9).
RESULTS AND DISCUSSION

Moreover, in contrast to many reactions performed in glacial acetic acid, little secondary oxidative byproduct can be detected in the crude reaction mixtures with PivOH.

6-anilino-5,8-quinazolinedione (92) was heated at 60 °C with Pd(OAc)$_2$, Cu(OAc)$_2$ and K$_2$CO$_3$ in PivOH under air for 12 h. TLC monitoring showed no reaction progress which prompted us to increase the temperature to 90 °C. Still, after continued stirring of 2 d no reaction progress could be observed.

Consequently, this route was abandoned in favour of the pathway outlined in scheme 18, used by Bernardo et al. as synthetic pathway for calothrixin B.

3. 15. 3 Attempted Synthesis via Cadogan Reaction

Since there are many reports on the synthesis of carbazoles using the Cadogan reaction we envisaged that an appropriate Cadogan precursor will allow access to the pyrimido[5,4-b]carbazoledione skeleton (Scheme 18). Hence, the nitro derivative should be synthesised using nitrophenylboronic acid in a Suzuki coupling reaction. The nitrophenylquinazoline should then be cyclised to the target system in a microwave assisted Cadogan reaction which refers to a deoxygenation of aromatic nitrocompounds by triethyl phosphite and subsequent cyclisation of the resulting intermediate nitrene.

Scheme 18. Attempted Synthesis of 53 via Cadogan Reaction
6-Bromo derivative 93 was furnished by refluxing 90 with N-bromosuccinimide (2 eq.) in dry THF for 2 h following a procedure described by Song and co-workers. Under these conditions only the 6-bromo derivative could be obtained as brown crystals, m.p. 125-127 °C, which could be determined by NMR spectroscopy and HRMS. In particular, a correlation between the H-7 proton and the -OCH$_3$ protons at C-8 can be observed in the NOESY experiment, whereas the -OCH$_3$ protons at C-5 indicate the spatial proximity only to the pyrimidine H-4 proton.

The 6-bromo derivative should be reacted with (2-nitrophenyl)boronic acid in a Suzuki coupling to yield the required Cadogan precursor. The reaction was carried out following the method of Bernardo et al. by heating 93 with Pd(OAc)$_2$, PPh$_3$, and K$_2$CO$_3$ in DMF at 150 °C for 16 h. After the workup procedure and NMR spectroscopic investigation it became evident that the reaction failed and only starting material 93 and the debrominated precursor 90 could be recovered. The reaction was repeated in the microwave reactor using 1,4-dioxane as solvent and heating only to 110 °C at 150 W, but no conversion into the desired compound could be observed. Then we followed the procedure developed by Prabakaran et al. in a microwave assisted ligand-free Suzuki coupling using PdCl$_2$(PPh$_3$)$_2$ and Na$_2$CO$_3$ as base in 1,4-dioxane. This reaction also failed and only led to debromination of the starting material. Replacing PdCl$_2$(PPh$_3$)$_2$ with Pd(PPh$_3$)$_4$ as catalyst was also unsuccessful.

In the following attempt we used the less reactive 6-chloro derivative with the boronic acid assuming it wouldn't lead to a dehalogenation. The 6-chloro derivative could be prepared in analogy to 93 with N-chlorosuccinimide in dry THF. When reacting it with (2-nitrophenyl)boronic acid no dechlorination could be observed, neither could any other reaction. PdCl$_2$(PPh$_3$)$_2$ or Pd(PPh$_3$)$_4$ served as palladium catalysts, Na$_2$CO$_3$ or K$_2$CO$_3$ was used as base, and different solvents were used (DMF or 1,4-dioxane). All reactions were heated by conventional means, but in all setups only the starting material could be recovered. As a consequence this route was discarded in favour to the one outlined in Scheme 19 on page 54.
RESULTS AND DISCUSSION

Scheme 19. Attempted Synthesis of 50 and 56 via Condensation Reaction
3.15.4 Attempted Synthesis via Condensation Reaction\textsuperscript{141}

This strategy employed the construction of a carbazole skeleton followed by condensation to form the pyrimidine unit. Hence it was necessary to introduce both amino and aldehyde function in positions 5 and 6 of the quinone system. The first step of this route comprised a non-regioselective addition of aniline to 2-methyl-1,4-benzoquinone, in which both isomers should be accessible. This procedure was described by Yogo et al.\textsuperscript{120} and was based on the works of Jacini.\textsuperscript{121} Aniline was added to 2-methyl-1,4-benzoquinone in a mixture of acetic acid/water under stirring at 50 °C to furnish both isomers 94a, m.p. 138-140 °C and 94b, m.p. 144-145 °C as violet crystals in a ratio of 1:1.7 (31 % and 53 %, respectively). The $^1$H and $^{13}$C NMR spectra of both isomers show very similar signal sets, which made it impossible to discriminate between them based on 1D spectra. Only 2D-NMR techniques such as HMBC allowed an unambiguous signal assignment for both compounds.

The ring closure was realised by a palladium promoted oxidative biaryl coupling reaction following the procedure of Yogo et al.\textsuperscript{120} 94a was dissolved in glacial acetic acid and refluxed under argon atmosphere in the presence of equimolar amounts of Pd(OAc)$_2$. After the workup procedure yields were comparably low, furnishing only 20 % of 95a.

\[1^H \text{ NMR (400 MHz, CDCl}_3\] $\delta$ 7.37 (m, H-3'/5', 2H), 7.31 (br s, H-8, 1H), 7.19 (m, H-2'/6', 2H), 7.17 (m, H-4', 1H), 6.51 (dq, $J = 2.5, 1.6$ Hz, H-3, 1H), 6.12 (d, $J = 2.5$ Hz, H-3, 1H), 2.07 (d, $J = 1.6$ Hz, H-7, 3H).

\[13^C \text{ NMR (100 MHz, CDCl}_3\] $\delta$ 186.8 (C-4), 184.4 (C-1), 143.0 (C-2), 141.2 (C-6), 137.6 (C-1'), 135.9 (C-5), 129.6 (C-3'/5'), 125.3 (C-4'), 122.1 (C-2'/6'), 100.7 (C-3), 15.5 (C-7).

\[15^N \text{ NMR (40 MHz, CDCl}_3\] $\delta$ -285.2 (N-8).

\[1^H \text{ NMR (400 MHz, CDCl}_3\] $\delta$ 7.38 (m, H-3'/5', 2H), 7.29 (br s, H-8, 1H), 7.19 (m, H-2'/6', 2H), 7.17 (m, H-4', 1H), 6.55 (q, $J = 1.6$ Hz, 1H), 6.16 (s, H-3, 1H), 2.08 (d, $J = 1.6$ Hz, H-7, 3H).

\[13^C \text{ NMR (100 MHz, CDCl}_3\] $\delta$ 186.6 (C-4), 183.7 (C-1), 149.6 (C-5), 142.9 (C-2), 137.5 (C-1'), 129.6 (C-3'/5'), 129.2 (C-6), 125.3 (C-4'), 122.0 (C-2'/6'), 100.9 (C-3), 16.4 (C-7).

\[15^N \text{ NMR (40 MHz, CDCl}_3\] $\delta$ -285.1 (N-8).
The procedure of Liegault et al.\textsuperscript{109} which was described before gave the prospect of higher yields by using pivalic acid (PivOH) instead of glacial acetic acid. 94a and 94b, respectively were suspended in PivOH, Pd(OAc)\textsubscript{2} (10 mol-\%) and K\textsubscript{2}CO\textsubscript{3} (10 mol-\%) were added and the resulting mixture was heated at 110 °C for 14 h under air. However, after the workup and purification procedure yields had not significantly improved. Consequently, we evaluated the benefits of Cu(OAc)\textsubscript{2} as co-catalyst to reoxidise Pd(0) to Pd(II)\textsuperscript{122}. After several reaction setups employing 50 mol-\% Pd(OAc)\textsubscript{2} and stirring at 70 °C for 21 h yields could be raised to 47 \% for 95a and 60 \% for 95b. However, the additive Cu(Oac)\textsubscript{2} seemed to have no influence in terms of yield. Instead, the amount of glacial acetic acid used seemed to be relevant. 28 mL solvent per mmol starting material resulted in the highest yields.\textsuperscript{141}

94a/94b were stirred with Pd(OAc)\textsubscript{2} (10 mol-\%) in glacial acetic acid at 70 °C for 21 h under air. After removal of the solvent under reduced pressure, the products were purified by column chromatography eluting with dichloromethane followed by recrystallisation from ethyl acetate to give red crystals of 95a, m.p. 237-239 °C (yielding 47 \%) or 95b, m.p. 240-242 °C (yielding 60 \%).

\textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) \delta 12.80 (s, H-9, 1H), 7.98 (m, H-5, 1H), 7.51 (m, H-8, 1H), 7.36 (m, H-7, 1H), 7.28 (m, H-6, 1H), 6.55 (q, J = 1.6 Hz, H-3, 1H), 2.02 (d, J = 1.6 Hz, H-10, 3H).

\textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}_6) \delta 183.3 (C-4), 180.2 (C-1), 144.0 (C-2), 137.6 (C-8a), 135.7 (C-9a), 134.8 (C-3), 126.3 (C-7), 123.7 (C-6), 123.2 (C-4b), 121.7 (C-5), 115.6 (C-4a), 113.7 (C-8), 14.9 (C-10).

\textsuperscript{15}N NMR (40 MHz, DMSO-\textit{d}_6) \delta -242.9 (N-9).

\textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) \delta 12.78 (s, H-9, 1H), 8.01 (d, J = 8.0 Hz, H-5, 1H), 7.51 (d, J = 8.2 Hz, H-8, 1H), 7.36 (m, H-7, 1H), 7.28 (m, H-6, 1H), 6.56 (q, J = 1.6 Hz, H-2, 1H), 2.03 (d, J = 1.6 Hz, H-10, 3H).

\textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}_6) \delta 183.0 (C-4), 180.0 (C-1), 147.9 (C-3), 137.4 (C-8a), 135.9 (C-9a), 131.5 (C-2), 126.2 (C-7), 123.7 (C-6), 123.5 (C-4b), 121.6 (C-5), 115.4 (C-4a), 113.8 (C-8), 15.6 (C-10).

\textsuperscript{15}N NMR (40 MHz, DMSO-\textit{d}_6) \delta -243.3 (N-9).
The next step comprised the functionalisation of the carbazole nitrogen of 95a and 95b following the procedure of Luo et al.\textsuperscript{123} which was - in contrast to previous alkylations employing sodium hydride - carried out in a biphasic mixture of chloroform/water in the presence of the phase-transfer catalyst tetrabutylammonium hydrogensulfate, Na\textsubscript{2}CO\textsubscript{3} and 2-chloro-N,N-dimethylethanamine hydrochloride. This mixture was vigorously stirred under reflux for 2 h. The reaction smoothly converted the starting material of both isomers into the desired compounds in good yields. The crude products could be easily isolated using column chromatography eluting with ethyl acetate/methanol (8:2, v/v) yielding 91 % of 96a, m.p. 109-11 °C and 67 % of 96b, m.p. 95-97 °C, both as red crystals. The successful reaction was readily confirmed in the $^1$H spectra, showing the absence of the carbazole proton. Instead, the introduced side chain shows two pseudo-triplets for H-11 (4.68 ppm) and H-12 (2.69 ppm) and a singlet for the six protons of the N-methyl groups at 2.37 ppm for 96a and 2.36 ppm for 96b, respectively.

$^1$H NMR (400 MHz, CDCl\textsubscript{3}) δ 8.26 (m, H-5, 1H), 7.45 (m, H-8, 1H), 7.41 (m, H-7, 1H), 7.33 (m, H-6, 1H), 6.41 (q, J = 1.6, H-2, 1H), 4.68 (m, H-11, 2H), 2.69 (m, H-12, 2H), 2.36 (s, H-14/14’ , 6H), 2.12 (d, J = 1.6 Hz, H-10, 3H).

$^{13}$C NMR (100 MHz, CDCl\textsubscript{3}) δ 183.5 (C-4), 181.1 (C-1), 147.5 (C-3), 138.6 (C-8a), 133.5 (C-9a), 126.7 (C-7), 124.4 (C-6), 123.8 (C-4b), 123.1 (C-5), 123.8 (C-4a), 117.0 (C-4a), 110.9 (C-8), 58.3 (C-12), 45.6 (C-14/14’), 42.9 (C-11), 15.7 (C-10).

$^{15}$N NMR (40 MHz, CDCl\textsubscript{3}) δ -240.5 (N-9), -358.4 (N-13).

$^1$H NMR (400 MHz, CDCl\textsubscript{3}) δ 8.23 (m, H-5, 1H), 7.44 (m, H-8, 1H), 7.41 (m, H-7, 1H), 7.32 (m, H-6, 1H), 6.48 (q, J = 1.6 Hz, 1H), 4.68 (m, H-11, 2H), 2.69 (m, H-12, 2H), 2.37 (s, H-14/14’ , 6H), 2.12 (d, J = 1.6 Hz, H-10, 3H).

$^{13}$C NMR (100 MHz, CDCl\textsubscript{3}) δ 183.6 (C-4), 181.2 (C-1), 144.6 (C-2), 138.8 (C-8a), 134.7 (C-3), 133.3 (C-9a), 126.8 (C-7), 124.3 (C-6), 123.3 (C-4b), 123.2 (C-5), 117.3 (C-4a), 110.8 (C-8), 58.4 (C-12), 45.7 (C-14/14’), 43.0 (C-11), 15.6 (C-10).

$^{15}$N NMR (40 MHz, CDCl\textsubscript{3}) δ -240.3 (N-9), -358.3 (N-13).
The amination of 96a and 96b should be performed using the same methodology with both isomers. 96a could be converted into the amino-derivative 97a by two different methods with varying success. Josey et al. employed NaN₃ in an acidic milieu for the synthesis of 2-amino-3-methyl-1,4-naphthoquinone, a route which could be adapted for the construction of 97a, but failed in the synthesis of regioisomer 97b.

96a was dissolved in methanol and treated with a solution of NaN₃ in water, which was adjusted to pH 3-4 by hydrochloric acid. After stirring at 50 °C for 5 h the reaction mixture was quenched with water, extracted with ethyl acetate and purified with column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to afford 97a in 15 % yield. The successful reaction could be confirmed by NMR spectroscopy. The quartet at 6.48 ppm of the proton in position 3 is absent and a broad singlet counting two protons appears at 5.09, indicative for exchangeable protons of the amino group. Moreover, in the 15N HMBC spectrum the introduced amino function is indicated by a correlation to the methyl protons H-10. HRMS analysis is also in agreement with the calculated mass.

The reaction was repeated for the amination of 96b in a modified procedure by Adams et al. who used NH₄Cl solution as acidifying agent. 96b was dissolved in methanol and treated with NaN₃ and NH₄Cl and refluxed for 4 h. After the extraction with dichloromethane and column chromatography 97b could not be isolated.

In order to raise yields of 97a and possibly open access to 97b we investigated the route of Allen et al. who employed a solution of methanol saturated with dry ammonia for the construction of 6-amino-1-ethyl-2-methyl-4,7-dioxo-3-indolecarboxaldehyde.

We modified this procedure by dissolving 96a in a solution of 7M ammonia in methanol and stirring for 12 h at room temperature in a closed reaction vessel. After evaporation of the volatiles the crude product was purified using column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to afford 97a as dark green solid, m.p. 167-169 °C in excellent 81 % yield. Unfortunately, this methodology failed in the construction of 97b.

Since the outcome of the final two steps was uncertain we postponed our efforts on the amination of 96b and focused on the final two steps of the synthesis of isomer a.
A prerequisite for the condensation of the pyrimidine ring to the existing framework with formamide was the presence of an aldehyde function in position 10. This should be realised by oxidation of the methyl group at position 2 with an excess of activated MnO$_2$. This strategy was applied by Takayanagi and co-workers$^{127}$ for the oxidation of Mitomycin C into its formyl derivative. This strategy seemed to be promising because of the structural similarity of Mitomycin C to 97a (Figure 15).

![Figure 15. Structural Similarity of Mitomycin and 97a](image)

97a was dissolved in ethyl acetate and treated with two eq. of activated MnO$_2$. The resulting suspension was vigorously stirred. TLC monitoring revealed that the starting material had not reacted. Two additional eq. of MnO$_2$ were added and stirring was continued. After a total of 4 hours with no noticeable effect the reaction mixture was heated at 70 °C for 12 h. After the workup procedure and NMR spectroscopic investigation the desired compound could not be determined. In the following setup the solvent was changed to dichloromethane and the reaction mixture stirred at room temperature for 2.5 days, showing no effect, only the starting material could be recovered. Changing the solvent to toluene and heating to 70 °C for 24 h in presence of 10 eq. MnO$_2$ also was uneffective.$^{141}$

A literature survey revealed that oxidation reactions of methyl groups to aldehydes is in most cases achieved by using SeO$_2$ as oxidant in 1,4-dioxane. The procedure of Goswami et al.$^{128}$ was used. 97a was dissolved in 1,4-dioxane and treated with an equimolar amount of SeO$_2$. The mixture was refluxed for 4 h after which TLC monitoring showed the complete consumption of the starting material. However, after the workup procedure the desired product could not be isolated. Repeating the setup and stirring at room temperature also consumed the starting material, but the desired product could not be isolated.$^{141}$

![Scheme 20 on page 60](image)

With the prospect of countless oxidation methods we abandoned this route and returned to a Friedel-Crafts hydroxyalkylation based approach. The strategy is based on the recent works of Ramkumar and co-workers$^{129}$ as outlined in Scheme 20 on page 60.
3. 16 Synthesis of the Isomeric Pyrimidocarbazolediones via Friedel-Crafts Hydroxyalkylation with Subsequent Ortho Metallation\textsuperscript{129, 143}

The latest strategy comprised the coupling of 1\textit{H}-indole-2-carboxylate in position 3 with pyrimidine-5-carbaldehyde via Friedel-Crafts hydroxyalkylation leading to 51 followed by conversion of the alcohol moiety into ketone 52 with IBX. The ring formation should then be achieved in a metallation step using LiTMP.

![Scheme 20. Synthesis of 53 via Friedel-Crafts Hydroxyalkylation](image)

3. 16. 1 Synthesis of Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1\textit{H}-indole-2-carboxylate (51)

Commercially available building blocks ethyl 1\textit{H}-indole-2-carboxylate and pyrimidine-5-carbaldehyde were reacted in a Friedel-Crafts hydroxylalkylation\textsuperscript{129, 143} in dry dichloromethane to furnish 51 in 35% yield as white crystals. It should be noted that pyrimidine-5-carbaldehyde is hygroscopic and had to be properly dried before usage, otherwise yields were comparably low. Intermediate 51 was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) followed by recrystallisation from ethyl acetate/light petroleum. The structure was elucidated via NMR spectroscopy and HRMS. The coupling product shows a consistent signal set in the \textsuperscript{1}H NMR spectrum. In particular, the pyrimidine protons give two singlets at 9.03 ppm (H-2') and 8.85 ppm (H-4'/6').
in the expected ratio of 1:2. Moreover, the proton of the newly formed hydroxy group (at 6.85 ppm) along with the coupling adjacent proton H-8 (at 6.30 ppm) give doublets in both cases ($J = 3.9$ Hz). Due to the chiral C-8 atom the stereotopic methylene protons of the ester function exhibit a complex coupling pattern (compare 6.2.52 on page 134).

3.16.2 Synthesis of Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (52)

![Chemical structure of 51 and 52](image)

Compound 51 was smoothly transformed into its respective ketone 52 by oxidation with 2-iodoxybenzoic acid (IBX). The title compound was afforded in excellent 84% yield. The successful reaction was confirmed via NMR and HRMS. The $^1$H spectrum reveals the absence of both OH group and the C-8 proton. In the $^{13}$C APT spectrum the carbinol C-8 at 64.3 ppm has disappeared and the newly formed C=O bond can be clearly identified at 189 ppm. The full spectroscopic characterisation is referenced in chapter 6.2.53 on page 135.

3.16.3 Synthesis of 5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (53)

![Chemical structure of 52 and 53](image)

The ring formation to obtain compound 53 was achieved by directed ortho metallation$^{129, 143}$ using 5 eq. LiTMP in THF. The title compound was afforded in only 5% yield and the purification process using column chromatography was tedious due to the poor solubility in most organic solvents and the similar $R_f$ of 53 and unreacted 52. A simple recrystallisation process was also out of question because unreacted 52 crystallised along with the product. Therefore, we envisaged a more practical approach with the alkylation preceding the cyclisation step. The alkylated nucleus would be easier to purify using column chromatography due its good solubility in the eluent. Moreover, the solid product would be easy to separate from the oily starting material by recrystallisation. The structure was elucidated via HRMS and NMR spectroscopy where the absence of the H-6’ pyrimidine proton and the methyl and methylene protons of the ester group indicate the successful ring formation. This can be also observed in the $^{13}$C spectrum with a shift to higher frequency of the ester
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carboxyl from 160.1 ppm to the newly formed quinone C-11 at 175.4 ppm as well as a shift to lower frequency of the ketone carbonyl from 189.0 ppm to 178.8 ppm of the second quinone C-5 which is in good agreement with the assignments made for the other compounds of this series and the correlations obtained from the $^{13}$C and $^{15}$N HMBC spectrum (compare 6. 2. 54 on page 136).

3. 16. 4 Synthesis of Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (54)

The alkylation of 52 was achieved using the routine alkylation procedure employing sodium hydride in DMF. The title compound 54 was afforded in 49 % yield as a yellow oil and was elucidated via NMR spectroscopy and HRMS (compare 6. 2. 55 on page 137).

3. 16. 5 Synthesis of 10-[2-(dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (56)

Compound 54 underwent cyclisation in analogy to 52 to obtain compound 56 as red needles in 7.5 % yield, which is 50 % higher than of compound 53, possibly due to the protected indole-nitrogen or a reduced loss during the purification procedure. As usual, HRMS and a full NMR spectroscopic signal assignment were performed (compare 6. 2. 57 on page 139).

Since the ortho lithiation procedure using the ester was successful we were interested to investigate if ester and the aldehyde would undergo the cyclisation process without adding the pyrimidine aldehyde to position 3 of the indole. It was conceivable that indole ester and the pyrimidine aldehyde would react by ortho lithiation and in situ oxidation following the same methodology as indole-3-carbaldehyde with the pyridine or quinoline amides. If successful, the synthesis of the target compound could be reduced from four steps to only two.
3. 16. 6 Synthesis of Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (55)

To avoid the tedious purification of the naked tetracycle we decided to attach the side chain to the indole ester before carrying out the ring formation step. Synthon 55 was prepared in the usual manner to give the title compound in 70 % yield as colourless oil. The full spectroscopic characterisation is in chapter 6. 2. 56 on page 138.


The ring formation step underwent as hoped following the usual procedure by ortho lithiation employing an in situ trapping technique. The building blocks reacted as planned and no dimerisation by-product of either synthon could be obtained, only some starting material was recovered. Yields were low at 3.6 % but this route and the preparation of the synthons was very convenient. NMR spectroscopy confirmed that this compound was identical to the compound synthesised earlier.

3. 17 Synthesis of 6-[2-(dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (50)
RESULTS AND DISCUSSION

With the successful preparation of 56 we were interested also in the preparation of the second pyrimidine isomer under similar conditions using the same aldehyde and subject it to the cyclisation step with amide 47. Position 2 of the indole should in theory be lithiated more easily than position 3, thus giving better yields. Indeed, the reaction proceeded as expected and afforded 50 in 7.5 % yield as red needles. We considered this route elegant since it opened access to both tetracyclic pyrimidine isomers using the same pyrimidine-5-carbaldehyde. The structure was elucidated by NMR spectroscopy and verified by HRMS. The unambiguous discrimination of isomers 50 and 56 was possible by 2D-NMR technique HMBC (compare 6. 2. 51 on page 133).

3. 17. 1 Synthesis of 5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (49)

In order to synthesise and spectroscopically characterise the mere skeleton, we referred to the known procedure by preparing the MOM-protected framework and cleavage of the protecting group after the ring formation process with BBr₃ as outlined in Scheme 21.

Scheme 21. Synthesis of 49

3. 17. 2 Synthesis of N,N-diethyl-1H-indole-3-carboxamide (45), N,N-diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (46) and 1-[2-(dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (47)

The requisite building blocks 46 and 47 were prepared accordingly by alkylation with the corresponding side chain starting from 45, which in turn was prepared in analogy to compound 61 in the modified procedure of Gilman and Spatz in a one-pot reaction from commercially available
1H-indole-3-carboxylic acid. The detailed procedure and full spectroscopic characterisation of all intermediates are in chapters 6.2.46 on page 128, 6.2.47 on page 129 and 6.2.48 on page 130.

Considering the ease of both methods we attempted to prepare compound 7 which was the first compound of this series starting from the available ester 55. If this method had succeeded the synthesis could have been considerably optimised reducing it by five steps (compare 3.1 on page 18). Surprisingly, both building blocks didn’t react as expected, but formed compound 98, which is a dimerisation product of 55 as illustrated in Figure 16. Compound 98 was isolated using column chromatography eluting with ethyl acetate/methanol (8:2, v/v) followed by recrystallisation from ethyl acetate to furnish orange crystals in 9 % yield, m.p. 203-205 °C.

The $^1$H spectrum shows the absence of ester group and indole proton H-3 while retaining the remaining indole protons and the side chain which is indicating a dimerisation product of 55. This evidence could be confirmed in the $^{13}$C spectrum showing signals of one half of the molecule only, with the characteristic quinone carbons giving a single peak, which reflects the symmetry of the molecule (Figure 17 on page 66). HRMS analysis confirmed the calculated mass.

Figure 16. Dimerisation of 55
RESULTS AND DISCUSSION

3.18 Synthesis of 6-[2-(dimethylamino)ethyl]-5H-pyrazino[4,5-b]carbazole-5,11(6H)-dione (81)

With the successful construction of 50 we were confident that the same procedure would be applicable to the construction of the missing pyridazine derivative. Commercially available, but expensive pyridazine-4-carbaldehyde underwent the cyclisation in the usual procedure by metallating 47 and subsequent trapping with the aldehyde. After the purification procedure the yield was acceptable furnishing 6.2 % of 81 as red needles. The structure was elucidated via NMR and HRMS. However, an unambiguous NMR spectroscopic signal assignment of C-4a and C-11a was not possible via HMBC experiment because correlations of C-4a and C-11a to adjacent pyridazine protons H-1 and H-4 are apparent, and can therefore not be discriminated. Moreover, the spectrum lacks correlation of C-4a/C-11a to protons of the carbazole system. The invariable part of 81 exhibits a consistent set of signals, which is in complete agreement to other compounds of the tetracyclic series. A full spectroscopic assignment is referenced in the experimental section (6.2.83 on page 165).

Figure 17. NMR Signal Assignment of 98

1H NMR (400 MHz, CDCl₃) δ 8.30 (m, H-1/7, 2H), 7.39 (m, H-4/10, 2H), 7.33 (m, H-3/9, 2H), 7.32 (m, H-2/8, 2H), 4.73 (m, H-13/13', 4H), 2.73 (m, H-14/14', 4H), 2.38 (s, H-16/16'/16"/16'''', 12H).

13C NMR (100 MHz, CDCl₃) δ 177.2 (C-6/12), 138.8 (C-4a/10a), 135.9 (C-5a/11a), 125.7 (C-3/9), 124.3 (C-2/8), 124.2 (C-6b/12b), 122.8 (C-1/7), 117.2 (C-6a/12a), 111.0 (C-4/10), 58.5 (C-14/14'), 45.8 (C-16/16'/16", 16'''), 43.2 (C-13/13').

15N NMR (40 MHz, CDCl₃) δ -237.5 (N-5/11), -357.5 (N-15/15').

With the successful construction of 50 we were confident that the same procedure would be applicable to the construction of the missing pyridazine derivative. Commercially available, but expensive pyridazine-4-carbaldehyde underwent the cyclisation in the usual procedure by metallating 47 and subsequent trapping with the aldehyde. After the purification procedure the yield was acceptable furnishing 6.2 % of 81 as red needles. The structure was elucidated via NMR and HRMS. However, an unambiguous NMR spectroscopic signal assignment of C-4a and C-11a was not possible via HMBC experiment because correlations of C-4a and C-11a to adjacent pyridazine protons H-1 and H-4 are apparent, and can therefore not be discriminated. Moreover, the spectrum lacks correlation of C-4a/C-11a to protons of the carbazole system. The invariable part of 81 exhibits a consistent set of signals, which is in complete agreement to other compounds of the tetracyclic series. A full spectroscopic assignment is referenced in the experimental section (6.2.83 on page 165).
3. 18. 1 Synthesis of 5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (83)

The construction of the naked pyridazine skeleton was achieved in analogy to 49 via the MOM-derivative 82 and subsequent MOM cleavage. The detailed procedure and spectroscopic reference is in chapters 6. 2. 84 on page 166 and 6. 2. 85 on page 167.
3. 19 Biological Evaluation - Viability Assay of the Second Set of Compounds, FACS Analysis and Conclusion

The second set of our compound library was tested against SW480 cells using the EZ4U® XTT assay (Figure 18). They also exhibited the highest cytotoxic properties of all compounds tested.

Figure 18. Second Set of Compounds.
The most cytotoxic compounds are depicted in red. Compounds of the first set are in Figure 19 on page 69).
RESULTS AND DISCUSSION

3. 19. 1 Discussion of the Biological Evaluation of the Tetracyclic Compounds

Viability assays revealed that all tested compounds are highly cytotoxic, but tetracyclic pyridazine derivative 81 showed an exceptional IC$_{50}$ value of approximately 19 nM against SW480 cells (Table 3 on page 70).

Similarly, both pyrimidine derivatives 50 and 56 showed excellent activity at about IC$_{50}$ = 0.2 µM (Table 3 on page 70 and Figure 21 on page 71).

Based on the observation that the N-oxid bearing calothrixin A derivative 66 showed almost two-fold increased activity compared with calothrixin B derivative 65, we synthesised 22 as tetracyclic N-oxide analogue of compound 20. As expected 22 showed a more than six-fold increased activity (IC$_{50}$ = 84 nM) compared with non-N-oxide species 20 (Table 3 on page 70).

Derivative 43 which shares the same side chain motif as mitoxantrone enhanced the activity significantly (Table 3 on page 70). This might be attributed to the secondary amino function which allows the formation of DNA adducts with cellular formaldehyde.

All other basic substituents do not show a consistent pattern of improvement on the pyridine- or the pyrazine nucleus. On the contrary, their contribution to the overall activity appears to be completely random and lies in a range of an IC$_{50}$ value of approximately 0.5 to 1.5 µM. Endowed with the same substituent in some cases the pyridine derivative shows higher activity and in others the pyrazine derivative.

On the other hand, the second nitrogen atom of the heterocycle and the polar N-oxide moiety seems to be highly beneficial in terms of activity. Especially the pyrazine unit seems to be contributing...
very much, presumably due to the strong dipole momentum of the two adjacent nitrogens in connection with the carbonyl groups of the quinone.

<table>
<thead>
<tr>
<th>Compound</th>
<th>SW480 (Colon) [95%-CI]</th>
<th>Compound</th>
<th>SW480 (Colon) [95%-CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>2.69 [1.13 - 6.41]</td>
<td>42</td>
<td>0.878 [0.650 - 1.18]</td>
</tr>
<tr>
<td>12</td>
<td>2.07 [1.16 - 3.69]</td>
<td>43</td>
<td>0.387 [0.355 - 0.421]</td>
</tr>
<tr>
<td>16</td>
<td>0.796 [0.696 - 0.911]</td>
<td>44</td>
<td>4.56 [4.05 - 5.12]</td>
</tr>
<tr>
<td>20</td>
<td>0.519 [0.499 - 0.539]</td>
<td>50</td>
<td>0.202 [0.164 - 0.247]</td>
</tr>
<tr>
<td>22</td>
<td>0.0845 [0.0788 - 0.0906]</td>
<td>56</td>
<td>0.169 [0.151 - 0.189]</td>
</tr>
<tr>
<td>26</td>
<td>0.498 [0.363 - 0.684]</td>
<td>60</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>29</td>
<td>0.354 [0.317 - 0.394]</td>
<td>65</td>
<td>1.34 [1.11 to 1.62]</td>
</tr>
<tr>
<td>30</td>
<td>0.672 [0.625 - 0.723]</td>
<td>66</td>
<td>0.772 [0.704 - 0.846]</td>
</tr>
<tr>
<td>32</td>
<td>1.54 [1.23 - 1.95]</td>
<td>72</td>
<td>0.732 [0.591 - 0.906]</td>
</tr>
<tr>
<td>33</td>
<td>1.08 [0.891 - 1.30]</td>
<td>76</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>35</td>
<td>0.733 [0.670 - 0.803]</td>
<td>80</td>
<td>1.02 [0.941 - 1.11]</td>
</tr>
<tr>
<td>36</td>
<td>0.376 [0.361 - 0.391]</td>
<td>81</td>
<td>0.0185 [0.00879 - 0.0391]</td>
</tr>
<tr>
<td>38</td>
<td>0.400 [0.378 - 0.423]</td>
<td>98</td>
<td>14.1 [12.6 - 15.7]</td>
</tr>
<tr>
<td>39</td>
<td>0.560 [0.528 - 0.594]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Antiproliferative XTT (EZ4U®) Assay of the Second Set of Compounds against SW480 cells. IC₅₀ values are expressed in µM. Error bars represent ± standard error mean (SEM) of at least 3 independent experiments in quadruplicate. Numbers in square brackets represent IC₅₀ values in the 95 % confidence interval. Results from the first set against SW480 cells are printed in red.

Surprisingly, there was a big difference in the activity of the carbamate bearing compounds. Pyrazine derivative 44 showed an IC₅₀ of only 4.56 µM whereas the IC₅₀ value of 42 is 0.88 µM. Since the carbamate unit should be susceptible for enzymatic hydrolysis we repeated viability assays after 48 h and 72 h in order to detect any delayed increase in activity. However, no significant improvement could be detected (Table 4 and Figure 20 on page 71).

<table>
<thead>
<tr>
<th>Compound (treatment)</th>
<th>SW480 (Colon) [95%-CI]</th>
<th>Compound (treatment)</th>
<th>SW480 (Colon) [95%-CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 (24 h)</td>
<td>0.878 [0.650 - 1.18]</td>
<td>44 (24 h)</td>
<td>4.56 [4.05 - 5.12]</td>
</tr>
<tr>
<td>42 (48 h)</td>
<td>0.914 [0.838 - 0.996]</td>
<td>44 (48 h)</td>
<td>4.21 [2.54 - 6.97]</td>
</tr>
<tr>
<td>42 (72 h)</td>
<td>0.907 [0.813 - 1.01]</td>
<td>44 (72 h)</td>
<td>3.29 [2.35-4.62]</td>
</tr>
</tbody>
</table>

Table 4. Antiproliferative XTT (EZ4U®) Assay of Carbamate Derivatives 42 and 44. Cells were treated for 24 h, 48 h and 72 h with compounds and viability was measured. IC₅₀ values are expressed in µM. Numbers in square brackets represent IC₅₀ values in the 95 % confidence interval.
Figure 20. Dose-Response Curve of Compounds 42 and 44 (XTT Assay). Cells were treated with compounds for 24, 48 and 72 h and viability was measured. Error bars represent ± standard error mean (SEM) of at least 2 independent experiments in quadruplicate.

Figure 21. Dose-Response Curve of Compounds 50 and 56 (XTT Assay). Dotted line indicate the respective IC_{50} value. Error bars represent ± standard error mean (SEM) of at least 3 independent experiments in quadruplicate.
In future works, the two remaining isomeric pyridazine derivatives are of major interest as well as several N-oxide derivatives (Figure 23 on page 73).

According to our previous optimisation strategy, the best structures resulting from biological testings should then again be modified with the mitoxantrone or even a pixantrone side chain motif.
3. 19. 2 Discussion of the Biological Evaluation of the Pentacyclic Compounds

Pentacyclic pyrazine derivative 72, which is a benzoannulated analogue of the highly active compound 26 (IC$_{50}$ = 0.50 µM) exhibits a slightly decreased activity (IC$_{50}$ = 0.73 µM) compared with 26. This is consistent with the observation that all pentacyclic (benzoannulated) derivatives showed at least slightly decreased activity compared with their tetracyclic counterparts. Interestingly, benzoannulated analogues of 7 and 12, (compounds 60 and 76) showed only weak activity (no activity at 10 µM). Nevertheless, the mere cytotoxic potential is not a paramount feature of a successful anticancer agent, which is why the synthesis of other pentacyclic structures will be pursued; isoquinoline based calothrixin analogues are of still of interest, as well as N-oxide derivatives thereof and the N-oxide derivative of synthesised compound 80 (Figure 24 on page 74).

Compound 98 which contains two basic side chains attached to a symmetric planar framework, was synthesised by coincidence, and showed only moderate activity against SW480 cells (IC$_{50}$ = 14.1 µM).
Cell Cycle (FACS®) Analysis of Compounds 20, 22, 26 and 43

The synthesis and duplication of nuclear DNA before division, and mitosis, the process of cellular division itself are the two most obvious features of the cell cycle. These two components of the cell cycle are usually termed "S phase" and "M phase". Later, a temporal gap between mitosis and the onset of DNA synthesis was discovered, and another gap between the completion of DNA synthesis and the onset of mitosis. In consequence, these gaps were termed G1 and G2, respectively (Figure 25). When cells are not in the preparation process for cell division, they remain in the G1 phase of the cell cycle, which is why the G1 phase is numerically the most predominant phase. Non-proliferating cells can enter a quiescent state from the G1 phase for a long period of time or indefinitely which is often the case in fully differentiated cells, such as neurons or muscle cells. This phase is termed G0.
cells can reenter the G1 phase, which is the case in liver, stomach or kidney cells.\textsuperscript{133} The G1 phase is characterised as a synthetic phase in which cell organelles, cytoplasm, RNA, replication enzymes and other molecules are produced before DNA replication. In the G2 phase, DNA damage of the preceding cell cycle phases is being repaired, and DNA structure is being reorganised which must take place before the DNA can be divided equally between daughters during mitosis.

FACS\textsuperscript{\textregistered}, a registered trademark of Becton Dickinson (BD) standing for "fluorescence activated cell sorting", has become a generic term in cell biology which employs flow cytometry to distinguish cells in different phases of the cell cycle. Cells are lysed and the extracted nuclei are stained with propidium iodide, an intercalating dye, which stains DNA in a stoichiometric manner (the amount of stain is directly proportional to the amount of DNA in the cell).

As the DNA content of cells duplicates during the S phase of the cell cycle, the relative amount of cells in the G0 phase and G1 phase (before S phase), in the S phase, and in the G2 phase and M phase (after S phase) can be determined, as the fluorescence of cells in the G2/M phase will be twice as high as that of cells in the G0/G1 phase.\textsuperscript{133}

Cell cycle analysis of highly cytotoxic compounds \textsuperscript{20} and \textsuperscript{26} of the first series and \textsuperscript{22} and \textsuperscript{43} of the second series was performed (\textit{Figure 26 on page 76}). After treatment of SW480 cells with compounds \textsuperscript{26}, \textsuperscript{22}, \textsuperscript{43} at their respective IC\textsubscript{50} and IC\textsubscript{25} concentrations for 24 h, cells were arrested in G2/M phase, whereas compound \textsuperscript{20} was causing an S-phase arrest. These results were not surprising and as similar to other compounds of our library. Similar reports are also reported for mitoxantrone\textsuperscript{134, 135}, which is known to cause a dose-dependent S-phase and G2/M-arrest. Still, the underlying mechanism needs further investigation.
A series of substituted tetracyclic ellipticine quinone and pentacyclic calothrixin analogues has been synthesised and tested for antiproliferative activity. Tetracyclic compounds 22, 43, 50, 56 and 81 showed the highest cytotoxic activity, particularly pyridazine derivative 81.

Ring expansion by one benzoannulation which leads to calothrixin isomers could not further improve cytotoxic activity.

Of all substituents the 2-[(2-Hydroxyethyl)amino]ethyl side chain was the most effective, presumably because of its capability to form formaldehyde-DNA adducts with its secondary amino function as could be demonstrated for mitoxantrone.

If this motif can improve the activity of other nuclei will be investigated in future works.

Figure 26. Cell Cycle FACS® Analysis.
Compounds 20 (P < 0.0001), 22 (P < 0.0005), 26 (P < 0.0001) and 43 (P < 0.0016). Calculated P Values of One-Way ANOVA are expressed in round brackets.

4 CONCLUSION

A series of substituted tetracyclic ellipticine quinone and pentacyclic calothrixin analogues has been synthesised and tested for antiproliferative activity. Tetracyclic compounds 22, 43, 50, 56 and 81 showed the highest cytotoxic activity, particularly pyridazine derivative 81.

Ring expansion by one benzoannulation which leads to calothrixin isomers could not further improve cytotoxic activity.

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If this motif can improve the activity of other nuclei will be investigated in future works.
5 EXPERIMENTAL PART - CELL CULTURE

5.1 Instrumentation And Materials Used For Cell Culture

Petri Dishes (10 cm and 6 cm): Sarstedt
24-well plates and 96-well plates: Sarstedt
Penicillin/Streptomycin (Penstrep): PAA
Fetal Calf Serum (FCS): Sigma-Aldrich
RPMI-1640 Medium 10x: Sigma-Aldrich
Trypsin/PBS 10x (for use diluted 1:10 in PBS): PAA
Eagles Minimal Essential Medium (MEM): Sigma-Aldrich
Bovine Serum Albumin (BSA): Sigma-Aldrich
Phosphate Buffered Saline (PBS): Sigma-Aldrich
DMSO: Sigma-Aldrich
PBS/EDTA (10mM EDTA in PBS): Merck
Neutral Red: Merck
EZ4U® Cell Proliferation & Cytotoxicity Assay: Biomedica

Plate Reader: TECAN Infinite® 200 PRO
Flow Cytometer: BD Sciences FACSCalibur®

5.2 Cell Lines

Viability assays were performed on 5 different standard cell lines, consisting of SW480, Hep3B, A549, U373 MG and HTB-65 which were obtained from the American Type Culture Collection (ATCC®).

5.2.1 SW480 (ATCC® CCl-228™)136

SW480 (ATCC® CCl-228™) cells were established from a primary adenocarcinoma of the colon from a 50-year-old Caucasian male. The chromosome number is hypotriploid. This line has a mutation in codon 12 of the ras proto-oncogene, and can be used as a positive control for PCR assays of mutation in this codon. It has an elevated level of p53 protein and is positive for expression of c-myc, K-ras, H-ras, N-ras, myb, sis and fos oncogene. The cells have been reported to produce GM-CSF. SW480 cells are cultivated in MEM containing 10 % FCS and 4 % Pen-Strep.
**EXPERIMENTAL PART**

5.2.2 **Hep3B (ATCC® HB-8064™)**

Hep3B (ATCC® HB-8064™) is a human hepatoma derived cell line from an 8-year-old black male. This line contains an integrated hepatitis B virus genome. Hep3B cells are cultivated in MEM containing 10 % FCS and 4 % Pen-Strep.

5.2.3 **A549 (ATCC® CCL-185™)**

A549 (ATCC® CCL-185™) was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. It is a hypotriplloid human cell line with the modal chromosome number of 66, occurring in 24 % of cells. The cells are positive for keratin by immunoperoxidase staining. Cells with 64 (22 %), 65, and 67 chromosome counts also occurred at relatively high frequencies. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40 % of 50 cells analysed. Cytogenetic information is based on initial seed stock at ATCC®. Cytogenetic instability has been reported in the literature for some cell lines. A549 cells are cultivated in MEM containing 10 % FCS and 4 % Pen-Strep.

5.2.4 **U373 MG (ATCC® HTB-17™)**

U373 MG (ATCC® HTB-17™) is a human glioblastoma astrocytoma cell line derived from a malignant tumour by explant technique. The ATCC® reported that their stock of U373 MG had been shown to have differing genetic properties to stock from the originator’s laboratory. (STR)-PCR-profiling, morphological and immunological analysis has revealed that U373 MG is identical to U251 MG. U373 MG cells are grown in MEM containing 10 % FCS, 1 % non-essential amino acids, and 1 mM sodium pyruvate.
5. 2. 5  MeWo (ATCC® HTB-65™)\textsuperscript{136}

MeWo (ATCC® HTB-65™) is a malignant melanoma cell line which was initiated by Y. Kodera and M. Bean in 1974 derived from lymph node tissue of a 78-year-old caucasian male. HTB-65 cells are cultivated in RPMI-1640 medium containing 10% FCS and 4% Pen-Strep.

5. 3  Passaging of Cells

SW480, Hep3B, A-549, U-373 MG HTB-65 cells were passaged when they reached a confluence of about 90%. Culture medium was removed and the cells were washed with 10 mL of PBS/EDTA. After removal of PBS/EDTA cells were trypsinised by adding 600 µL of trypsin/PBS and incubating for 5-10 min at 37 °C. The detached cells were taken up in 10 mL culture medium and passaged into new 10 cm petri dishes (PD). All cell lines were usually split by a ratio of 1:5 to 1:10.
5. 4  Cell Viability Assays

5. 4. 1  XTT Cell Proliferation & Cytotoxicity Assay – EZ4U®

The non-radioactive cell proliferation and cytotoxicity assay EZ4U® is based on the capability of living cells to reduce slightly coloured or uncoloured tetrazolium salts in the mitochondria into intensely coloured formazan derivatives. This water soluble formazan is secreted into the culture medium and can be measured with a standard colorimetric reader.\textsuperscript{137}

Cells were seeded at a density of 1x10\textsuperscript{4} cells (SW480, Hep3B and A549 cell lines) or 6x10\textsuperscript{3} cells (U373 MG, HTB-65) per well into 96-well plates and after 24 hour treatment with active compounds in increasing concentrations, the supernatant was replaced with 100 μl of freshly prepared EZ4U® solution. The cells were incubated at 37 °C for 3 hours and the absorbance was measured at 450 nm with 620 nm as reference in a microplate reader Tecan Infinite\textsuperscript{a} 200 Pro. Absorbance from a substrate blank in serum free medium without cells was subtracted from all other values.

**EZ4U® Solution**

Dissolve 1 vial SUB (substrate) in 2.5 mL prewarmed (37 °C) ACT (activator) solution. This procedure yields a yellow solution which was added to serum free medium (1:10, v/v).

5. 4. 2  Neutral Red Assay

This colorimetric assay is based on the ability of viable cells to incorporate and bind Neutral Red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride). Viable cells incorporate neutral red into their lysosomes. As cells begin to die, their ability to incorporate neutral red diminishes. Thus, loss of Neutral red uptake corresponds to loss of cell viability.\textsuperscript{138}

Cells were seeded at a density of 1x10\textsuperscript{4} cells (SW480, Hep3B and A549 cell lines) or 6x10\textsuperscript{3} cells (U373 MG and HTB-65 cell lines) per well into 96-well plates and treated with active compounds in increasing concentrations for 24 h.

The supernatant from each well was removed and 200 μl Neutral red solution was added in each well. After incubation at 37 °C for 2 h, the Neutral red solution was removed and the cells were washed with 200 μL PBS. Then, the dye was extracted by adding 100 μL of a mixture of 70 % ethanol + 1 % acetic acid and shaking gently for 5 minutes at room temperature on a titer-plate shaker. The absorbance was measured at 562 nm with 620 nm as reference in a microplate reader Tecan Infinite\textsuperscript{a} 200 Pro.

**Neutral Red Solution:**

Dissolve 50 μg/mL Neutral Red in serum free medium (SFM) at 37 °C for 1 hour followed by filtering.
5.5 Cell Cycle Distribution (FACS®)

Cells were seeded at a density of 1 x 10^6 cells per 6 cm PD. Cells were treated with active compounds for 24 h at 37 °C. The supernatant of treated cells was removed and the cells were washed with 3 mL PBS/EDTA. Cells were trypsinised by adding 250 µL trypsin/EDTA and incubation at 37 °C for 5-10 minutes. 3 mL MEM containing 10 % FCS was added and the cells were passaged into a 15 mL Falcon® tube. After centrifugation at 1200 rpm for 5 minutes the cell pellet was resuspended in 1 mL PBS and transferred into a 1.5 mL tube. Cells were lysed by adding 1 mL of nuclear isolation buffer, mixed well and incubated for 5 minutes on ice. Nuclei were prepared and separated using a syringe. Nuclei were collected by centrifugation for 5 minutes at 4 °C at 2000 rpm, resuspended in 0.5 mL propidium iodide staining solution and transferred into light protected FACS vials. Cell cycle was measured using a BD Sciences FACSCalibur®.

Nuclear Isolation Buffer:

10.5 g citric acid and 0.5 g Tween 20 are added to to 100 mL aqua bidest.

RNAse Stock Solution:

Dissolve 10 mg RNAse A in 10 mL 1x PBS. Incubate for 15 min at 100 °C in heating block. Put on ice immediately and store at -20 °C.

Propidium Iodide Stock Solution:

Dissolve 5 mg propidium iodide in 10 mL 1x PBS. Store protected from light (wrap in aluminium foil).

Propidium Iodide Staining Solution: 0.5 mL necessary for 1 sample

Mix 0.05 mL RNAse stock solution and 0.05 mL propidium iodide stock solution with 5 mL 1x PBS.
6 EXPERIMENTAL PART - SYNTHESIS

6.1 Instrumentation and Chemicals

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 293 K (400.23 MHz for $^1$H, 100.65 MHz for $^{13}$C, 40.56 MHz for $^{15}$N) or on a Bruker Avance 500 spectrometer at 293 K (500.13 MHz for $^1$H, 125.77 MHz for $^{13}$C, 50.68 MHz for $^{15}$N). The center of the residual undeuterated solvent signal was used as an internal standard, which was related to TMS with $\delta$ 7.26 ppm ($^1$H in CDCl$_3$), $\delta$ 2.49 ppm ($^1$H in DMSO-$d_6$), $\delta$ 77.0 ppm ($^{13}$C in CDCl$_3$), and $\delta$ 39.5 ppm ($^{13}$C in DMSO-$d_6$). $^{15}$N NMR spectra were referenced against external nitromethane with a ‘directly’ detecting broadband observe probe (BBFO). Digital resolutions were 0.25 Hz/data point in the $^1$H spectra and 0.4 Hz/data point in the $^{13}$C NMR spectra. Assignments of signals was carried out by the combined application of standard NMR spectroscopic techniques such as $^1$H coupled $^{13}$C-NMR spectra, APT, HSQC, HMBC, COSY, and NOESY spectroscopy. Chemical shift values ($\delta$) are reported in ppm, coupling constants ($J$) in Hz.

HRESIMS spectra were obtained on a Bruker maXis HD Qq-TOF mass spectrometer. Samples were dissolved in MeOH (1-100 µg/mL) and directly infused into the ESI source at a flow rate of 3 µL/min with a syringe pump. The ESI ion source was operated as follows: capillary voltage: 1.5 to 4.0 kV (individually optimised), nebuliser: 0.4 bar (N$_2$), dry gas flow: 4 L/min (N$_2$), and dry temperature: 200 °C. Mass spectra were recorded in the range of m/z 50 – 1550 in the positive-ion mode. For one sample, an APCI source was used instead of ESI, with comparable parameter settings. The sum formulas were determined using Bruker Compass DataAnalysis 4.2 based on the mass accuracy ($\Delta m/z \leq 2$ ppm) and isotopic pattern matching (SmartFormula algorithm).

Microwave reactions were performed with a CEM Discover® Labmate microwave reactor.

For TLC, Merck Millipore aluminum sheets pre-coated with Silica gel 60 F254 were used and developed under UV light. For column chromatography, Silica gel 60 (63-200 µm) or LiChroprep® RP-18 (40-63 µm) by Merck Millipore was used.

Melting points were determined on a Reichert–Kofler® hot-stage microscope and are uncorrected. Systematic names were generated with ACD/Name® according to the IUPAC recommendations.

All chemicals and solvents were purchased from Sigma-Aldrich, Fisher Scientific, Alfa Aesar, TCI Europe N.V. and VWR except of pyridazine-4-carbaldehyde (purchased from Activate Scientific) and pyrimidine-5-carbaldehyde (purchased from Frontier Scientific).
6. 2  Synthesis

6. 2. 1  Furo[3,4-\textit{b}]pyridin-5,7-dione (1)

Pyridine-2,3-dicarboxylic acid (10 g, 0.06 mol) was dissolved in freshly distilled acetic anhydride (30 mL, 0.32 mol) and the solution was refluxed for 2 h. The excess of acetic anhydride was removed in vacuo (70 °C at 20 mbar). The wet brown residue was then transferred in a recystallising dish and completely dried under reduced pressure in a desiccator overnight, ground and purified by sublimation (120 °C) to afford 6.7 g (75.0 %) of \textit{1} as white crystals, m.p. 135-136 °C.

\begin{center}
\includegraphics[width=0.5\textwidth]{furo-b-pyridin-5-7-dione.png}
\end{center}

H NMR (400 MHz, DMSO-\textit{d}_6) \delta 9.13 (dd, \textit{J} = 4.9, 1.5 Hz, H-2, 1H), 8.53 (dd, \textit{J} = 7.8, 1.5 Hz, H-4, 1H), 7.93 (dd, \textit{J} = 7.8, 4.9 Hz, H-3, 1H).

\begin{center}
\includegraphics[width=0.4\textwidth]{furo-b-pyridin-5-7-dione_nmr.png}
\end{center}

\begin{center}
\begin{tabular}{c}
\textbf{13}C NMR (100 MHz, DMSO-\textit{d}_6) \delta 161.7 (C-7), 161.5 (C-5), 156.9 (C-2), 150.8 (C-7a), 133.6 (C-4), 129.1 (C-3), 127.3 (C-4a).
\end{tabular}
\end{center}

\begin{center}
\begin{tabular}{c}
\textbf{15}N NMR (40 MHz, DMSO-\textit{d}_6) -71.5 (N-1).
\end{tabular}
\end{center}
6. 2. 2  Furo[3,4-\(b\)]pyridin-5(7)-one (2)

To a solution of 1 (5.0 g, 0.2 mol) in dry THF (40 mL) was added NaBH\(_4\) (1.25 g, 0.03 mol) at 15 °C under argon atmosphere, then CH\(_3\)COOH (4.0 g, 3.3 mL) was added dropwise along with evolving H\(_2\) gas at 15 °C under argon. After stirring at 15 °C for 4 h under argon, the mixture was concentrated in vacuo. CH\(_3\)COOH (14 mL) and acetic anhydride (14 mL) were added to the residue and the solution was stirred at 100 °C for 3 h. The mixture was concentrated in vacuo and to the residue were added H\(_2\)O (40 mL) and NaCl (7 g). The water layer was extracted with CH\(_2\)Cl\(_2\) (2 x 40 mL). The combined organic layers were dried over Na\(_2\)SO\(_4\) and the solvents removed in vacuo. The residue was purified by column chromatography eluting with ethyl acetate to afford 1.76 g (38.0 %) of 2 as colourless crystals, m.p. 152-154 °C. Alternatively, 2 can be purified sufficiently by recrystallising twice from isopropanol to give beige needles.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.84 (dd, \(J = 4.9, 1.6\) Hz, H-2, 1H), 8.18 (dd, \(J = 7.8, 1.6\) Hz, H-4, 1H), 7.47 (dd, \(J = 7.8, 4.9\) Hz, H-3, 1H), 5.31 (s, H-7, 2H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 168.9 (C-5), 166.4 (C-7a), 155.3 (C-2), 134.0 (C-4), 123.9 (C-3), 119.6 (C-4a), 70.5 (C-7).

\(^{15}\)N NMR (40 MHz, CDCl\(_3\)) \(\delta\) -79.6 (N-1).
6. 2. 3 1-(Phenylsulfonyl)-1H-indole (3)

To an ice-cold mixture of powdered NaOH (50.0 g, 1.25 mol) and tetra-n-butylammonium hydrogen sulfate (3.50 g, 0.10 mol) in dry CH₂Cl₂ (500 mL) under argon was added indole (46.75 g, 0.40 mol) in one portion, followed by a solution of benzenesulfonyl chloride (63.8 mL, 0.50 mol) in CH₂Cl₂ (300 mL) at such a rate as to keep the internal temperature below 20 °C. The mixture was then vigorously stirred at room temperature for 2 h, filtered, and evaporated in vacuo to produce a thick orange oil. Trituration with methanol afforded 77 g (74.9 %) of 3 as colourless crystals, m.p. 77-79 °C.

¹H NMR (400 MHz, DMSO-d₆) δ 7.97 (m, H-2'/6', 2H), 7.95 (m, H-7, 1H), 7.81 (d, J = 3.7 Hz, H-2, 1H), 7.64 (m, H-4', 1H), 7.59 (m, H-4, 1H), 7.55 (m, H-3'/5', 2H), 7.33 (m, H-6, 1H), 7.23 (m, H-5, 1H), 6.84 (dd, J = 3.7, 0.8 Hz, H-3, 1H).

¹³C NMR (100 MHz, DMSO-d₆) δ 137.0 (C-1'), 134.5 (C-4'), 134.1 (C-7a), 130.4 (C-3a), 129.7 (C-3'/5'), 126.9 (C-2), 126.6 (C-2'/6'), 124.7 (C-6), 123.5 (C-5), 121.6 (C-4), 113.0 (C-7), 109.5 (C-3).

¹⁵N NMR (40 MHz, DMSO-d₆) δ -207.9 (N-1).
6. 2. 4 [2-(Hydroxymethyl)-3-pyridinyl][1-(phenylsulfonyl)-1H-indol-2-yl] methanone (4)

\[
\begin{align*}
\text{N} & \quad \text{SO}_2 \\
\text{O} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\text{SO}_2 & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{N} & \quad \text{SO}_2 \\
\text{O} & \quad \text{N} \\
\end{align*}
\]

To a solution of 2 (4.0 g, 15.5 mol) in dry THF (200 mL) at -78 °C was added \(n\)-BuLi (9.8 mL of 1.6M-solution in \(n\)-hexanes) in one portion. The mixture was allowed to warm to room temperature during 1 h, and then warmed to 40 °C for 5 min. After recooling to -78 °C furo[3,4-b]pyrindin-5(7H)-one 3 (2.0 g, 14.8 mmol) in dry THF (200 mL) was added rapidly in one portion. The cooling bath was removed and the mixture allowed to warm to room temperature, then brought to 40 °C for 5 min. The solvent was then removed in vacuo at 20 °C. The residue was partitioned between water (60 mL) and Et\(_2\)O (4 x 200 mL) and the product extracted from the organic layer with 2M HCl. Basification of the aqueous extract with solid K\(_2\)CO\(_3\), extraction with CH\(_2\)Cl\(_2\), and evaporation of the solvent under reduced pressure affords 2 g (34 %) of 4 as orange-red amorphous solid which can be used without purification in the next step. After purification with column chromatography using ethyl acetate as eluent the ketone appears as colourless irregular prisms (1.27 g, 21.8 %), m.p. 139-140 °C.

HRMS: m/z calculated for C\(_{21}\)H\(_{17}\)N\(_2\)O\(_4\)S ([M+H]\(^+\)): 393.0904; found: 393.0909.

HRMS: m/z calculated for C\(_{21}\)H\(_{16}\)N\(_2\)NaO\(_4\)S ([M+Na]\(^+\)): 415.0723; found: 415.0727.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.76 (dd, \(J = 4.9, 1.6\) Hz, H-6', 1H), 8.09 (m, H-7, 1H), 7.97 (m, H-4'/2''/6'', 3H), 7.58 (m, H-4'', 1H), 7.56 (m, H-4, 1H), 7.48 (m, H-6'/3'', 3H), 7.36 (dd, \(J = 7.8, 4.9\) Hz, H-5'', 1H), 7.31 (m, H-5, 1H), 6.97 (d, \(J = 0.7\) Hz, H-3, 1H).

\(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 187.0 (C-8), 159.9 (C-2'), 150.4 (C-6'), 137.9 (C-2), 137.7 (C-7a), 137.4 (C-1''), 134.2 (C-4''), 131.0 (C-3'), 129.1 (C-5''), 128.5 (C-3a), 127.7 (C-6), 127.4 (C-6'/5'', 3H), 122.9 (C-4), 122.0 (C-5''), 118.3 (C-3), 115.2 (C-7), 63.2 (C-9).

\(^15\)N NMR (40 MHz, CDCl\(_3\)) \(\delta\) not found (N-1/1').
6. 2. 5  \textit{6H-Pyrido[3',2':5,6]oxepino[3,2-b]indol-5(12H)-one (5)}

\begin{equation}
\text{Ketone 4 (2.0 g, 0.005 mol) was dissolved in methanol (200 mL) under reflux. Aqueous 3M NaOH (100 mL) was added in one portion to the colourless solution which turned red instantly. The mixture was refluxed for further 2-5 minutes. Prolonged heating must be avoided. The red mixture was poured into ice water (1500 mL) and the yellow precipitate was collected by filtration. The filtrate was extracted with CH}_2Cl_2 (3 x) to extract additional product from the aqueous layer. The collected crystals and the CH}_2Cl_2 extract were purified by column chromatography using ethyl acetate as eluent affording 420 mg of 5 (32.9 \% of unpurified compound 4) as bright yellow crystals, m.p. 195-197 °C. Compound 5 is LIGHT SENSITIVE and should be stored accordingly.}

\textit{HRMS: m/z calculated for C}_{15}H_{11}N_2O_2 ([M+H]^+): 251.0815; found: 251.0811.}

\textit{HRMS: m/z calculated for C}_{15}H_{10}N_2NaO_2 ([M+Na]^+): 273.0634; found: 273.0632.}

\begin{equation}
\text{\textbf{1}H NMR (400 MHz, DMSO-\textit{d}_6) \delta 11.39 (s, H-6, 1H), 8.80 (dd, J = 4.8, 1.8 Hz, H-2, 1H), 8.36 (dd, J = 7.9, 1.8 Hz, H-4, 1H), 7.66 (m, H-10, 1H), 7.65 (dd, J = 7.9, 4.8 Hz, H-3, 1H), 7.39 (m, H-7, 1H), 7.35 (m, H-8, 1H), 7.05 (m, H-9, 1H), 5.50 (s, H-12, 2H).}
\end{equation}

\begin{equation}
\text{\textbf{13}C NMR (100 MHz, DMSO-\textit{d}_6) \delta 178.2 (C-5), 153.1 (C-12a), 152.5 (C-2), 147.2 (C-10b), 136.8 (C-6a), 136.5 (C-4), 132.8 (C-4a), 127.9 (C-8), 124.7 (C-3), 122.1 (C-5a), 120.1 (C-10), 119.7 (C-9), 117.2 (C-10a), 112.9 (C-7), 78.0 (C-12).}
\end{equation}

\begin{equation}
\text{\textbf{15}N NMR (40 MHz, DMSO-\textit{d}_6) \delta -64.9 (N-1), -263.3 (N-6).}
\end{equation}
Oxepinoindole 5 (420 mg, 1.7 mmol) from the previous step was refluxed in methanol (120 mL) and 3M NaOH (60 mL) for 12 h with passage of air. The mixture was poured into water (1500 mL) and the reddish-brown precipitate was filtered off and dried in a desiccator in vacuo. The crude product was purified by sublimation (200 °C at < 0.01 Torr) to afford 395 mg (95.0 %) of 6 as yellow crystals, m.p. >350 °C.

HRMS: m/z calculated for C_{15}H_{9}N_{2}O_{2} ([M+H]^+): 249.0659; found: 249.0660.
HRMS: m/z calculated for C_{15}H_{8}N_{2}NaO_{2} ([M+Na]^+): 271.0478; found: 271.0481.

\[^{1}\text{H NMR (400 MHz, DMSO-d}_6\)] \( \delta \) 13.15 (br s, H-6, 1H), 8.99 (dd, \( J = 4.7, 1.7 \) Hz, H-2, 1H), 8.45 (dd, \( J = 7.8, 1.7 \) Hz, H-4, 1H), 8.24 (m, H-10, 1H), 7.80 (dd, \( J = 7.8, 4.7 \) Hz, H-3, 1H), 7.61 (m, H-7, 1H), 7.46 (m, H-8, 1H), 7.38 (m, H-9, 1H).

\[^{13}\text{C NMR (100 MHz, DMSO-d}_6\)] \( \delta \) 178.6 (C-11), 177.1 (C-5), 153.7 (C-2), 149.9 (C-11a), 138.5 (C-6a, via HMBC), 134.0 (C-4), 129.8 (C-4a), 127.1 (C-8), 127.0 (C-3), 124.11 (C-9), 124.05 (C-10, via HMBC), 122.5 (C-10), 118.1 (C-10b), 114.1 (C-7), not found (C-5a).

\[^{15}\text{N NMR (40 MHz, DMSO-d}_6\)] \( \delta \) not found (N1/6).
6.2.7 6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (7)

NaH (380 mg of a 60 % dispersion in mineral oil, 9.5 mmol) was suspended in dry DMF (13 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 6 (396 mg, 1.6 mmol) in dry DMF (24 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-N,N-dimethylethanamine hydrochloride (460 mg, 3.19 mmol) in dry DMF (20 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (250 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (8:2, v/v) to obtain 134 mg (26.3 %) of 7 as orange-brown crystals, m.p. 187-189 °C.

HRMS: m/z calculated for C₁₉H₁₈N₃O₂ ([M+H]+): 320.1394; found: 320.1395.
HRMS: m/z calculated for C₁₉H₁₇N₃NaO₂ ([M+Na]+): 342.1213; found: 342.1215.

¹H NMR (400 MHz, CDCl₃) δ 9.01 (dd, J = 4.7, 1.7 Hz, H-2, 1H), 8.54 (m, H-10, 1H), 8.49 (dd, J = 7.8, 1.7 Hz, H-4, 1H), 7.62 (dd, J = 7.8, 4.7 Hz, H-3, 1H), 7.51 (m, H-7, 1H), 7.50 (m, H-8, 1H), 7.42 (m, H-9, 1H), 4.83 (m, H-12, 2H), 2.75 (m, H-13, 2H), 2.38 (s, H-15/15’, 6H).

¹³C NMR (100 MHz, CDCl₃) δ 179.1 (C-11), 177.7 (C-5), 154.0 (C-2), 149.8 (C-11a), 139.7 (C-6a), 134.5 (C-4), 134.2 (C-5a), 130.3 (C-4a), 127.9 (C-8), 126.5 (C-3), 124.9 (C-9), 124.4 (C-10), 124.2 (C-10a), 119.8 (C-10b), 111.0 (C-7), 58.6 (C-13), 45.9 (C-15/15’, 43.6 (C-12).

¹⁵N NMR (40 MHz, CDCl₃) δ -71.7 (N-1), -238.4 (N-6), -358.0 (N-14).
**EXPERIMENTAL PART**

6. 2. 8  1-(Methoxymethyl)-1\(H\)-indole-3-carbaldehyde (8)

\[
\begin{align*}
\text{NaH/THF} & \quad -23 \, ^{\circ}\text{C} \to \text{RT} \\
\text{NaH} & \quad \text{THF} \\
\text{1-(Methoxymethyl)-1}\text{-indole-3-carbaldehyde} (8)
\end{align*}
\]

NaH (60 % dispersion in mineral oil) (1.26 g, 31.5 mmol) was suspended in dry THF (20 mL). The mixture was cooled to -23 °C and 1\(H\)-indole-3-carbaldehyde (4.35 g, 30 mmol) in dry THF (70 mL) was added dropwise. After warming to room temperature and stirring for 30 min, the solution was cooled to -23 °C and chloro(methoxy)methane (2.50 mL, 33 mmol) was added, and the mixture was allowed to warm to room temperature. The mixture was poured into a vigorously stirred cold 5 % NaHCO\(_3\) solution (30 mL) and extracted with diethyl ether. The combined organic layers were washed with brine and dried over MgSO\(_4\). The mixture was filtered and concentrated in vacuo to give a solid. The crude product was purified by recrystallisation from ethyl acetate/light petroleum (1:1, v/v) to afford 4 g (70.5 %) of 8 as beige crystals, m.p. 77-78 °C.

\[
\begin{align*}
\text{1H NMR (400 MHz, CDCl}_3) & \quad \delta 9.96 \text{ (s, H-8, 1H), 8.29 (m, H-4, 1H), 7.72 (s, H-2, 1H), 7.47 (m, H-7, 1H), 7.31 (m, H-6, 1H), 7.30 (m, H-5, 1H), 5.40 (s, H-9, 2H), 3.23 (s, H-10, 3H).} \\
\text{13C NMR (100 MHz, CDCl}_3) & \quad \delta 184.7 \text{ (C-8), 138.5 (C-2), 136.9 (C-7a), 125.2 (C-3a), 124.2 (C-6), 123.1 (C-5), 121.7 (C-4), 118.6 (C-3), 110.5 (C-7), 77.9 (C-9), 56.0 (C-10).} \\
\text{15N NMR (40 MHz, CDCl}_3) & \quad \delta -228.6 \text{ (N-1).}
\end{align*}
\]
6. 2. 9  \(N,N\)-Diethylpyridine-2-carboxamide (9)

To a solution of pyridine-2-carboxylic acid (24.5 g, 0.2 mol) and diethylamine (74 mL, 0.7 mol) in dry toluene (100 mL) was added phosphoric anhydride (31.2 g, 0.22 mol) in portions (exothermic). Then the mixture was refluxed for 4 h, cooled in an ice-bath while an excess of aqueous 10 % NaOH was added. The toluene layer was separated, and the aqueous layer was thoroughly extracted with a mixture of ether and toluene (1:1, v/v). The combined organic layers were dried over Na\(_2\)SO\(_4\) and the solvents removed in vacuo. The dark brown, oily residue was purified by ball-tube distillation (b.p. 102-110 °C at < 1 Torr) or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to afford 20 g (69.0 %) of a viscous yellow oil.

\(^{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.49 (m, H-6, 1H), 7.69 (m, H-4, 1H), 7.48 (m, H-3, 1H), 7.23 (m, H-5, 1H), 3.49 (q, \(J = 7.1\) Hz, H-10, 2H), 3.29 (q, \(J = 7.1\) Hz, H-10', 2H), 1.19 (t, \(J = 7.1\) Hz, H-11, 3H), 1.07 (t, \(J = 7.1\) Hz, H-11', 3H).

\(^{13}C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\) 168.4 (C-7), 155.0 (C-2), 148.2 (C-6), 136.7 (C-4), 123.8 (C-5), 122.7 (C-3), 43.0 (C-10'), 39.9 (C-10), 14.1 (C-11'), 12.7 (C-11).

\(^{15}N\) NMR (40 MHz, CDCl\(_3\)) \(\delta\) -73.5 (N-1), -252.0 (N-9).
EXPERIMENTAL PART

6.2.10 10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (10)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 9 (1.00 g, 5.61 mmol, 1 eq.) and 8 (1.06 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish an orange residue. The residue was purified by recrystallisation from ethyl acetate to give 579 mg (35.3 %) of 10 as orange needles, m.p. 196-198 °C.

HRMS: m/z calculated for C₁₁₇H₁₇₃N₂NaO₃ ([M+Na]⁺): 315.0740; found: 315.0739.

¹H NMR (400 MHz, CDCl₃) δ 8.93 (dd, J = 4.7, 1.7 Hz, H-2, 1H), 8.44 (dd, J = 7.8, 1.7 Hz, H-4, 1H), 8.31 (m, H-6, 1H), 7.61 (dd, J = 7.8, 4.7 Hz, H-3, 1H), 7.56 (m, H-9, 1H), 7.43 (m, H-8, 1H), 7.34 (m, H-7, 1H), 6.12 (s, H-12, 2H), 3.36 (s, H-13, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 179.8 (C-5), 176.6 (C-11), 153.4 (C-2), 148.9 (C-11a), 139.8 (C-9a), 135.0 (C-10a), 134.2 (C-4), 130.3 (C-4a), 128.2 (C-8), 127.3 (C-3), 125.2 (C-7), 123.6 (C-6), 123.5 (C-5b), 119.6 (C-5a), 112.1 (C-9), 75.3 (C-12), 56.5 (C-13).

¹⁵N NMR (40 MHz, CDCl₃) δ -71.3 (N-1), not found (N-10).
6.2. 11  5H-Pyrido[2,3-b]carbazole-5,11(10H)-dione (11)

To an ice-cold solution of 10 (547 mg, 1.87 mmol) in dry CH₂Cl₂ (120 mL) a solution of BBr₃ (1M in CH₂Cl₂, 2.24 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated NaHCO₃ solution (120 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH₂Cl₂. Recrystallisation from ethyl acetate afforded 542 mg (95.0 %) of 11 as orange crystals, m.p. 317-319 °C.

HRMS: m/z calculated for C₁₅H₉N₂O₂ ([M+H]+): 249.0659; found: 249.0659.

¹H NMR (400 MHz, DMSO-d₆) δ 13.19 (s, H-10, 1H), 8.95 (dd, J = 4.7, 1.7 Hz, H-2, 1H), 8.45 (dd, J = 7.8, 1.7 Hz, H-4, 1H), 8.18 (m, H-6, 1H), 7.82 (dd, J = 7.8, 4.7 Hz, H-3, 1H), 7.59 (m, H-9, 1H), 7.46 (m, H-8, 1H), 7.37 (m, H-7, 1H).

¹³C NMR (100 MHz, DMSO-d₆) δ 179.4 (C-5), 175.8 (C-11), 153.0 (C-2), 148.8 (C-11a), 138.3 (C-9a), 137.8 (C-10a), 134.1 (C-4), 131.2 (C-4a), 127.8 (C-3), 127.2 (C-8), 124.2 (C-7), 123.7 (C-5b), 122.4 (C-6), 116.9 (C-5a), 113.9 (C-9).

¹⁵N NMR (40 MHz, DMSO-d₆) δ -66.6 (N-1), not found (N-10).
6.2.12 10-[(2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (12)

NaH (528 mg of a 60 % dispersion in mineral oil, 13.2 mmol) was suspended in dry DMF (18 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 11 (547 mg, 2.2 mmol) in dry DMF (24 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-N,N-dimethylethanamine hydrochloride (634 mg, 4.4 mmol) in dry DMF (30 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (350 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na$_2$SO$_4$ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (8:2, v/v) to obtain 240 mg (29.4 %) of 12 as orange-brown crystals, m.p. 136-138 °C.

HRMS: m/z calculated for C$_{19}$H$_{18}$N$_3$O$_2$ ([M+H]$^+$): 320.1394; found: 320.1396.
HRMS: m/z calculated for C$_{19}$H$_{17}$N$_3$NaO$_2$ ([M+Na]$^+$): 342.1213; found: 342.1214.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.93 (dd, $J = 4.6, 1.7$ Hz, H-2, 1H), 8.47 (dd, $J = 7.8, 1.6$ Hz, H-4, 1H), 8.34 (m, H-6, 1H), 7.62 (dd, $J = 7.8, 4.6$ Hz, H-3, 1H), 7.51 (m, H-9, 1H), 7.45 (m, H-8, 1H), 7.35 (m, H-7, 1H), 4.84 (m, H-12, 2H), 2.78 (m, H-13, 2H), 2.41 (s, H-15/15', 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 179.5 (C-5), 176.6 (C-11), 153.3 (C-2), 149.0 (C-11a), 139.6 (C-9a), 134.7 (C-10a), 134.3 (C-4), 130.7 (C-4a), 128.0 (C-8), 127.3 (C-3), 124.9 (C-7), 123.8 (C-6), 123.6 (C-5b), 118.7 (C-5a), 111.1 (C-9), 58.0 (C-13), 45.4 (C-15'/15), 43.1 (C-12).

$^{15}$N NMR (50 MHz, CDCl$_3$) $\delta$ -71.9 (N-1), -237.5 (N-10), -357.5 (N-14).
6. 2. 13 *N,N*-Diethylnicotinamide (13)

![Chemical Structure](image)

To a solution of nicotinic acid (24.5 g, 0.2 mol) and diethylamine (74 mL, 0.7 mol) in dry toluene (100 mL) was added phosphoric anhydride (31.2 g, 0.22 mol) in portions (exothermic). Then the mixture was refluxed for 4 h, cooled in an ice-bath while an excess of aqueous 10% NaOH was added. The toluene layer was separated, and the aqueous layer was thoroughly extracted with a mixture of ether and toluene (1/1, v/v). The combined organic layers were dried over Na₂SO₄ and the solvents removed in vacuo. The dark brown, oily residue was purified by ball-tube distillation (b.p. 115 °C at < 0.5 Torr) or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) affording 30 g (84.2 %) of 13 as viscous yellow oil.

**1H NMR (400 MHz, CDCl₃)** δ 8.55 (m, H-6, 1H), 7.63 (m, H-4, 1H), 7.26 (m, H-5, 1H), 3.47 (br s, H-9, 2H), 3.18 (br s, H-9', 2H), 1.17 (br s, H-10, 3H), 1.05 (br s, H-10', 3H).

**13C NMR (100 MHz, CDCl₃)** δ 168.3 (C-7), 150.1 (C-6), 147.0 (C-2), 134.0 (C-4), 132.8 (C-3), 123.2 (C-5), 43.2 (C-9'), 39.3 (C-9), 14.1 (C-10'), 12.7 (C-10).

**15N NMR (40 MHz, CDCl₃)** δ -69.1 (N-1), not found (N-8).
6. 2. 14 10-(Methoxymethyl)-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (14)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 13 (1.00 g, 5.61 mmol, 1 eq.) and 8 (1.06 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish a dark orange residue. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (1:1, v/v) followed by recrystallisation from ethyl acetate to give 526 mg (32.1 %) of 14 as orange needles, m.p. 178-180 °C.

HRMS: m/z calculated for C₁₇H₁₂N₂NaO₃ ([M+Na]⁺): 315.0740; found: 315.0736.

1H NMR (400 MHz, CDCl₃) δ 9.37 (d, J = 0.7 Hz, H-4, 1H), 9.03 (d, J = 4.9 Hz, H-2, 1H), 8.40 (m, H-10, 1H), 7.97 (dd, J = 4.9, 0.7 Hz, H-1, 1H), 7.63 (m, H-7, 1H), 7.50 (m, H-8, 1H), 7.43 (m, H-9, 1H), 6.13 (s, H-12, 2H), 3.39 (s, H-13, 3H).

13C NMR (100 MHz, CDCl₃) δ 180.0 (C-5), 178.3 (C-11), 155.7 (C-3), 148.4 (C-1), 139.8 (C-9a), 139.1 (C-4a), 134.5 (C-10a), 128.4 (C-8), 126.4 (C-11a), 125.4 (C-7), 123.73 (C-6), 123.68 (C-5b), 120.2 (C-5a), 118.6 (C-4), 112.1 (C-9), 75.4 (C-12), 56.6 (C-13).

15N NMR (40 MHz, CDCl₃) δ -56.6 (N-2), not found (N-10).
6.2.15 5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (15)

To an ice-cold solution of 14 (223 mg, 0.76 mmol) in dry CH₂Cl₂ (40 mL) a solution of BBr₃ (1M in CH₂Cl₂, 0.92 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated NaHCO₃ solution (40 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH₂Cl₂. Recrystallisation from ethyl acetate afforded 150 mg (79.5 %) of 15 as orange crystals, m.p. 318-320 °C.

HRMS: m/z calculated for C₁₅H₉N₂O₂ ([M+H]⁺): 249.0659; found: 249.0655.

¹H NMR (400 MHz, DMSO-d₆) δ 13.17 (s, H-10, 1H), 9.15 (s, H-1, 1H), 9.03 (d, J = 4.9 Hz, H-3, 1H), 8.11 (m, H-6, 1H), 7.87 (d, J = 4.9 Hz, H-4, 1H), 7.54 (m, H-9, 1H), 7.42 (m, H-8, 1H), 7.33 (m, H-7, 1H).

¹³C NMR (100 MHz, DMSO-d₆) δ 179.0 (C-5), 177.1 (C-11), 155.8 (C-3), 147.0 (C-1), 139.6 (C-4a), 138.1 (C-9a), 136.9 (C-10a), 127.2 (C-8), 125.8 (C-11a), 124.3 (C-7), 123.7 (C-5b), 122.2 (C-6), 118.7 (C-4), 117.5 (C-5a), 113.9 (C-9).

¹⁵N NMR (40 MHz, DMSO-d₆) δ -54.1 (N-2), -240.2 (N-10).
NaH (296 mg of a 60% dispersion in mineral oil, 7.2 mmol) was suspended in dry DMF (15 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 15 (463 mg, 1.9 mmol) in dry DMF (27 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-$N,N$-dimethylethanamine hydrochloride (533 mg, 3.7 mmol) in dry DMF (22 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over $\text{Na}_2\text{SO}_4$ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (8:2, v/v) to obtain 260 mg (43.6%) of 16 as orange crystals m.p. 149-151 °C.

HRMS: m/z calculated for $\text{C}_{19}\text{H}_{18}\text{N}_3\text{O}_2$ ([M+H]$^+$): 320.1394; found: 320.1393.

HRMS: m/z calculated for $\text{C}_{19}\text{H}_{17}\text{N}_3\text{NaO}_2$ ([M+Na]$^+$): 342.1213; found: 342.1211.

$^1$H NMR (500 MHz, CDCl$_3$) δ 9.37 (d, $J = 0.6$ Hz, H-1, 1H), 9.02 (d, $J = 4.9$ Hz, H-3, 1H), 8.40 (m, H-6, 1H), 7.98 (dd, $J = 4.9$, 0.7 Hz, H-4, 1H), 7.53 (m, H-9, 1H), 7.50 (m, H-8, 1H), 7.41 (m, H-7, 1H), 4.84 (m, H-12, 2H), 2.77 (m, H-13, 2H), 2.40 (s, H-15/15', 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 179.6 (C-5), 178.1 (C-11), 155.5 (C-3), 148.3 (C-1), 139.5 (C-9a/4a), 134.3 (C-10a), 128.0 (C-8), 126.5 (C-11a), 125.1 (C-7), 123.9 (C-6), 123.8 (C-5b), 119.2 (C-5a), 118.6 (C-4), 111.2 (C-9), 58.5 (C-13), 45.7 (C-15'/15), 43.5 (C-12).

$^{15}$N NMR (50 MHz, CDCl$_3$) δ -57.9 (N-2), -237.2 (N-10), -358.5 (N-14).
6.2.17 10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (16)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 27 (1.21 g, 5.61 mmol, 1 eq.) and 17 (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below −65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na₂SO₄ and the solvent was evaporated to furnish a orange-brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to obtain 618 mg (34.5 %) of 16 as orange crystals, m.p. 149-151°C.

HRMS: m/z calculated for C₁₉H₁₈N₃O₂ ([M+H]⁺): 320.1394; found: 320.1393.
HRMS: m/z calculated for C₁₉H₁₇N₃NaO₂ ([M+Na]⁺): 342.1213; found: 342.1211.

1H NMR (500 MHz, CDCl₃) δ 9.37 (d, J = 0.6 Hz, H-1, 1H), 9.02 (d, J = 4.9 Hz, H-3, 1H), 8.40 (m, H-6, 1H), 7.98 (dd, J = 4.9, 0.7 Hz, H-4, 1H), 7.53 (m, H-9, 1H), 7.50 (m, H-8, 1H), 7.41 (m, H-7, 1H), 4.84 (m, H-12, 2H), 2.77 (m, H-13, 2H), 2.40 (s, H-15/15', 6H).

13C NMR (125 MHz, CDCl₃) δ 179.6 (C-5), 178.1 (C-11), 155.5 (C-3), 148.3 (C-1), 139.5 (C-9a/4a), 134.3 (C-10a), 128.0 (C-8), 126.5 (C-11a), 125.1 (C-7), 123.9 (C-6), 123.8 (C-5b), 119.2 (C-5a), 118.6 (C-4), 111.2 (C-9), 58.5 (C-13), 45.7 (C-15'/15), 43.5 (C-12).

15N NMR (50 MHz, CDCl₃) δ -57.9 (N-2), -237.2 (N-10), -358.5 (N-14).
6. 2. 18  *N,N*-Diethylisonicotinamide (17)

To a solution of isonicotinic acid (24.5 g, 0.2 mol) and diethylamine (74 mL, 0.7 mol) in dry toluene (100 mL) was added phosphoric anhydride (31.2 g, 0.22 mol) in portions (exothermic). The mixture was refluxed for 4 h, and then cooled in an ice-bath while an excess of aqueous 10 % NaOH was added. The toluene layer was separated, and the aqueous layer was thoroughly extracted with a mixture of ether and toluene (1:1, v/v). The combined organic layers were dried over Na$_2$SO$_4$ and the solvents removed in vacuo. The dark brown, oily residue was purified by ball-tube distillation (b.p. 105 °C at < 0.5 Torr) or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) affording 28.2 g (78.5 %) of 17 as viscous orange oil.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.62 (m, H-2/6, 2H), 7.21 (m, H-3/5, 2H), 3.49 (q, $J$ = 7.1 Hz, H-9, 2H), 3.15 (q, $J$ = 7.0 Hz, H-9', 2H), 1.19 (t, $J$ = 7.1 Hz, H-10, 3H), 1.05 (t, $J$ = 7.1 Hz, H-10', 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 168.5 (C-7), 150.1 (C-2/6), 144.6 (C-4), 120.5 (C-3/5), 43.0 (C-9'), 39.2 (C-9), 14.1 (C-10'), 12.7 (C-10).

$^{15}$N NMR (40 MHz, CDCl$_3$) $\delta$ -67.4 (N-1), -250.3 (N-8).
6. 2. 19 6-(Methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (18)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of 17 (1.00 g, 5.61 mmol, 1 eq.) and 8 (1.06 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish a dark orange residue. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (1:1, v/v) followed by recrystallisation from ethyl acetate to give 540 mg (32.9 %) of 18 as orange needles, m.p. 199-201 °C.

HRMS: m/z calculated for C₁₇H₁₂N₂NaO₃ ([M+Na]⁺): 315.0740; found: 315.0740.

¹H NMR (400 MHz, CDCl₃) δ 9.39 (d, J = 0.7 Hz, H-1, 1H), 9.02 (d, J = 5.0 Hz, H-3, 1H), 8.41 (m, H-10, 1H), 7.92 (dd, J = 5.0, 0.8 Hz, H-4, 1H), 7.60 (m, H-7, 1H), 7.50 (m, H-8, 1H), 7.41 (m, H-9, 1H), 6.09 (s, H-12, 2H), 3.37 (s, H-13, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 180.8 (C-11), 177.7 (C-5), 155.0 (C-3), 148.3 (C-1), 140.1 (C-6a), 138.9 (C-4a), 134.3 (C-5a), 128.6 (C-8), 125.9 (C-11a), 125.4 (C-9), 124.0 (C-10), 123.6 (C-10a), 120.2 (C-10b), 118.6 (C-4), 111.9 (C-7), 75.4 (C-12), 56.6 (C-13).

¹⁵N NMR (40 MHz, CDCl₃) δ -54.2 (N-2), not found (N-6).
6. 2. 20 5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione (19)

To an ice-cold solution of 18 (540 mg, 1.85 mmol) in dry CH₂Cl₂ (120 mL) a solution of BBr₃ (1M in CH₂Cl₂, 2.24 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated NaHCO₃ solution (120 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH₂Cl₂. Recrystallisation from ethyl acetate afforded 365 mg (79.6 %) of 19 as orange crystals, m.p. 345-347 °C.

HRMS: m/z calculated for C₁₅H₈N₂O₂ ([M+H]+): 249.0659; found: 249.0656.

¹H NMR (400 MHz, DMSO-d₆) δ 13.21 (s, 1H), 9.24 (d, J = 0.7 Hz, H-1, 1H), 9.06 (d, J = 4.9 Hz, H-3, 1H), 8.20 (m, H-10, 1H), 7.92 (dd, J = 4.9, 0.8 Hz, H-4, 1H), 7.59 (m, H-7, 1H), 7.47 (m, H-8, 1H), 7.38 (m, H-9, 1H).

¹³C NMR (100 MHz, DMSO-d₆) δ 180.2 (C-11), 176.8 (C-5), 155.1 (C-3), 147.4 (C-1), 138.5 (C-6a), 138.4 (C-4a), 136.9 (C-5a), 127.5 (C-8), 126.5 (C-11a), 124.3 (C-9), 123.7 (C-10a), 122.4 (C-10), 118.3 (C-4), 117.4 (C-10b), 113.9 (C-7).

¹⁵N NMR (40 MHz, DMSO-d₆) δ -51.0 (N-2), not found (N-6).
**EXPERIMENTAL PART**

6. 2. 21  6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (20)

NaH (288 mg of a 60 % dispersion in mineral oil, 7.2 mmol) was suspended in dry DMF (15 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 19 (446 mg, 1.8 mmol) in dry DMF (26 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-N,N-dimethylethanamine hydrochloride (513 mg, 3.6 mmol) in dry DMF (22 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (280 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (8:2, v/v) to obtain 170 mg (29.6 %) of 20 as orange crystals, m.p. 157-159 °C.

HRMS: m/z calculated for C₁₉H₁₈N₃O₂ ([M+H]⁺): 320.1394; found: 320.1394.
HRMS: m/z calculated for C₁₉H₁₉N₃NaO₂ ([M+Na]⁺): 342.1213; found: 342.1213.

**¹H NMR (500 MHz, CDCl₃)** δ 9.40 (d, J = 0.7 Hz, H-1, 1H), 9.01 (d, J = 4.9 Hz, H-3, 1H), 8.42 (m, H-10, 1H), 7.92 (dd, J = 4.9, 0.8 Hz, H-4, 1H), 7.49 (m, H-7, 1H), 7.48 (m, H-8, 1H), 7.40 (m, H-9, 1H), 4.80 (m, H-12, 2H), 2.73 (m, H-13, 2H), 2.37 (s, H-15/15', 6H).

**¹³C NMR (125 MHz, CDCl₃)** δ 180.5 (C-11), 177.6 (C-5), 154.8 (C-3), 148.3 (C-1), 139.8 (C-6a), 139.0 (C-4a), 134.2 (C-5a), 128.2 (C-8), 126.3 (C-11a), 125.0 (C-9), 124.1 (C-10), 123.7 (C-10a), 119.1 (C-10b), 118.5 (C-4), 111.1 (C-7), 58.5 (C-13), 45.8 (C-15'/15), 43.5 (C-12).

**¹⁵N NMR (50 MHz, CDCl₃)** δ -55.0 (N-2), -237.2 (N-6), -358.7 (N-14).
6. 2. 22 5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (21)

To a suspension of 19 (400 mg, 1.61 mmol) in CH₂Cl₂ (500 mL), was added in one portion m-CPBA (57-86 % peracid, 2.1 g, excess) in CH₂Cl₂ (250 mL). The mixture was refluxed overnight. The resulting orange mixture was washed with a saturated NaHCO₃ solution (3 x 15 mL). The aqueous layer was back-extracted with CH₂Cl₂ (3 x) and the combined organic layers were dried over Na₂SO₄ and evaporated to give an orange-red solid which was purified by column chromatography eluting with ethyl acetate to afford 410 mg (96.3 %) of 21 as orange-red crystals, m.p. >350 °C.

HRMS: m/z calculated for C₁₅H₉N₂O₃ ([M+H]+): 265.0608; found: 265.0607.

¹H NMR (400 MHz, DMSO-ｄ₆) δ 13.24 (s, H-6, 1H), 8.48 (dd, J = 6.6, 1.9 Hz, H-3, 1H), 8.42 (d, J = 1.9 Hz, H-1, 1H), 8.09 (m, H-10, 1H), 7.90 (d, J = 6.6, H-4, 1H), 7.55 (m, H-7, 1H), 7.43 (m, H-8, 1H), 7.35 (m, H-9, 6H).

¹³C NMR (100 MHz, DMSO-ｄ₆) δ 177.4 (C-11), 174.3 (C-5), 142.3 (C-3), 138.2 (C-6a), 137.4 (C-5a), 135.9 (C-1), 131.9 (C-11a), 127.4 (C-4a), 127.3 (C-8), 124.5 (C-9), 123.6 (C-10a), 123.0 (C-4), 122.1 (C-10), 117.2 (C-10b), 114.0 (C-7).

¹⁵N NMR (40 MHz, DMSO-ｄ₆) δ -76.9 (N-2), not found (N-6).
6. 2. 23  6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (22)

NaH (242 mg of a 60% dispersion in mineral oil, 6.0 mmol) was suspended in dry DMF (15 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 21 (400 mg, 1.51 mmol) in dry DMF (25 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-N,N-dimethylethanamine hydrochloride (435 mg, 3.0 mmol) in dry DMF (25 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (250 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (9:1, v/v) to obtain 131 mg (26.0%) of 22 as orange crystals, m.p. 154-156 °C.


HRMS: m/z calculated for C₁₉H₁₇N₃NaO₃ ([M+Na]⁺): 358.1162; found: 358.1166.

³¹H NMR (400 MHz, CDCl₃) δ 8.67 (dd, J = 1.9, 0.5 Hz, H-1, 1H), 8.32 (m, H-10, 1H), 8.30 (dd, J = 6.7, 1.9 Hz, H-3, 1H), 7.93 (dd, J = 6.7, 0.5 Hz, H-4, 1H), 7.48 (m, H-7/8, 2H), 7.38 (m, H-9, 1H), 4.79 (m, H-12, 2H), 2.72 (m, H-13, 2H), 2.35 (s, H-15/15', 6H).

¹³C NMR (100 MHz, CDCl₃) δ 177.3 (C-11), 174.8 (C-5), 142.2 (C-3), 139.5 (C-6a), 136.8 (C-1), 134.6 (C-5a), 131.5 (C-11a), 128.3 (C-4a), 128.1 (C-8), 125.3 (C-9), 123.7 (C-10), 123.6 (C-10a), 122.9 (C-4), 118.9 (C-10b), 111.2 (C-7), 58.6 (C-13), 45.8 (C-15/15'), 43.7 (C-12).

¹⁵N NMR (40 MHz, CDCl₃) δ -77.7 (N-2), -235.4 (N-6), -359.2 (N-14).
EXPERIMENTAL PART

6. 2. 24  \( N,N\)-Diethyl-2-pyrazinecarboxamide (23)

\[
\begin{align*}
\text{N} & \quad \text{O} \\
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{N}
\end{align*}
\]

To a solution of 2-pyrazinecarboxylic acid (6 g, 48.3 mmol) and diethylamine (17.6 mL, 0.17 mol) in dry toluene (250 mL) was added phosphoric anhydride (7.54 g, 0.53 mol) in portions (exothermic). The mixture was refluxed for 4 h, and then cooled in an ice-bath while an excess of aqueous 10 % NaOH was added. The toluene layer was separated, and the aqueous layer was thoroughly extracted with a mixture of ether and toluene (1:1, v/v). The combined organic layers were dried over \( \text{Na}_2\text{SO}_4 \) and the solvents removed in vacuo. The dark brown, oily residue was purified by ball-tube distillation (b.p. 110 °C at < 0.5 Torr) or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) affording 5.56 g (64.2 %) of 23 as viscous yellow oil.

\( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.81 (d, \( J = 1.5 \) Hz, H-3, 1H), 8.53 (d, \( J = 2.6 \) Hz, H-5, 1H), 8.46 (dd, \( J = 2.6, 1.5 \) Hz, H-6, 1H), 3.50 (q, \( J = 7.1 \) Hz, H-10, 2H), 3.32 (q, \( J = 7.1 \) Hz, H-10', 2H), 1.19 (t, \( J = 7.1 \) Hz, H-11, 3H), 1.12 (t, \( J = 7.1 \) Hz, H-11', 3H).

\( ^13C \) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 165.9 (C-7), 150.2 (C-2), 144.8 (C-5), 144.7 (C-3), 142.4 (C-6), 43.1 (C-9'), 40.3 (C-9), 14.2 (C-10'), 12.6 (C-10).

\( ^15N \) NMR (40 MHz, CDCl\(_3\)) \( \delta \) -55.4 (N-1), -57.2 (N-4), -250.0 (N-8).
6. 2. 25 6-(Methoxymethyl)-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (24)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 23 (1.01 g, 5.61 mmol, 1 eq.) and 8 (1.06 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na$_2$SO$_4$ and the solvent was evaporated to furnish a dark residue. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (1:1, v/v) followed by recrystallisation from ethyl acetate to give 180 mg (10.9 %) of 24 as orange crystals, m.p. 250-252 °C.

HRMS: m/z calculated for C$_{16}$H$_{11}$N$_3$NaO$_3$ ([M+Na]$^+$): 316.0693; found: 316.0691.

$^1$H NMR (500 MHz, CDCl$_3$) δ 9.01 (d, $J$ = 2.2 Hz, H-2*/3*, 1H), 8.98 (d, $J$ = 2.2 Hz, H-2*/3*, 1H), 8.53 (m, H-10, 1H), 7.68 (m, H-7, 1H), 7.56 (m, H-8, 1H), 7.48 (m, H-9, 1H), 6.21 (s, H-12, 2H), 3.41 (s, H-13, 3H).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 178.1 (C-11), 175.7 (C-5), 148.1 (C-2*/3*), 147.6 (C-2*/3*), 145.5 (C-4a*/11a*), 145.4 (C-4a*/11a*), 140.3 (C-6a), 134.9 (C-5a), 129.0 (C-8), 125.8 (C-9), 124.3 (C-10), 123.9 (C-10a), 120.9 (C-10b), 112.3 (C-7), 75.6 (C-12), 56.7 (C-13).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -50.4 (N-1*/4*), -50.8 (N-1*/N-4*), not found (N-6).
6. 2. 26 5H-Pyrazino[2,3-b]carbazole-5,11(6H)-dione (25)

To an ice-cold solution of 24 (500 mg, 1.70 mmol) in dry CH₂Cl₂ (150 mL) a solution of BBr₃ (1M in CH₂Cl₂, 2.1 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated NaHCO₃ solution (150 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH₂Cl₂. Recrystallisation from ethyl acetate afforded 320 mg (75.3 %) of 25 as orange crystals, m.p. >350 °C.

HRMS: m/z calculated for C₁₄H₇N₃NaO₂ ([M+Na]+): 272.0430; found: 272.0431.

^1H NMR (400 MHz, DMSO-d₆) δ 13.28 (br s, H-10, 1H), 9.03 (d, J = 2.3 Hz, H-2*/3*, 1H), 9.01 (d, J = 2.3 Hz, H-2*/3*, 1H), 8.23 (m, H-10, 1H), 7.62 (m, H-7, 1H), 7.49 (m, H-8, 1H), 7.40 (m, H-9, 1H).

^13C NMR (100 MHz, DMSO-d₆) δ 177.8 (C-11), 175.3 (C-5), 147.6 (C-3*/2*), 147.1 (C-2*/3*), 146.4 (C-4a*/11a*), 145.4 (C-4a*/11a*), 138.4 (C-6a), 137.6 (C-5a), 127.5 (C-8), 124.4 (C-9), 123.8 (C-10a), 122.5 (C-10), 117.9 (C-10b), 114.0 (C-7).

^15N NMR (40 MHz, DMSO-d₆) δ -50.2 (N-1), -49.4 (N-4), not found (N-6).
6. 2. 27 6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (26)

NaH (206 mg of a 60 % dispersion in mineral oil, 5.14 mmol) was suspended in dry DMF (10 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 25 (320 mg, 1.28 mmol) in dry DMF (30 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-N,N-dimethylethanamine hydrochloride (369 mg, 2.56 mmol) in dry DMF (25 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (250 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to obtain 105 mg (25.4 %) of 26 as orange crystals, m.p. 213-215 °C.


HRMS: m/z calculated for C₁₈H₁₆N₄NaO₂ ([M+Na]+): 343.1165; found: 343.1168.

$^1$H NMR (400 MHz, CDCl₃) δ 8.95 (d, $J = 2.3$ Hz, H-2*/3*, 1H), 8.92 (d, $J = 2.3$ Hz, H-2*/3*, 1H), 8.43 (m, H-10, 1H), 7.49 (m, H-7, 1H), 7.46 (m, H-8, 1H), 7.38 (m, H-9, 1H), 4.84 (m, H-12, 2H), 2.76 (m, H-13, 2H), 2.37 (s, H-15/15', 6H).

$^{13}$C NMR (100 MHz, CDCl₃) δ 177.5 (C-11), 175.5 (C-5), 147.9 (C-2*/3*), 147.3 (C-2*/3*), 145.8 (C-4a*/11a*), 145.3 (C-4a*/11a*), 139.9 (C-6a), 134.6 (C-5a), 128.4 (C-8), 125.3 (C-9), 124.3 (C-10), 123.8 (C-10a), 119.7 (C-10b), 111.2 (C-7), 58.4 (C-13), 45.7 (C-15'/15), 43.6 (C-12).

$^{15}$N NMR (40 MHz, CDCl₃) δ -51.6 (N-1*/4*), -51.0 (N-1*/4*), -236.3 (N-6), -359.2 (N-14).
6. 2. 28 1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (27)

NaH (8.27 g of a 60 % dispersion in mineral oil, 206.7 mmol) was suspended in dry DMF (50 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 1H-Indole-3-carbaldehyde (10 g, 68.9 mmol) in dry DMF (200 mL) was added. The cooling bath was removed and after stirring for further 60 min solid 2-chloro-N,N-dimethylethanamine hydrochloride (14.9 g, 103.4 mmol) was added in portions and the mixture was stirred at 75 °C overnight. After cooling the reaction mixture was quenched with water (500 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na$_2$SO$_4$ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to afford 11.45 g (76.8 %) of 27 as brown oil.

HRMS: m/z calculated for C$_{13}$H$_{17}$N$_2$O ([M+H]$^+$): 217.1335; found: 217.1339.
HRMS: m/z calculated for C$_{13}$H$_{16}$N$_2$NaO ([M+Na]$^+$): 239.1155; found: 239.1160.

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.96 (s, H-8, 1H), 8.30 (m, H-4, 1H), 7.80 (s, H-2, 1H), 7.35 (m, H-7, 1H), 7.31 (m, H-6, 1H), 7.29 (m, H-5, 1H), 4.21 (t, J = 6.6 Hz, H-9, 2H), 2.72 (t, J = 6.6 Hz, H-10, 2H), 2.28 (s, H-12/12', 6H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 184.5 (C-8), 139.0 (C-2), 137.1 (C-7a), 125.2 (C-3a), 123.8 (C-6), 122.7 (C-5), 122.0 (C-4), 118.0 (C-3), 109.7 (C-7), 58.3 (C-10), 45.5 (C-12/12'), 44.9 (C-9).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -234.3 (N-1), -359.3 (N-11).
6. 2. 29 1-[3-(Dimethylamino)propyl]-1\textit{H}-indole-3-carbaldehyde (28)

1\textit{H}-Indole-3-carbaldehyde (5.0 g, 34.4 mmol), anhydrous K$_2$CO$_3$ (14.3 g, 103.3 mmol) and N,N-di-
dimethylpropan-1-amine hydrochloride (6.53 g, 41.3 mmol) were dissolved in DMF (100 mL) and stirred at 100 °C for 24 h. After cooling down the reaction mixture was diluted with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na$_2$SO$_4$ and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) followed by ethyl acetate/methanol/triethylamine (7:2:1, v/v) to afford 5.58 g (70.3 %) of 28 as viscous dark orange oil.

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.96 (s, H-8, 1H), 8.29 (m, H-4, 1H), 7.77 (s, H-2, 1H), 7.41 (m, H-7, 1H), 7.31 (m, H-6, 1H), 7.30 (m, H-5, 1H), 4.27 (t, $J$ = 6.8 Hz, H-9, 2H), 2.30 (t, $J$ = 6.8 Hz, H-11, 2H), 2.26 (s, H-13/13', 6H), 2.05 (m, H-10, 2H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 184.5 (C-8), 138.8 (C-2), 137.1 (C-7a), 125.3 (C-3a), 123.9 (C-6), 122.8 (C-5), 122.0 (C-4), 118.0 (C-3), 110.1 (C-7), 55.6 (C-11), 45.0 (C-13'/13), 44.5 (C-9), 27.1 (C-10).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -232.1 (N-1), -356.5 (N-12).
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 28 (1.29 g, 5.61 mmol, 1 eq.) and 17 (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below −65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na₂SO₄ and the solvent was evaporated to furnish a orange-brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to give 178 mg (9.5 %) of 29 as orange crystals, m.p. 133-135 °C.

HRMS: m/z calculated for C₂₀H₂₀N₃O₂ ([M+H]+): 334.1550; found: 334.1549.

¹H NMR (400 MHz, CDCl₃) δ 9.37 (d, J = 0.7 Hz, H-1, 1H), 8.98 (d, J = 5.0 Hz, H-3, 1H), 8.38 (m, H-10, 1H), 7.89 (dd, J = 5.0, 0.7 Hz, H-4, 1H), 7.54 (m, H-7, 1H), 7.45 (m, H-8, 1H), 7.36 (m, H-9, 1H), 4.72 (m, H-12, 2H), 2.34 (m, H-14, 2H), 2.23 (s, H-16/16’, 6H), 2.02 (m, H-13, 2H).

¹³C NMR (100 MHz, CDCl₃) δ 180.4 (C-11), 177.4 (C-5), 154.7 (C-3), 148.2 (C-1), 139.9 (C-6a), 138.9 (C-4a), 134.1 (C-5a), 128.0 (C-8), 126.3 (C-11a), 124.9 (C-9), 123.9 (C-10), 123.6 (C-10a), 119.0 (C-10b), 118.4 (C-4), 111.3 (C-7), 56.3 (C-14), 45.3 (C-16’/16), 43.5 (C-12), 28.0 (C-13).

¹⁵N NMR (40 MHz, CDCl₃) δ -54.9 (N-2), -234.0 (N-6), -357.2 (N-15).
6.2.31 6-[(3-Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (30)

[Diagram of the molecule]

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 28 (1.29 g, 5.61 mmol, 1 eq.) and 23 (1.01 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na$_2$SO$_4$ and the solvent was evaporated to furnish a dark residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to give 160 mg (8.5 %) of 30 as orange crystals, m.p. 171-173 °C.

HRMS: m/z calculated for C$_{19}$H$_{19}$N$_4$O$_2$ ([M+H]$^+$): 335.1503; found: 335.1506.
HRMS: m/z calculated for C$_{19}$H$_{19}$N$_4$NaO$_2$ ([M+Na]$^+$): 357.1322; found: 357.1326.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.95 (d, J = 2.3 Hz, H-2*/3*, 1H), 8.92 (d, J = 2.3 Hz, H-2*/3*, 1H), 8.42 (m, H-10, 1H), 7.54 (m, H-7, 1H), 7.43 (m, H-8, 1H), 7.36 (m, H-9, 1H), 4.76 (m, H-12, 2H), 2.34 (t, J = 6.7 Hz, H-14, 2H), 2.20 (s, H-16/16', 6H) 2.03 (m, H-13, 2H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 177.5 (C-11), 175.3 (C-5), 147.8 (C-2*/3*), 147.2 (C-2*/3*), 145.8 (C-4a*/11a*), 145.3 (C-4a*/11a*), 140.0 (C-6a), 134.6 (C-5a), 128.2 (C-8), 125.3 (C-9), 124.1 (C-10), 123.7 (C-10a), 119.6 (C-10b), 111.4 (C-7), 56.3 (C-14), 45.3 (C-16/16'), 43.7 (C-12), 27.9 (C-13).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -51.1 (N1*/4*), -51.7 (N1*/4*), -232.0 (N-6), -357.6 (N-15).
6. 2. 32 1-[2-(Morpholin-4-yl)ethyl]-1H-indole-3-carbaldehyde (31)

NaH (5.5 g of a 60 % dispersion in mineral oil, 137.6 mmol) was suspended in dry DMF (30 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 1H-Indole-3-carbaldehyde (5.0 g, 34.4 mmol) in dry DMF (50 mL) was added. The cooling bath was removed and after stirring for further 60 min solid 4-(2-chloroethyl)morpholine hydrochloride (9.6 g, 51.6 mmol) was added in portions and the mixture was stirred at 90 °C overnight. After cooling the reaction mixture was quenched with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na$_2$SO$_4$ and the solvent was removed in vacuo to give the crude product as dark oil which was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to afford 5.6 g (62.9 %) of 31 as brown solid, m.p. 63-64 °C.

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.98 (s, H-8, 1H), 8.30 (m, H-4, 1H), 7.79 (s, H-2, 1H), 7.36 (m, H-7, 1H), 7.33 (m, H-6, 1H), 7.31 (m, H-5, 1H), 4.25 (t, J = 6.4 Hz, H-9, 2H), 3.68 (m, H-2’/6’, 4H), 2.78 (t, J = 6.4 Hz, H-10, 2H), 2.47 (m, H-3’/5’, 4H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 184.4 (C-8), 138.8 (C-2), 137.1 (C-7a), 125.2 (C-3a), 123.9 (C-6), 122.8 (C-5), 122.1 (C-4), 118.1 (C-3), 109.7 (C-7), 66.8 (C-2’/6’), 57.5 (C-10), 53.7 (C-3’/5’), 44.4 (C-9).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -234.6 (N-1), -340.3 (N-4’).
6. 2. 33 6-[2-(Morpholin-4-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (32)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 31 (1.45 g, 5.61 mmol, 1 eq.) and 17 (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below −65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na₂SO₄ and the solvent was evaporated to furnish a brown oily residue that was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) to give 280 mg (9.5 %) of 32 as orange crystals, m.p. 189-191 °C.

HRMS: m/z calculated for C₂₁H₂₄N₃O₃ ([M+H]⁺): 362.1499; found: 362.1496.

¹H NMR (400 MHz, CDCl₃) δ 9.41 (d, J = 0.8 Hz, H-1, 1H), 9.01 (d, J = 5.0 Hz, H-3, 1H), 8.43 (m, H-10, 1H), 7.91 (dd, J = 5.0, 0.8 Hz, H-4, 1H), 7.48 (m, H-7/8, 2H), 7.40 (m, H-9, 1H), 4.82 (m, H-12, 2H), 3.62 (m, H-2'/6', 4H), 2.76 (m, H-13, 2H), 2.55 (m, H-3'/5', 4H).

¹³C NMR (100 MHz, CDCl₃) δ 180.5 (C-11), 177.7 (C-5), 154.9 (C-3), 148.3 (C-1), 139.8 (C-6a), 139.0 (C-4a), 134.5 (C-5a), 128.1 (C-8), 126.3 (C-11a), 125.0 (C-9), 124.1 (C-10), 123.7 (C-10a), 119.1 (C-10b), 118.4 (C-4), 111.1 (C-7), 66.9 (C-2'/6'), 57.8 (C-13), 53.9 (C-3'/5'), 42.9 (C-12).

¹⁵N NMR (40 MHz, CDCl₃) δ -54.6 (N-2), -238.1 (N-6), -340.6 (N-4').
6. 2. 34 6-[2-(Morpholin-4-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (33)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of 31 (1.45 g, 5.61 mmol, 1 eq.) and 23 (1.01 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na$_2$SO$_4$ and the solvent was evaporated to furnish a brown oily residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to give 42 mg (2.1 %) of 33 as orange crystals, m.p. 180-182 °C.

HRMS: m/z calculated for C$_{20}$H$_{19}$N$_4$O$_3$ ([M+H]$^+$): 363.1452; found: 363.1450.
HRMS: m/z calculated for C$_{20}$H$_{18}$N$_4$NaO$_3$ ([M+Na]$^+$): 385.1271; found: 385.1272.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.97 (d, $J = 2.3$ Hz, H-2*/3*, 1H), 8.94 (d, $J = 2.2$ Hz, H-2*/3*, 1H), 8.47 (m, H-10, 1H), 7.48 (m, H-7, 1H), 7.47 (m, H-8, 1H), 7.40 (m, H-9, 1H), 4.87 (t, $J = 6.9$ Hz, H-12, 2H), 3.60 (m, H-2'/6', 4H), 2.78 (t, $J = 6.9$ Hz, H-13, 2H), 2.57 (m, H-3'/5', 4H).

$^13$C NMR (100 MHz, CDCl$_3$) δ 177.6 (C-11), 175.6 (C-5), 148.0 (C-2*/3*), 147.3 (C-2*/3*), 145.8 (C-4a*/11a*), 145.3 (C-4a*/11a*), 139.9 (C-6a), 135.0 (C-5a), 128.4 (C-8), 125.4 (C-9), 124.3 (C-10), 123.9 (C-10a), 119.7 (C-10b), 111.2 (C-7), 66.9 (C-2'/6'/), 57.7 (C-13), 53.9 (C-3'/5'), 42.9 (C-12).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -51.2 (N-1/4), -236.3 (N-6), -340.8 (N-4').
6. 2. 35 1-[2-(Piperidin-1-yl)ethyl]-1H-indole-3-carbaldehyde (34)

NaH (5.5 g of a 60 % dispersion in mineral oil, 137.6 mmol) was suspended in dry DMF (30 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 1H-Indole-3-carbaldehyde (5.0 g, 34.4 mmol) in dry DMF (50 mL) was added. The cooling bath was removed and after stirring for further 60 min solid 1-(2-chloroethyl)piperidine hydrochloride (9.5 g, 51.6 mmol) was added in portions and the mixture was stirred at 90-100 °C overnight. After cooling the reaction mixture was quenched with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na$_2$SO$_4$ and the solvent was removed in vacuo to give the crude product as oily residue which was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to afford 8.1 g (91.7 %) of 34 as beige solid, m.p. 80-81 °C.

HRMS: m/z calculated for C$_{16}$H$_{21}$N$_2$O ([M+H$^+$]): 257.1648; found: 257.1652.
HRMS: m/z calculated for C$_{16}$H$_{20}$N$_2$NaO ([M+Na$^+$]): 279.1468; found: 279.1469.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.97 (s, H-8, 1H), 8.30 (m, H-4, 1H), 7.79 (s, H-2, 1H), 7.36 (m, H-7, 1H), 7.31 (m, H-6, 1H), 7.30 (m, H-5, 1H), 4.22 (t, $J = 6.7$ Hz, H-9, 2H), 2.71 (t, $J = 6.7$ Hz, H-10, 2H), 2.42 (m, H-2'/6', 4H), 1.56 (m, H-3'/5', 4H), 1.43 (m, H-4', 2H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 184.4 (C-8), 139.1 (C-2), 137.2 (C-7a), 125.2 (C-3a), 123.7 (C-6), 122.7 (C-5), 122.0 (C-4), 118.0 (C-3), 109.8 (C-7), 57.9 (C-7), 54.7 (C-2'/6'), 44.7 (C-9), 25.9 (C-3'/5'), 24.1 (C-4').

$^{15}$N NMR (40 MHz, CDCl$_3$) $\delta$ -233.7 (N-1), -335.7 (N-1').
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 34 (1.01 g, 3.93 mmol, 0.7 eq.) and 17 (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to give 429 mg (30.4 %) of 35 as orange crystals, m.p. 168-170 °C.

HRMS: m/z calculated for C₂₂H₂₂N₃O₂ ([M+H]⁺): 360.1707; found: 360.1707.
HRMS: m/z calculated for C₂₂H₂₁N₃NaO₂ ([M+Na]⁺): 382.1526; found: 382.1525.

¹H NMR (400 MHz, CDCl₃) δ 9.36 (d, J = 0.8 Hz, H-1, 1H), 8.98 (d, J = 4.9 Hz, H-3, 1H), 8.37 (m, H-10, 1H), 7.88 (dd, J = 4.9, 0.8 Hz, H-4, 1H), 7.44 (m, H-7, 1H), 7.43 (m, H-8, 1H), 7.35 (m, H-9, 1H), 4.77 (m, H-12, 2H), 2.69 (m, H-13, 2H), 2.49 (m, H-2’/6’, 4H), 1.52 (m, H-3’/5’, 4H), 1.40 (m, H-4’, 2H).

¹³C NMR (100 MHz, CDCl₃) δ 180.4 (C-11), 177.4 (C-5), 154.7 (C-3), 148.2 (C-1), 139.8 (C-6a), 138.9 (C-4a), 134.4 (C-5a), 127.9 (C-8), 126.2 (C-11a), 124.9 (C-9), 123.9 (C-10), 123.6 (C-10a), 118.9 (C-10b), 118.4 (C-4), 111.2 (C-7), 58.1 (C-13), 54.9 (C-2’/6’), 43.2 (C-12), 26.0 (C-3’/5’), 24.1 (C-4’).

¹⁵N NMR (100 MHz, CDCl₃) δ -55.0 (N-2), -237.1 (N-6), -336.0 (N-1’).
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 34 (1.44 g, 5.61 mmol, 1 eq.) and 23 (1.01 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) and recrystallisation from ethyl acetate to afford 180 mg (8.9 %) of 36 as orange crystals, m.p. 127-129 °C.

HRMS: m/z calculated for C₂₁H₂₁N₄O₂ ([M+H]+): 361.1659; found: 361.1658.


1H NMR (400 MHz, CDCl₃) δ 8.96 (d, J = 2.2 Hz, H-2*/3*, 1H), 8.92 (d, J = 2.2 Hz, H-2*/3*, 1H), 8.44 (d, J = 8.0 Hz, H-10, 1H), 7.50 (m, H-7, 1H), 7.45 (m, H-8, 1H), 7.38 (m, H-9, 1H), 4.84 (m, H-12, 2H), 2.73 (m, H-13, 2H), 2.52 (m, H-2'/6', 4H), 1.50 (m, H-3'/5', 4H), 1.39 (m, H-4', 2H).

13C NMR (100 MHz, CDCl₃) δ 177.5 (C-11), 175.4 (C-5), 147.9 (C-2*/3*), 147.2 (C-2*/3*), 145.8 (C-4a*/11a*), 145.3 (C-4a*/11a*), 140.0 (C-6a), 134.8 (C-5a), 128.3 (C-8), 125.3 (C-9), 124.2 (C-10), 123.8 (C-10a), 119.6 (C-10b), 111.3 (C-7), 58.0 (C-13), 54.8 (C-2'/6'), 43.2 (C-12), 25.9 (C-3'/5'), 24.0 (C-4').

15N NMR (40 MHz, CDCl₃) δ -51.1 (N-1*/4*), -51.6 (N-1*/4*), -235.5 (N-6), -336.0 (N-1*).
6. 2.38 1-[2-(Pyrrolidin-1-yl)ethyl]-1H-indole-3-carbaldehyde (37)

1H-Indole-3-carbaldehyde (5.0 g, 34.4 mmol), anhydrous K2CO3 (14.3 g, 103.5 mmol) and 1-(2-chloroethyl)pyrrolidine hydrochloride (8.79 g, 51.7 mmol) were dissolved in DMF (100 mL) and stirred at 90 °C for 24 h. After cooling down the reaction mixture was diluted with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na2SO4 and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) to afford 7.3 g (70.3 %) of 28 as beige solid, m.p. 73-75 °C.


1H NMR (400 MHz, CDCl3) δ 9.96 (s, H-8, 1H), 8.29 (m, H-4, 1H), 7.78 (s, H-2, 1H), 7.36 (m, H-7, 1H), 7.30 (m, H-6, 1H), 7.29 (m, H-5, 1H), 4.25 (t, J = 6.9 Hz, H-9, 2H), 2.90 (t, J = 6.9 Hz, H-10, 2H), 2.54 (m, H-2'/5', 4H), 1.77 (m, H-3'/4', 4H).

13C NMR (100 MHz, CDCl3) δ 184.4 (C-8), 138.8 (C-2), 137.1 (C-7a), 125.2 (C-3a), 123.8 (C-6), 122.7 (C-5), 122.0 (C-4), 118.0 (C-3), 109.8 (C-7), 55.1 (C-10), 54.2 (C-2'/5'), 46.2 (C-9), 23.5 (C-3'/4').

15N NMR (40 MHz, CDCl3) δ -233.8 (N-1'), -331.2 (N-1).
6. 2. 39  6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (38)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidide (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 37 (1.36 g, 5.61 mmol, 1 eq.) and 17 (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na\textsubscript{2}SO\textsubscript{4} and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to give 296 mg (15.3 %) of 38 as orange-brown crystals, m.p. 125-127 °C.

HRMS: m/z calculated for C\textsubscript{21}H\textsubscript{20}N\textsubscript{3}O\textsubscript{2} ([M+H]\textsuperscript{+}): 346.1550; found: 346.1546.
HRMS: m/z calculated for C\textsubscript{21}H\textsubscript{19}N\textsubscript{3}O\textsubscript{2}Na ([M+Na]\textsuperscript{+}): 368.1369; found: 368.1363.

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) δ 9.38 (d, J = 0.8 Hz, H-1, 1H), 8.99 (d, J = 5.0 Hz, H-3, 1H), 8.39 (m, H-10, 1H), 7.90 (dd, J = 5.0, 0.8 Hz, H-4, 1H), 7.50 (m, H-7, 1H), 7.46 (m, H-8, 1H), 7.37 (m, H-9, 1H), 4.82 (m, H-12, 2H), 2.90 (m, H-13, 2H), 2.67 (m, H-2'/5', 4H), 1.80 (m, H-3'/4', 4H).

\textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) δ 180.4 (C-11), 177.5 (C-5), 154.8 (C-3), 148.3 (C-1), 139.8 (C-6a), 138.9 (C-4a), 134.1 (C-5a), 128.1 (C-8), 126.3 (C-11a), 125.0 (C-9), 124.0 (C-10), 123.7 (C-10a), 119.1 (C-10b), 118.4 (C-4), 111.1 (C-7), 55.3 (C-13), 54.4 (C-2'/5'), 44.5 (C-12), 23.6 (C-3'/4').

\textsuperscript{15}N NMR (40 MHz, CDCl\textsubscript{3}) δ -54.9 (N-2), -237.7 (N-6), -330.9 (N-1').
6. 2. 40 6-[2-(Pyrrolidin-1-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (39)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 37 (1.36 g, 5.61 mmol, 1 eq.) and 23 (1.01 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below −65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to give 39 mg (2.0 %) of 39 as orange crystals, m.p. 176-177 °C.

HRMS: m/z calculated for C₂₀H₁₉N₄O₂ ([M+H]⁺): 347.1503; found: 347.1504.
HRMS: m/z calculated for C₂₀H₁₈N₄NaO₂ ([M+Na]⁺): 369.1322; found: 369.1325.

¹H NMR (400 MHz, CDCl₃) δ 8.94 (d, J = 2.2 Hz, H-2*/3*, 1H), 8.92 (d, J = 2.2 Hz, H-2*/3*, 1H), 8.42 (m, H-10, 1H), 7.50 (m, H-7, 1H), 7.45 (m, H-8, 1H), 7.37 (m, H-9, 1H), 4.85 (m, H-12, 2H), 2.91 (m, H-13, 2H), 2.67 (m, H-2'/5', 4H), 1.76 (m, H-3'/4', 4H).

¹³C NMR (100 MHz, CDCl₃) δ 177.5 (C-11), 175.4 (C-5), 147.9 (C-2*/3*), 147.3 (C-2*/3*), 145.7 (C-4a*/11a*), 145.3 (C-4a*/11a*), 139.9 (C-6a), 134.6 (C-5a), 128.4 (C-8), 125.3 (C-9), 124.2 (C-10), 123.8 (C-10a), 119.6 (C-10b), 111.2 (C-7), 55.2 (C-13), 54.3 (C-2'/5'), 44.6 (C-12), 23.6 (C-3'/4').

¹⁵N NMR (40 MHz, CDCl₃) δ -51.1 (N-1*/4*), -51.8 (N-1*/4*), -235.8 (N-6), -331.2 (N-1').
To a solution of diethanolamine (31 g, 29.4 mmol) and diethyl carbonate (44.0 mL, 36.3 mmol) was added methoxide (0.14 g, 2.6 mmol) in one portion and the resulting mixture was refluxed for 3 h. Ethanol formed in the reaction was removed under reduced pressure to give 38 g (98 %) of the crude product 40 as colourless oil which was used for the following reaction step. A small amount was purified by ball-tube distillation (b.p. 150-155 °C at 0.3 Torr) for NMR spectroscopic purposes.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.28 (m, H-5, 2H), 3.68 (m, H-7, 2H), 3.64 (m, H-4, 2H), 3.31 (m, H-6, 2H), not found (H-8).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 159.2 (C-2), 62.1 (C-5), 59.8 (C-7), 46.5 (C-6), 45.3 (C-4).

$^{15}$N NMR (40 MHz, CDCl$_3$) $\delta$ -201.5 (N-3).
6.2.42 3-(2-Chloroethyl)-1,3-oxazolidin-2-one (41)

To a solution of alcohol 40 (30 g, 228.8 mmol) in dry CH₂Cl₂ (100 mL) were slowly added thionyl chloride (29.9 g, 18.3 mL, 251.6 mmol) and 3 drops of dry DMF. The mixture was refluxed for 4 h. To the cooled reaction mixture was carefully added saturated Na₂CO₃ solution (250 mL). The organic layer was collected and the aqueous layer was extracted with CH₂Cl₂ (3 x). The combined organic layers were washed with small amounts of water and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude product was distilled (b.p. 110 °C at 0.1 Torr) to obtain 27.4 g (80 %) of 41 as a pale yellow oil.

\[ \text{H NMR (400 MHz, CDCl₃)} \delta 4.32 (\text{m}, \text{H-5, 2H}), 3.69 (\text{m}, \text{H-4, 2H}), 3.65 (\text{m}, \text{H-7, 2H}), 3.56 (\text{m}, \text{H-6, 2H}). \]

\[ \text{C NMR (100 MHz, CDCl₃)} \delta 158.3 (\text{C-2}), 62.0 (\text{C-5}), 46.0 (\text{C-6}), 45.5 (\text{C-4}), 41.8 (\text{C-7}). \]

\[ \text{N NMR (40 MHz, CDCl₃)} \delta -202.4 (\text{N-3}). \]
6. 2. 43 6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (42)

NaH (36 mg of a 60 % dispersion in mineral oil, 0.89 mmol) was suspended in dry DMF (5 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 19 (147 mg, 0.59 mmol) in dry DMF (10 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 41 (179 mg, 1.2 mmol) in dry DMF (5 mL) was added dropwise and the mixture was stirred at 75 °C for 6 d. After cooling down the reaction mixture was quenched with water (30 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate to obtain 124 mg (57.9 %) of 42 as bright red needles, m.p. 224-226 °C.

**HRMS:** m/z calculated for C₂₀H₁₆N₃O₄ ([M+H]+): 362.1135; found: 362.1135.
**HRMS:** m/z calculated for C₂₀H₁₅N₃NaO₄ ([M+Na]+): 384.0955; found: 384.0955.

**¹H NMR (400 MHz, CDCl₃)** δ 9.46 (s, H-1, 1H), 9.06 (d, J = 5.0 Hz, H-3, 1H), 8.47 (d, J = 8.0 Hz, H-10, 1H), 7.95 (d, J = 5.0 Hz, H-4, 1H), 7.63 (d, J = 8.4 Hz, H-7, 1H), 7.56 (m, H-8, 1H), 7.46 (m, H-9, 1H), 4.92 (t, J = 6.3 Hz, H-12, 2H), 4.09 (m, H-5’, 2H), 3.75 (t, J = 6.3 Hz, H-13, 2H), 3.32 (m, H-4’, 2H).

**¹³C NMR (100 MHz, CDCl₃)** δ 180.5 (C-11), 178.0 (C-5), 158.4 (C-2’), 155.0 (C-3), 148.5 (C-1), 140.3 (C-6a), 138.8 (C-4a), 133.8 (C-5a), 128.9 (C-8), 126.3 (C-11a), 125.5 (C-9), 124.2 (C-10), 123.6 (C-10a), 119.8 (C-10b), 118.4 (C-4), 110.9 (C-7), 62.0 (C-5’), 45.8 (C-4’), 44.3 (C-13), 43.2 (C-12).

**¹⁵N NMR (40 MHz, CDCl₃)** δ -53.6 (N-2), -240.4 (N-6), -304.5 (N-3’).
Carbamate 42 (218 mg, 0.60 mmol) was dissolved in 2N NaOH in methanol (50 mL) and refluxed (approximately 4 h) until no starting material could be detected by TLC (ethyl acetate). The cooled solution was neutralised with 6M HCl. Precipitated NaCl was removed by filtration through a Buchner funnel. The filter cake was washed with small amounts of cold ethanol and the filtrate was concentrated in vacuo. The residue was basified with saturated Na$_2$CO$_3$ solution and then the water evaporated in vacuo. The crude product was taken up in acetonitrile and filtered to remove the inorganic salts, the filtrate was concentrated in vacuo and the residue subjected to RP-chromatography eluting with methanol/water (6:4, v/v) to afford 23 mg (11.3 %) of 43 as brown crystals, m.p. 100-102 °C.

HRMS: m/z calculated for C$_{19}$H$_{18}$N$_3$O$_3$ ([M+H]$^+$): 336.1343; found: 336.1347.
HRMS: m/z calculated for C$_{19}$H$_{17}$N$_3$NaO$_3$ ([M+Na]$^+$): 358.1162; found: 358.1165.

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.43 (d, $J=0.8$ Hz, H-1, 1H), 9.03 (d, $J=5.0$ Hz, H-3, 1H), 8.46 (m, H-10, 1H), 7.94 (dd, $J=5.0$, 0.8 Hz, H-4, 1H), 7.54 (m, H-7, 1H), 7.52 (m, H-8, 1H), 7.43 (m, H-9, 1H), 4.84 (t, $J=6.5$ Hz, H-12, 2H), 3.60 (m, H-16, 2H), 3.16 (t, $J=6.5$ Hz, H-13, 2H), 2.83 (m, H-15, 2H), not found (H-14/17).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 180.5 (C-11), 177.9 (C-5), 154.9 (C-3), 148.4 (C-1), 140.0 (C-6a), 139.0 (C-4a), 134.3 (C-5a), 128.3 (C-8), 126.3 (C-11a), 125.2 (C-9), 124.2 (C-10), 123.8 (C-10a), 119.3 (C-10b), 118.5 (C-4), 111.1 (C-7), 61.0 (C-16), 50.9 (C-15), 48.9 (C-13), 45.5 (C-12).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -54.2 (N-2), -237.7 (N-6), -355.0 (N-14).
6.2.45 6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (44)

NaH (147 mg of a 60 % dispersion in mineral oil, 3.68 mmol) was suspended in dry DMF (10 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 25 (612 mg, 2.46 mmol) in dry DMF (30 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 41 (735 mg, 4.91 mmol) in dry DMF (5 mL) was added dropwise and the mixture was stirred at 75 °C for 3 d. After cooling down the reaction mixture was quenched with water (100 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate to obtain 253 mg (28.4 %) of 44 as orange crystals, m.p. 288-290 °C.

HRMS: m/z calculated for C₁₉H₁₅N₄O₄ ([M+H]+): 363.1088; found: 363.1087.
HRMS: m/z calculated for C₁₉H₁₄N₄NaO₄ ([M+Na]+): 385.0907; found: 385.0908.

¹H NMR (400 MHz, CDCl₃) δ 9.02 (d, J = 2.3 Hz, H-2*/3*, 1H), 8.97 (d, J = 2.3 Hz, H-2*/3*, 1H), 8.53 (m, H-10, 1H), 7.67 (m, H-7, 1H), 7.59 (m, H-8, 1H), 7.48 (m, H-9, 1H), 4.96 (m, H-12, 2H), 4.17 (m, H-5', 2H), 3.78 (m, H-13, 2H), 3.50 (m, H-4', 2H).

¹³C NMR (100 MHz, CDCl₃) δ 177.6 (C-11), 176.0 (C-5), 158.4 (C-2'), 148.2 (C-2*/3*), 147.4 (C-2*/3*), 145.9 (C-4a*/11a*), 145.3 (C-4a*/11a*), 140.4 (C-6a), 134.2 (C-5a), 129.3 (C-8), 125.9 (C-9), 124.5 (C-10), 123.8 (C-10a), 120.4 (C-10b), 111.1 (C-7), 62.1 (C-5'), 45.6 (C-4'), 44.1 (C-13), 43.1 (C-12).

¹⁵N NMR (40 MHz, CDCl₃) δ -50.1 (N-1*/4*), -51.4 (N-1*/4*), -239.2 (N-6), -304.4 (N-3*).
6. 2. 46  \( \text{N,\text{N}-Diethyl-1H-indole-3-carboxamide (45)} \)

To a solution of 1\( \text{H-Indole-3-carboxylic acid (25 g, 155.13 mmol)} \) in dry CH\(_2\)Cl\(_2\) (500 mL) were slowly added oxalyl chloride (18.6 mL, 209.7 mmol) and 3 drops of dry DMF. The mixture was continuously stirred at room temperature until the formed CO\(_2\) was completely gone (approximately 1h). The mixture was ice-cooled, then diethylamine (40.5 mL, 387.8 mmol) was added slowly. The reaction mixture was allowed to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated Na\(_2\)CO\(_3\) solution (400 mL). The organic layer was collected and the aqueous layer was extracted with CH\(_2\)Cl\(_2\). The combined organic layers were washed with water, dried over Na\(_2\)SO\(_4\) and the solvent was removed in vacuo. The crude product was purified by column chromatography eluting with ethyl acetate as eluent to obtain 18.12 g (54.0 \%) of 45 as colourless crystals, m.p. 151-152 °C.

\[^1\text{H} \text{NMR (400 MHz, DMSO-d₆)} \delta 11.45 \text{ (br s, H-1, 1H)}, 7.69 \text{ (m, H-4, 1H)}, 7.58 \text{ (s, H-2, 1H)}, 7.41 \text{ (m, H-7, 1H)}, 7.13 \text{ (m, H-6, 1H)}, 7.06 \text{ (m, H-5, 1H)}, 3.46 \text{ (q, J = 7.1 Hz, H-10/10’, 4H)}, 1.14 \text{ (t, J = 7.1 Hz, H-11/11’, 6H).}

\[^1\text{C} \text{NMR (100 MHz, DMSO-d₆)} \delta 165.6 \text{ (C-8)}, 135.5 \text{ (C-7a)}, 126.5 \text{ (C-3a)}, 125.8 \text{ (C-2)}, 121.7 \text{ (C-6)}, 120.3 \text{ (C-4)}, 119.8 \text{ (C-5)}, 111.7 \text{ (C-7)}, 110.4 \text{ (C-3)}, 40.8 \text{ (C-10/10’)}, 13.8 \text{ (C-11/11’).}

\[^{15}\text{N} \text{NMR (40 MHz, DMSO-d₆)} \delta -244.1 \text{ (N-1), not found (N-9).} \)
6. 2. 47  \textit{N,N}-Diethyl-1-(methoxymethyl)-1\textit{H}-indole-3-carboxamide (46)

NaH (444 mg of a 60 % dispersion in mineral oil, 11.09 mmol) was suspended in dry DMF (10 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 45 (2.0 g, 9.24 mmol) in dry DMF (30 mL) was added. The cooling bath was removed and after stirring for further 60 min the mixture was cooled to -10 °C and chloro(methoxy)methane (0.967 mg, 931µL, 12.01 mmol) was added slowly. The mixture was allowed to reach room temperature and was stirred for 2 h. The reaction was quenched by pouring onto a cold saturated NaHCO₃ solution (100 mL) and the product was extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product as yellowish oil which was purified by trituration under heating with ethyl acetate/light petroleum (1:1, v/v) followed by cooling to afford 1.38 g (58.0 %) of 46 as colourless crystals, m.p. 76-77 °C.

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.75 (s, H-2, 1H), 7.70 (m, H-4, 1H), 7.58 (m, H-7, 1H), 7.22 (m, H-6, 1H), 7.14 (m, H-5, 1H), 5.57 (s, H-12, 2H), 3.46 (q, $J$ = 7.1 Hz, H-10/10', 4H), 3.17 (s, H-13, 3H), 1.14 (t, $J$ = 7.1 Hz, H-11/11', 6H).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 165.0 (C-8), 135.5 (C-7a), 129.1 (C-2), 127.3 (C-3a), 122.4 (C-6), 120.8 (C-5), 120.6 (C-4), 110.8 (C-3), 110.7 (C-7), 76.7 (C-12), 55.3 (C-13), 40.8 (C-10/10'), 13.8 (C-11/11').

$^{15}$N NMR (40 MHz, DMSO-$d_6$) $\delta$ -235.0 (N-1), -252.9 (N-9).
6. 2. 48 1-[2-(Dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (47)

NaH (5.825 g of a 60% dispersion in mineral oil, 145.6 mmol) was suspended in dry DMF (30 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 45 (9.0 g, 41.6 mmol) in dry DMF (70 mL) was added. The cooling bath was removed and after stirring for further 60 min solid 2-chloro-N,N-dimethylethanamine hydrochloride (11.98 g, 83.2 mmol) was added in portions and the mixture was stirred at 90-100 °C overnight. After cooling the reaction mixture was quenched with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to afford 7.71 g (64.5%) of 47 as yellow oil.


HRMS: m/z calculated for C₁₇H₂₅N₃NaO ([M+Na]⁺): 310.1890; found: 310.1892.

¹H NMR (400 MHz, CDCl₃) δ 7.78 (m, H-4, 1H), 7.42 (s, H-2, 1H), 7.34 (m, H-7, 1H), 7.24 (m, H-6, 1H), 7.18 (m, H-5, 1H), 4.21 (m, H-12, 2H), 3.57 (q, J = 7.1 Hz, H-10/10’, 4H), 2.70 (m, H-13, 2H), 2.29 (s, H-15/15’, 6H), 1.22 (t, J = 7.1 Hz, H-11/11’, 6H).

¹³C NMR (100 MHz, CDCl₃) δ 166.7 (C-8), 135.7 (C-7a), 128.7 (C-2), 126.8 (C-3a), 122.2 (C-6), 121.0 (C-4), 120.6 (C-5), 111.3 (C-3), 109.3 (C-7), 58.8 (C-13), 45.6 (C-15/15’), 44.7 (C-12), 41.3 (C-10/10’, via HMBC), 13.8 (C-11/11’).

¹⁵N NMR (40 MHz, CDCl₃) δ -244.5 (N-1), -252.6 (N-9), -358.5 (N-14).
6.2.49 6-(Methoxymethyl)-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (48)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 46 (1.46 g, 5.61 mmol, 1 eq.) in dry THF (40 mL) was added dropwise over 10 min. After stirring the mixture at -75 °C for 30 min a solution of pyrimidine-5-carbaldehyde (606 mg, 5.61 mmol, 1 eq.) in dry THF (30 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na$_2$SO$_4$ and the solvent was evaporated to furnish a dark orange residue which was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) followed by recrystallisation from ethyl acetate to give 150 mg (9.1 %) of 48 as bright orange crystals, m.p. 238-240 °C.

HRMS: m/z calculated for C$_{16}$H$_{12}$N$_3$O$_3$ ([M+H]$^+$): 294.0873; found: 294.0873.

HRMS: m/z calculated for C$_{16}$H$_{11}$N$_3$NaO$_3$ ([M+Na]$^+$): 316.0693; found: 316.0696.

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.65 (s, H-2, 1H), 9.54 (s, H-4, 1H), 8.50 (m, H-10, 1H), 7.66 (m, H-7, 1H), 7.55 (m, H-8, 1H), 7.47 (m, H-9, 1H), 6.14 (s, H-12, 2H), 3.40 (s, H-13, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 178.3 (C-11), 176.6 (C-5), 162.8 (C-2), 156.8 (C-4), 154.8 (C-11a), 140.1 (C-6a), 133.8 (C-5a), 129.0 (C-8), 125.9 (C-9), 125.5 (C-10), 123.8 (C-10a), 121.1 (C-10b), 112.1 (C-7), 75.6 (C-12), 56.7 (C-13).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -77.1 (N-3), -95.7 (N-1), not found (N-6).
6. 2. 50  5H-Pyrimido[5,4-b]carbazole-5,11(6H)-dione (49)

To an ice-cold solution of 48 (138 mg, 0.47 mmol) in dry CH$_2$Cl$_2$ (15 mL) a solution of BBr$_3$ (1M in CH$_2$Cl$_2$, 0.56 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated NaHCO$_3$ solution (15 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH$_2$Cl$_2$. The filter cake was subjected to column chromatography eluting with ethyl acetate/methanol (8:2, v/v) followed by recrystallisation from methanol afforded 51 mg (43.5 %) of 49 as orange crystals, m.p. >350 °C.

HRMS: m/z calculated for C$_{14}$H$_8$N$_3$O$_2$ ([M+H]$^+$): 250.0611; found: 250.0610.
HRMS: m/z calculated for C$_{14}$H$_7$N$_3$NaO$_2$ ([M+Na]$^+$): 272.0430; found: 272.0433.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.32 (br s, H-6, 1H), 9.63 (s, H-2, 1H), 9.43 (s, H-4, 1H), 8.21 (m, H-10, 1H), 7.61 (m, H-7, 1H), 7.48 (m, H-8, 1H), 7.40 (m, H-9, 1H).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 177.8 (C-11), 176.1 (C-5), 162.2 (C-2), 155.58 (C-11a), 155.56 (C-4), 138.2 (C-6a), 136.5 (C-5a), 127.6 (C-8), 125.5 (C-4a), 124.6 (C-9), 123.8 (C-10a), 122.4 (C-10), 118.5 (C-10b), 114.1 (C-7).

$^{15}$N NMR (40 MHz, DMSO-$d_6$) δ -77.4 (N-3), -92.8 (N-1).
6. 2. 51  6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (50)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (-70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to -78 °C and a solution of 47 (1.61 g, 5.61 mmol, 1 eq.) in dry THF (40 mL) was added dropwise over 10 min. After stirring the mixture at -75 °C for 30 min a solution of pyrimidine-5-carbaldehyde (606 mg, 5.61 mmol, 1 eq.) in dry THF (30 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na$_2$SO$_4$ and the solvent was evaporated to furnish a brown residue, which was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) followed by column chromatography eluting with CH$_2$Cl$_2$/methanol (9:1, v/v) and recrystallisation from ethyl acetate to give 135 mg (7.5 %) of 50 as red needles, m.p. 195-197 °C.

HRMS: m/z calculated for C$_{18}$H$_{17}$N$_4$O$_2$ ([M+H]$^+$): 321.1346; found: 321.1350.
HRMS: m/z calculated for C$_{18}$H$_{16}$N$_4$NaO$_2$ ([M+Na]$^+$): 343.1165; found: 343.1167.

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.61 (s, H-2, 1H), 9.50 (s, H-4, 1H), 8.45 (m, H-10, 1H), 7.48 (m, H-7/8, 2H), 7.40 (m, H-9, 1H), 4.81 (m, H-12, 2H), 2.73 (m, H-13, 2H), 2.35 (s, H-15/15', 6H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 177.7 (C-11), 176.3 (C-5), 162.7 (C-2), 156.6 (C-4), 155.1 (C-11a), 139.7 (C-6a), 133.7 (C-5a), 128.4 (C-8), 125.52 (C-4a), 125.48 (C-9), 124.2 (C-10), 123.9 (C-10a), 120.0 (C-10b), 111.2 (C-7), 58.5 (C-13), 45.8 (C-15/15'), 43.7 (C-12).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -78.0 (N-3), -95.9 (N-1), -235.9 (N-6), -359.3 (N-14).
**EXPERIMENTAL PART**

6. 2. 52  **Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (51)**

![Chemical Structure](image)

To a solution of ethyl 1H-indole-2-carboxylate (8.76 g, 46.3 mmol) in dry CH₂Cl₂ (250 mL) was added anhydrous AlCl₃ (6.79 g, 50.9 mmol), and the mixture was stirred at room temperature for 30 min under argon. A solution of pyrimidine-5-carbaldehyde (hygroscopic, must therefore be dried properly in a desiccator before use) (5 g, 46.3 mmol) in dry CH₂Cl₂ (600 mL) was added rapidly via dropping funnel to the mixture at 0 °C, brought back to room temperature and stirred for 2−4 h. The completion of the reaction was monitored by TLC (ethyl acetate). The mixture was then poured on ice water (300 mL), the organic layer was collected and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) followed by recrystallisation from ethyl acetate/light petroleum to afford 4.86 g (35.3 %) of 51 as colourless crystals, m.p. 216-218 °C.

**HRMS:** m/z calculated for C₁₆H₁₆N₃O₃ ([M+H]⁺): 298.1186; found: 298.1186.

**HRMS:** m/z calculated for C₁₆H₁₅N₃NaO₃ ([M+Na]⁺): 320.1006; found: 320.1007.

**¹H NMR (400 MHz, DMSO-d₆)** δ 11.79 (s, H-1, 1H), 9.03 (s, H-2', 1H), 8.85 (s, H-4'/6', 2H), 7.86 (m, H-4, 1H), 7.45 (m, H-7, 1H), 7.23 (m, H-6, 1H), 7.00 (m, H-5, 1H), 6.85 (d, J = 3.9 Hz, H-8, 1H), 6.30 (d, J = 3.9 Hz, H-9, 1H), 4.34-4.46 (m, H-11, 2H), 1.35 (t, J = 7.1 Hz, H-12, 3H).

**¹³C NMR (100 MHz, DMSO-d₆)** δ 161.6 (C-10), 156.9 (C-2'), 154.5 (C-4'/6'), 138.1 (C-5'), 136.6 (C-7a), 125.1 (C-3a), 124.9 (C-6), 124.0 (C-3), 122.7 (C-4), 122.5 (C-2), 119.9 (C-5), 112.7 (C-7), 64.3 (C-8), 60.7 (C-11), 14.3 (C-12).

**¹⁵N NMR (40 MHz, DMSO-d₆)** δ -87.1 (N-1'/3'), -247.1 (N-1).
6.2.53 Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (52)

To a solution of 51 (195 mg, 0.66 mmol) in DMSO (10 mL) was added 2-iodoxybenzoic acid (IBX stabilised, containing 45 wt. % IBX, 492.8 mg, 0.79 mmol). The reaction mixture was stirred at room temperature for 2-4 h, the reaction progress was monitored by TLC (ethyl acetate). The reaction mixture was quenched with ice-cold water (75 mL) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) to afford 163 mg of 52 (84.2 %) as colourless crystals, m.p. 224-226 °C.

HRMS: m/z calculated for C₁₆H₁₄N₃O₃ ([M+H]⁺): 296.1030; found: 296.1030.

¹H NMR (400 MHz, DMSO-d₆) δ 12.77 (s, H-1, 1H), 9.38 (s, H-2', 1H), 9.09 (s, H-4'/6', 2H), 7.82 (m, H-4, 1H), 7.60 (m, H-7, 1H), 7.39 (m, H-6, 1H), 7.25 (m, H-5, 1H), 4.01 (q, J = 7.1 Hz, H-10, 2H), 0.90 (t, J = 7.1 Hz, H-11, 3H).

¹³C NMR (100 MHz, DMSO-d₆) δ 189.0 (C-8), 160.4 (C-2'), 160.1 (C-9), 156.7 (C-4'/6'), 136.0 (C-7a), 133.1 (C-5'), 129.0 (C-2), 126.8 (C-3a), 125.7 (C-6), 122.6 (C-5), 121.3 (C-4), 116.6 (C-3), 113.1 (C-7), 61.3 (C-10), 13.4 (C-11).

¹⁵N NMR (40 MHz, DMSO-d₆) δ -87.2 (N-1'/3'), not found (N-1).
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperide (1.89 mL, 11.22 mmol) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 4.49 mL, 11.22 mmol) in dry THF (60 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 52 (1.66 g, 5.61 mmol) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (30 mL). The aqueous layer was extracted with ethyl acetate, dried over Na₂SO₄ and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/ light petroleum (4:6, v/v) followed by recrystallisation from ethyl acetate to give 74 mg (5.3 %) of 53 as orange crystals, m.p. >350 °C.

HRMS: m/z calculated for C₁₄H₈N₃O₂ ([M+H]⁺): 250.0611; found: 250.0612.

HRMS: m/z calculated for C₁₄H₇N₃NaO₂ ([M+Na]⁺): 272.0430; found: 272.0432.
6. 2. 55  Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (54)

To an ice-cooled solution of 52 (500 mg, 1.69 mmol) in dry DMF (60 mL) was first added NaH (237 mg of a 60 % dispersion in mineral oil, 5.92 mmol), then solid 2-chloro-\(N,N\)-dimethylethanamine hydrochloride (487.8 mg, 3.39 mmol) and the mixture was stirred at 80 °C overnight. After cooling the reaction mixture was quenched with water (100 mL) and extracted with ethyl acetate. The combined organic layers were dried over \(\text{Na}_2\text{SO}_4\) and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate/methanol (95:5, v/v) to afford 305 mg (49.1 %) of 54 as colourless oil.

HRMS: \(m/z\) calculated for \(\text{C}_{20}\text{H}_{23}\text{N}_4\text{O}_3\) ([M+H]+): 367.1765; found: 367.1770.

HRMS: \(m/z\) calculated for \(\text{C}_{20}\text{H}_{22}\text{N}_4\text{NaO}_3\) ([M+Na]+): 389.1584; found: 389.1589.

\(^1\text{H} \text{NMR (400 MHz, CDCl}_3\}) \delta 9.34 (s, H-2', 1H), 9.08 (s, H-4'/6', 2H), 7.80 (m, H-4, 1H), 7.50 (m, H-7, 1H), 7.43 (m, H-6, 1H), 7.28 (m, H-5, 1H), 4.63 (m, H-12, 2H), 3.96 (q, \(J = 7.2\) Hz, H-10, 2H), 2.67 (m, H-13, 2H), 2.28 (s, H-15/15', 6H), 0.96 (t, \(J = 7.2\) Hz, H-11, 3H).

\(^{13}\text{C} \text{NMR (100 MHz, CDCl}_3\}) \delta 188.7 (C-8), 160.8 (C-9), 160.6 (C-2'), 157.0 (C-4'/6'), 137.3 (C-7a), 133.0 (C-5'), 130.5 (C-2), 126.0 (C-6), 125.8 (C-3a), 123.2 (C-5), 121.8 (C-4), 118.0 (C-3), 110.7 (C-7), 61.8 (C-10), 58.7 (C-13), 45.8 (C-15/15'), 43.5 (C-12), 13.5 (C-11).

\(^{15}\text{N} \text{NMR (40 MHz, CDCl}_3\}) \delta -89.1 (N-1'/3'), -238.1 (N-1), -359.1 (N-14).
EXPERIMENTAL PART

6. 2. 56 Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (55)

To an ice-cooled solution of ethyl 1H-indole-2-carboxylate (10 g, 52.9 mmol) in dry DMF (150 mL) was first added NaH (7.41 g of a 60 % dispersion in mineral oil, 185.2 mmol), then solid 2-chloro-N,N-dimethylethanamine hydrochloride (15.24 g, 105.8 mmol) and the mixture was stirred at 60 °C for 4 h. After cooling the reaction mixture was quenched with water (100 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate to afford 9.59 g (69.6 %) of 55 as yellow oil.

¹H NMR (400 MHz, CDCl₃) δ 7.67 (m, H-4, 1H), 7.43 (m, H-7, 1H), 7.35 (m, H-6, 1H), 7.31 (s, H-3, 1H), 7.15 (m, H-5, 1H), 4.69 (m, H-11, 2H), 4.38 (q, J = 7.1 Hz, H-9, 2H), 2.67 (m, H-12, 2H), 2.36 (s, H-14/14', 6H), 1.41 (t, J = 7.1 Hz, H-10, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 161.9 (C-8), 139.0 (C-7a), 127.6 (C-2), 126.0 (C-3a), 125.0 (C-6), 122.6 (C-4), 120.6 (C-5), 110.5 (C-3), 110.3 (C-7), 60.5 (C-9), 58.9 (C-12), 45.8 (C-14/14'), 42.9 (C-11), 14.4 (C-10).

¹⁵N NMR (40 MHz, CDCl₃) δ -246.4 (N-1), -358.1 (N-13).
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (0.83 mL, 4.84 mmol) dropwise (over less than 1 min) to a cold (–70 °C) solution of n-BuLi (2.5M in hexanes, 3.87 mL, 9.68 mmol) in dry THF (20 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to -78 °C and a solution of 54 (355 mg, 0.96 mmol) in dry THF (10 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (30 mL). The aqueous layer was extracted with ethyl acetate and dried over Na$_2$SO$_4$ and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) to give 23 mg (7.4 %) of 56 as red needles, m.p. 180-182 °C.

HRMS: m/z calculated for C$_{18}$H$_{17}$N$_4$O$_2$ ([M+H]$^+$): 321.1346; found: 321.1349.

HRMS: m/z calculated for C$_{18}$H$_{16}$N$_4$NaO$_2$ ([M+Na]$^+$): 343.1165; found: 343.1170.

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.63 (s, H-2, 1H), 9.58 (s, H-4, 1H), 8.43 (m, H-6, 1H), 7.55 (m, H-8/9, 2H), 7.45 (m, H-7, 1H), 4.87 (m, H-12, 2H), 2.76 (m, H-13, 2H), 2.38 (s, H15/15', 6H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 178.5 (C-5), 175.8 (C-11), 162.2 (C-2), 156.9 (C-4), 154.6 (C-11a), 140.1 (C-9a), 134.7 (C-10a), 128.7 (C-8), 125.6 (C-7), 125.5 (C-4a), 124.2 (C-6), 123.6 (C-5b), 111.3 (C-9), 58.5 (C-13), 45.8 (C-15/15'), 43.7 (C-12).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -359.1 (N-14), -235.6 (N-10), -96.9 (N-1), -75.0 (N-3).
6. 2. 58 10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (56)

A lithium 2,2,6,6-tetramethylpiperidine solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (-70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to -78 °C and a solution of 55 (1.46 g, 5.61 mmol, 1 eq.) in dry THF (25 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After stirring the mixture at -75 °C for 15 min a solution of pyrimidine-5-carbaldehyde (606 mg, 5.61 mmol, 1 eq.) in dry THF (25 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) to afford 65 mg (3.6 %) of 56 as red needles, m.p. 180-182 °C.


¹H NMR (400 MHz, CDCl₃) δ 9.63 (s, H-2, 1H), 9.58 (s, H-4, 1H), 8.43 (m, H-6, 1H), 7.55 (m, H-8/9, 2H), 7.45 (m, H-7, 1H), 4.87 (m, H-12, 2H), 2.76 (m, H-13, 2H), 2.38 (s, H15/15', 6H).

¹³C NMR (100 MHz, CDCl₃) δ 178.5 (C-5), 175.8 (C-11), 162.2 (C-2), 156.9 (C-4), 154.6 (C-11a), 140.1 (C-9a), 134.7 (C-10a), 128.7 (C-8), 125.6 (C-7), 125.5 (C-4a), 124.2 (C-6), 123.6 (C-5b), 111.3 (C-9), 58.5 (C-13), 45.8 (C-15/15'), 43.7 (C-12).

¹⁵N NMR (40 MHz, CDCl₃) δ -359.1 (N-14), -235.6 (N-10), -96.9 (N-1), -75.0 (N-3).
6. 2. 59  **Furo[3,4-b]quinolin-1(3H)-one (57)**

2-aminobenzaldehyde (1.05 g, 8.7 mmol) and tetronic acid (0.87 g, 8.7 mmol) were dissolve in dry ethanol (12 mL). The mixture was stirred at room temperature for 5 h. The precipitate was filtered and recrystallised from dry ethanol to afford 57 as pale yellow crystals (1.15 g, 71.0 %), m.p. 215-217 °C.

**NMR Spectra**

**1H NMR** (400 MHz, CDCl3) δ 8.78 (s, H-9, 1H), 8.18 (d, J = 8.6 Hz, H-5, 1H), 8.05 (m, H-8, 1H), 7.93 (m, H-6, 1H), 7.69 (m, H-7, 1H), 5.48 (s, H-3, 2H).

**13C NMR** (100 MHz, CDCl3) δ 168.8 (C-1), 163.4 (C-3a), 151.2 (C-4a), 136.1 (C-9), 133.1 (C-6), 130.0 (C-8), 129.3 (C-5), 127.6 (C-7), 127.3 (C-8a), 117.0 (C-9a), 70.7 (C-3).

**15N NMR** (40 MHz, CDCl3) δ -87.4 (N-4).
6. 2. 60  6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (58)

To a solution of 3 (6.0 g, 23.3 mmol) in dry THF (200 mL) at -78 °C was slowly added n-BuLi (1.6 M in hexane, 15.2 mL). The mixture was allowed to reach room temperature for 1 h then warmed to 40 °C for 5 min. The mixture was cooled to -78 °C, then lactone 57 (3 g, 16.2 mmol) in dry THF (250 mL) was added in one portion. The ice bath was removed and the mixture was allowed to reach room temperature then brought to 40 °C for 5 min. The solvent was removed in vacuo. Water (100 mL) was added to the residue and extracted with diethyl ether (4 x 150 mL). The combined etheric layers were extracted with 2N hydrochloric acid (3 x), then the aqueous layer was basified using solid K$_2$CO$_3$. Extraction with CH$_2$Cl$_2$ and evaporation gave a brown foam (1.2 g) which was dissolved in a 3:1 (v/v) mixture of methanol (120 mL) and 3M NaOH (40 mL). The resulting mixture was refluxed for 10 minutes. The yellow precipitate was filtered from the cooled solution, washed with water and recrystallised from methanol, affording 600 mg (12.3 %) of 58 as yellow flakes, m.p. 290-292 °C.

HRMS: m/z calculated for C$_{19}$H$_{13}$N$_2$O$_2$ ([M+H]$^+$): 301.0972; found: 301.0974.
HRMS: m/z calculated for C$_{19}$H$_{12}$N$_2$NaO$_2$ ([M+Na]$^+$): 323.0791; found: 323.0794.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.45 (s, H-12, 1H), 9.06 (s, H-14, 1H), 8.28 (m, H-1, 1H), 8.16 (m, H-4, 1H), 7.94 (m, H-3, 1H), 7.74 (m, H-2, 1H), 7.69 (m, H-8, 1H), 7.40 (m, H-11, 1H), 7.36 (m, H-10, 1H), 7.07 (m, H-9, 1H), 5.65 (s, H-6, 2H).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 177.9 (C-13), 153.3 (C-5a), 148.2 (C-4a), 147.3 (C-7a), 138.3 (C-14), 136.8 (C-11a), 132.3 (C-3), 130.5 (C-13a), 129.5 (C-1), 128.7 (C-4), 128.0 (C-2), 127.9 (C-10), 127.1 (C-14a), 122.1 (12a), 120.1 (C-8), 119.7 (C-9), 117.3 (C-7b), 112.9 (C-11), 78.7 (C-6).

$^{15}$N NMR (40 MHz, DMSO-$d_6$) δ -69.1 (N-5), -263.0 (N-12).
6. 2. 61 6H-Indolo[2,3-b]acridine-6,12(11H)-dione (59)

Oxepinoindole 58 (300 mg, 1.00 mmol) dissolved in methanol (400 mL) and 3M NaOH (80 mL) was refluxed for 7 days. Then most of the solvent was evaporated and the reaction mixture poured into cold water. The precipitate was collected by filtration, purified by column chromatography eluting with ethyl acetate and recrystallising from ethyl acetate to afford 120 mg (40.2 %) of 59 as yellow crystals, m.p. >350 °C.

HRMS: m/z calculated for C_{19}H_{11}N_{2}O_{2} ([M+H]^+): 299.0815; found: 299.0816.
HRMS: m/z calculated for C_{19}H_{10}N_{2}NaO_{2} ([M+Na]^+): 321.0634; found: 321.0638.

^1\text{H} \text{NMR} (400 MHz, DMSO-d_6) \delta 13.22 (br s, H-11, 1H), 9.13 (s, H-13, 1H), 8.33 (m, H-1, 1H), 8.30 (m, H-7, 1H), 8.28 (m, H-4, 1H), 7.98 (m, H-3, 1H), 7.79 (m, H-2, 1H), 7.61 (m, H-10, 1H), 7.47 (m, H-9, 1H), 7.39 (m, H-8, 1H).

^13\text{C} \text{NMR} (100 MHz, DMSO-d_6) \delta 178.3 (C-6), 176.4 (C-12), 149.4 (C-5a), 148.5 (C-4a), 138.4 (C-10a), 137.9 (C-11a), 136.2 (C-13), 133.0 (C-3), 130.4 (C-4), 130.0 (C-1), 129.3 (C-2), 127.8 (C-13a), 127.5 (C-9), 127.2 (C-12a), 124.17 (C-8), 124.14 (C-6b), 122.7 (C-7), 119.6 (C-6a), 113.9 (C-10).

^15\text{N} \text{NMR} (40 MHz, DMSO-d_6) \delta \text{not found} (N-5/11).
EXPERIMENTAL PART

6.2.62 11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (60)

A solution of 59 (92.5 mg, 0.3 mmol) in dry DMF (8 mL) was added dropwise to a cooled (-5 to -10 °C) suspension of NaH (40 mg, 0.93 mmol, 60 % in paraffin) in dry DMF (4 mL). After 30 min of stirring 2-chloro-N,N-dimethylethanamine hydrochloride (69 mg, 0.46 mmol) in DMF (6 mL) was added slowly and the reaction mixture was stirred at 75 °C for 4 h. After quenching with 30 mL of water the mixture was extracted with CH₂Cl₂ and the combined organic layers were dried over Na₂SO₄. The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with CH₂Cl₂/methanol (95:5, v/v) affording compound 60 (50 mg, 43.8 %) as yellow flakes, m.p. 165-167 °C.

HRMS: m/z calculated for C₂₃H₂₀N₃O₂ ([M+H]⁺): 370.1550; found: 370.1545.
HRMS: m/z calculated for C₂₃H₁₉N₃NaO₂ ([M+Na]⁺): 392.1369; found: 392.1368.

¹H NMR (400 MHz, CDCl₃) δ 9.01 (s, H-13, 1H), 8.60 (m, H-7, 1H), 8.45 (m, H-4, 1H), 8.02 (m, H-1, 1H), 7.89 (m, H-3, 1H), 7.70 (m, H-2, 1H), 7.53 (m, H-10, 1H), 7.50 (m, H-9, 1H), 7.43 (m, H-8, 1H), 4.91 (m, H-14, 2H), 2.83 (m, H-15, 2H), 2.44 (s, H-17/17′, 6H).

¹³C NMR (100 MHz, CDCl₃) δ 178.9 (C-6), 177.3 (C-12), 149.5 (C-4a), 148.9 (C-5a), 139.8 (C-10a), 136.8 (C-13), 135.2 (C-11a), 132.7 (C-3), 131.5 (C-4), 129.4 (C-1), 129.3 (C-2), 128.3 (C-13a), 128.1 (C-9), 127.6 (C-12a), 124.9 (C-8), 124.6 (C-7), 124.3 (C-6b), 121.3 (C-6a), 111.0 (C-10), 58.5 (C-15), 45.7 (C-17/17′), 43.5 (C-14).

¹⁵N NMR (40 MHz, CDCl₃) δ -69.2 (N-5), -237.9 (N-11), -358.1 (N-16).
6. 2. 63 *N,N*-Diethylquinoline-4-carboxamide (61)

To a solution of quinoline-4-carboxylic acid (5.07 g, 29.3 mmol) in dry CH₂Cl₂ (75 mL) were slowly added oxalyl chloride (3.4 mL, 39.6 mmol) and 3 drops of dry DMF. The mixture was continuously stirred at room temperature until the formed CO₂ was completely gone (approximately 1 h). The mixture was ice-cooled then diethylamine (7.6 mL, 73.4 mmol) was added slowly. The reaction mixture was allowed to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated Na₂CO₃ solution (50 mL). The organic layer was collected and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were washed with water, dried over Na₂SO₄ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography eluting with ethyl acetate/light petroleum (4:1, v/v) to afford 6.52 g (97.5 %) of 61 as yellow oil.

^1H NMR (400 MHz, CDCl₃) δ 8.90 (d, *J* = 4.3 Hz, H-2, 1H), 8.09 (m, H-8, 1H), 7.74 (m, H-5, 1H), 7.70 (m, H-7, 1H), 7.53 (m, H-6, 1H), 7.26 (d, *J* = 4.3 Hz, H-3, 1H), 3.78 (br s, H-11, 1H), 3.50 (br s, H-11, 1H), 3.04 (br s, H-11', 2H), 1.32 (t, *J* = 7.1 Hz, H-12, 3H), 0.96 (t, *J* = 7.1 Hz, H-12', 3H).

^13C NMR (100 MHz, CDCl₃) δ 167.6 (C-9), 149.9 (C-2), 148.2 (C-8a), 143.1 (C-4), 129.9 (C-7), 129.8 (C-8), 127.4 (C-6), 124.6 (C-5), 124.2 (C-4a), 117.3 (C-3), 42.9 (C-11'), 39.0 (C-11), 14.1 (C-12'), 12.9 (C-12).

^15N NMR (40 MHz, CDCl₃) δ -70.8 (N-1), -246.7 (N-10).
6. 2. 64 12-(Methoxymethyl)-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (62)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.6 mL, 21.3 mmol) to a cold (-78 °C) solution of n-BuLi (2.5 M in hexane, 8.5 mL, 21.3 mmol) in dry THF (60 mL), under Argon atmosphere. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to -78 °C and a solution of 61 (1.20 g, 5.26 mmol) in dry THF (30 mL) was added dropwise over 10 min. The mixture was stirred at -78 °C for a total of 30 min and then a solution of aldehyde 8 (400 mg, 2.11 mmol) in dry THF (18 mL) was added over 5 min. The flask was then taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL) and extracted with CH₂Cl₂ (3 x 70 mL). The combined organic layers were dried over Na₂SO₄ and the solvent was removed under reduced pressure to give a brown residue which was purified by column chromatography eluting with ethyl acetate/light petroleum (4:6, v/v) followed by recrystallisation from ethyl acetate to afford 200 mg (27.6 %) of 62 as orange crystals, m.p. 236-237 °C.

HRMS: m/z calculated for C₂₁H₁₄N₂O₃ ([M+H]+): 343.1077; found: 343.1081.

¹H NMR (400 MHz, CDCl₃) δ 9.78 (s, H-6, 1H), 9.59 (m, H-1, 1H), 8.44 (m, H-8, 1H), 8.20 (m, H-4, 1H), 7.85 (m, H-3, 1H), 7.77 (m, H-2, 1H), 7.65 (m, H-11, 1H), 7.52 (m, H-10, 1H), 7.45 (m, H-9, 1H), 6.16 (s, H-14, 2H), 3.44 (s, H-15, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 182.2 (C-7*/13*), 181.4 (C-7*/13*), 152.3 (C-4a), 147.8 (C-6), 140.2 (C-11a), 135.5 (C-12a), 133.3 (C-13a), 131.5 (C-3), 130.4 (C-4), 130.2 (C-2), 128.3 (C-10), 127.7 (C-1), 125.4 (C-9), 124.2 (C-6a), 123.9 (C-8), 123.2 (C-7b), 123.1 (C-13b), 118.6 (C-7a), 111.9 (C-11), 75.5 (C-14), 56.7 (C-15).

¹⁵N NMR (40 MHz, CDCl₃) δ not found (N-5/12).
6. 2. 65  7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (63)

To an ice-cold solution of 62 (620 mg, 1.81 mmol) in dry CH₂Cl₂ (150 mL) a solution of BBr₃ (1 M in CH₂Cl₂, 2.26 mL) was added dropwise. The solution was allowed to reach room temperature and continued stirring for a total of 1 h. The mixture was quenched with saturated NaHCO₃ solution (120 mL) and vigorously stirred for 1 h at 60 °C. The cooled mixture was filtrated, the filter cake was washed with water and cold CH₂Cl₂. Recrystallisation from ethyl acetate afforded 500 mg (92.6 %) of 63 as orange crystals, m.p. 273-275 °C.

HRMS: m/z calculated for C₁₉H₁₁N₂O₂ ([M+H]⁺): 299.0815; found: 299.0814.
HRMS: m/z calculated for C₁₉H₁₀N₂NaO₂ ([M+Na]⁺): 321.0634; found: 321.0634.

¹H NMR (400 MHz, DMSO-d₆) δ 13.09 (br s, H-12, 1H), 9.56 (s, H-6, 1H), 9.52 (m, H-1, 1H), 8.11 (m, H-4/8, 2H), 7.90 (m, H-3, 1H), 7.83 (m, H-2, 1H), 7.56 (m, H-10, 1H), 7.42 (m, H-11, 1H), 7.34 (m, H-9, 1H).

¹³C NMR (100 MHz, DMSO-d₆) δ 180.8 (C-7*/13*), 180.2 (C-7*/13*), 151.2 (C-4a), 147.4 (C-6), 138.4 (C-11a), 138.0 (C-12a), 132.5 (C-13a), 131.5 (C-3), 130.2 (C-2), 129.8 (C-4), 127.14 (C-1), 127.08 (C-10), 124.8 (C-6a), 124.3 (C-9), 123.3 (C-7b), 122.5 (C-13b), 122.2 (C-8), 115.5 (C-7a), 113.9 (C-11).

¹⁵N NMR (40 MHz, DMSO-d₆) δ -50.6 (N-5), not found (N-12).
6. 2. 66  

**7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (64)**

To a suspension of 63 (88 mg, 0.3 mmol) in CH₂Cl₂ (50 mL), was added in one portion m-CPBA (57-86 % peracid, 400 mg, excess) in CH₂Cl₂ (125 mL). The mixture was refluxed until no starting material could be detected by TLC (ethyl acetate/light petroleum 1:1, v/v) (approx. 14 h). The resulting mixture was diluted with 375 mL of CH₂Cl₂, which was washed with saturated NaHCO₃ solution (3 x 15 mL). The aqueous layer was back-extracted with CH₂Cl₂ (3 x). The combined organic layers were dried over Na₂SO₄ and evaporated to give an orange-red solid. The product was purified by column chromatography eluting with ethyl acetate to give 89 mg (96.3 %) of 64 as amorphous orange-red solid, m.p. 284-286 °C.

**HRMS:** m/z calculated for C₁₉H₁₁N₂O₃ ([M+H]+): 315.0764; found: 315.0767.

**HRMS:** m/z calculated for C₁₉H₁₀N₂NaO₃ ([M+Na]+): 337.0584; found: 337.0585.

**¹H NMR (400 MHz, DMSO-d₆)** δ 13.16 (s, H-12, 1H), 9.63 (m, H-1, 1H), 8.82 (s, H-6, 1H), 8.56 (m, H-4, 1H), 8.06 (m, H-8, 1H), 7.95 (m, H-2, 1H), 7.94 (m, H-3, 1H), 7.55 (m, H-11, 1H), 7.41 (m, H-10, 1H), 7.33 (m, H-9, 1H).

**¹³C NMR (100 MHz, DMSO-d₆)** δ 178.1 (C-13), 177.7 (C-7), 143.0 (C-4a), 138.6 (C-12a), 138.0 (C-11a), 131.9 (C-3), 131.8 (C-2), 131.7 (C-6), 129.8 (C-6a), 128.1 (C-1), 127.0 (C-10), 126.8 (C-13b), 124.4 (C-9), 123.4 (C-7b), 122.0 (C-8), 121.8 (C-13a), 119.1 (C-4), 115.0 (C-7a), 113.9 (C-11).

**¹⁵N NMR (40 MHz, DMSO-d₆)** δ -238.6 (N-12), not found (N-5).
A solution of 63 (450 mg, 1.51 mmol) in dry DMF (20 mL) was added dropwise to a cooled (-5 to -10 °C) suspension of NaH (160 mg, 4.00 mmol, 60 % in paraffin) in dry DMF (15 mL). After 30 min of stirring 2-chloro-N,N-dimethylethanamine hydrochloride (330 mg, 2.27 mmol) in DMF (20 mL) was added slowly and the reaction mixture was stirred at 75 °C for 5 h. After quenching with 50 mL of water the mixture was extracted with CH$_2$Cl$_2$ and the combined organic layers were dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with ethyl acetate/light petroleum (4:6, v/v) followed by ethyl acetate/methanol (95:5, v/v) to afford 65 (200 mg, 39.5 %) as orange-brown crystals, m.p. 208-209 °C.

HRMS: m/z calculated for C$_{23}$H$_{20}$N$_3$O$_2$ ([M+H]$^+$): 370.1550; found: 370.1551.

HRMS: m/z calculated for C$_{23}$H$_{19}$N$_3$NaO$_2$ ([M+Na]$^+$): 392.1369; found: 392.1371.

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.68 (s, H-6, 1H), 9.51 (m, H-1, 1H), 8.32 (m, H-8, 1H), 8.12 (m, H-4, 1H), 7.78 (m, H-3, 1H), 7.71 (m, H-2, 1H), 7.41 (m, H-11, 1H), 7.40 (m, H-10, 1H), 7.32 (m, H-9, 1H), 4.75 (m, H-14, 2H), 2.74 (m, H-15, 2H), 2.39 (s, H-17/17’, 6H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 181.8 (C-7*/13*), 180.7 (C-7*/13*), 152.0 (C-4a), 147.8 (C-6), 139.6 (C-11a), 135.3 (C-12a), 133.1 (C-13a), 131.2 (C-3), 130.3 (C-4), 130.0 (C-2), 127.64 (C-10), 127.58 (C-1), 124.9 (C-9), 124.4 (C-6a), 123.8 (C-8), 123.2 (C-7b), 123.0 (C-13b), 117.2 (C-7a), 111.0 (C-11), 58.5 (C-15), 45.9 (C-17/17‘), 43.6 (C-14).

$^{15}$N NMR (40 MHz, CDCl$_3$) δ -53.7 (N-5), -237.6 (N-12), -358.8 (N-16).
6.2.68 12-[(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (66)

A solution of 64 (89 mg, 0.28 mmol) in dry DMF (7 mL) was added dropwise to a cooled (-5 to -10 °C) suspension of NaH (30 mg, 0.73 mmol, 60 % in paraffin) in dry DMF (2 mL). After 30 min of stirring 2-chloro-\( N,N \)-dimethylethanamine hydrochloride (61 mg, 0.42 mmol) in DMF (20 mL) was added slowly and the reaction mixture was stirred at 75 °C for 4 h. After quenching with 50 mL of water the mixture was extracted with CH\(_2\)Cl\(_2\) and the combined organic layers were dried over Na\(_2\)SO\(_4\). The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with ethyl acetate/light petroleum (4:6, v/v) to ethyl acetate/methanol (95:5, v/v) affording compound 66 (23 mg, 21.1 %) as red crystals, m.p. 204-205 °C.

HRMS: m/z calculated for C\(_{23}\)H\(_{20}\)N\(_3\)O\(_3\) ([M+H]+): 386.1499; found: 386.1491.

HRMS: m/z calculated for C\(_{23}\)H\(_{19}\)N\(_3\)NaO\(_3\) ([M+Na]+): 408.1319; found: 408.1311.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.68 (m, H-1, 1H), 9.06 (s, H-6, 1H), 8.71 (m, H-4, 1H), 8.28 (m, H-8, 1H), 7.80 (m, H-2/3, 2H), 7.45 (m, H-11, 1H), 7.42 (m, H-10, 1H), 7.34 (m, H-9, 1H), 4.80 (m, H-14, 2H), 2.76 (m, H-15, 2H), 2.40 (s, H-17/17’, 6H).

\(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 179.2 (C-13), 178.0 (C-7), 144.0 (C-4a), 139.4 (C-11a), 135.8 (C-12a), 132.3 (C-6), 131.6 (C-3), 131.5 (C-2), 129.2 (C-6a), 128.7 (C-1), 127.6 (C-10), 127.3 (C-13b), 125.2 (C-9), 123.5 (C-8), 123.2 (C-7b), 122.7 (C-13a), 119.8 (C-4), 116.8 (C-7a), 111.1 (C-11), 58.5 (C-15), 45.9 (C-17/17’), 43.7 (C-14).

\(^15\)N NMR (40 MHz, CDCl\(_3\)) \(\delta\) -88.3 (N-5), -235.6 (N-12), -358.7 (N-16).
6. 2. 69  1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (67)

\[
\text{D-fructose} + \text{NH}_2\text{NH}_2 \xrightarrow{10 \% \text{aq. CH}_3\text{COOH}} \text{67}
\]

Benzene-1,2-diamine (50.0 g, 0.46 mol) was placed in a round bottom flask then aqueous acetic acid (225 mL, 10 \% solution) was added and the mixture was stirred for 30 min. Then D-Fructose (83 g, 0.46 mol) was added portionwise over 10 min. The mixture was heated to 80 \(^\circ\)C and stirred for 18 h. The reaction was cooled to -2 \(^\circ\)C for 4 h. The brown solids were collected by filtration, washed with water (2 x 100 mL) and recrystallised twice from ethanol to afford 38.5 g (33.5 \%) of 67 as beige crystals, m.p. 194-196 \(^\circ\)C.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 9.12 (s, H-3, 1H), 8.07 (m, H-5, 1H), 8.04 (m, H-8, 1H), 7.82 (m, H-7, 1H), 7.78 (m, H-6, 1H), 5.63 (d, \(J = 6.2\) Hz, H-9, 1H), 5.18 (d, \(J = 6.2\) Hz, H-1', 1H), 4.75 (m, H-11, 1H), 4.66 (m, H-10, 1H), 4.43 (t, \(J = 5.5\) Hz, H-12, 1H), 3.70 (m, H-2'/3', 2H), 3.68 (m, H-4', 1H), 3.49 (m, H-4', 1H).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 159.5 (C-2), 145.3 (C-3), 141.0 (C-4a), 140.9 (C-8a), 130.0 (C-7), 129.3 (C-6), 128.9 (C-5), 128.6 (C-8), 74.4 (C-2'), 72.5 (C-1'), 71.3 (C-3'), 63.6 (C-4').

\(^{15}\)N NMR (40 MHz, DMSO-\(d_6\)) \(\delta\) -53.3 (N-4), -62.8 (N-1).
6. 2. 70  2-Quinoxalinecarboxylic acid (68)

67 (38.5 g, 0.15 mol) was added portionwise into a solution of NaOH (93.2 g, 2.33 mol) in water (945 mL) then stirred for 45 min. A 30% solution of H₂O₂ (72 mL) was added to the reaction mixture over 10 min. The mixture was slowly heated to 60 °C over a period 3 h. Then the remainder of the 30% H₂O₂ solution (62.75 mL) was added slowly over 20 min (maximum temperature reached 91 °C) and stirring was continued for further 30 min. The reaction mixture was then refluxed for 15 min. The reaction was cooled to 80 °C, and the liquid was decanted away from the tar-like material. The tar-like material was discarded and the separated liquid was allowed to cool to room temperature. Then the solution was adjusted to pH 2 with concentrated hydrochloric acid. The mixture was allowed to stand overnight at room temperature. The pale brown solids were collected by filtration and washed with water (2 x 50 mL) and dried in a desiccator in vacuo. 17.3 g (50.3%) of 68 were obtained as off-white solid, m.p. 206-207 °C.

⁻¹H NMR (400 MHz, DMSO-đ₆) δ 13.93 (s, H-10, 1H), 9.38 (s, H-3, 1H), 8.17 (m, H-8, 1H), 8.11 (m, H-5, 1H), 7.94 (m, H-6, 1H), 7.91 (m, H-7, 1H).

⁻¹C NMR (100 MHz, DMSO-đ₆) δ 165.2 (C-9), 145.1 (C-3), 143.6 (C-2), 142.7 (C-4a), 140.7 (C-8a), 132.3 (C-6), 131.2 (C-7), 130.0 (C-8), 128.9 (C-5).

⁻¹N NMR (40 MHz, DMSO-đ₆) δ -48.3 (N-4), -48.8 (N-1).
6. 2. 71  \( \text{N,N-Diethylquinoxaline-2-carboxamide (69)} \)

\[
\begin{align*}
\text{OH} \quad &\xrightarrow{1. \text{ C}_2\text{O}_2\text{Cl}_2/\text{CH}_2\text{Cl}_2/\text{DMF}}\quad \text{N} \\
\text{O} \quad &\xrightarrow{2. \text{ Diethylamine}}\quad \text{N} \\
\end{align*}
\]

To a solution of 68 (15.0 g, 86.1 mmol) in dry \( \text{CH}_2\text{Cl}_2 \) (225 mL) were added slowly oxaly chloride (8.1 mL, 94.7 mmol) and 3 drops of dry DMF. The mixture was stirred at room temperature until the formed \( \text{CO}_2 \) was completely gone (approximately 1 h). The mixture was ice-cooled, then diethylamine (27 mL, 258.4 mmol) was added slowly. The mixture was allowed to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated \( \text{Na}_2\text{CO}_3 \) solution (100 mL). The organic layer was collected and the aqueous layer was extracted with \( \text{CH}_2\text{Cl}_2 \). The combined organic layers were washed with water, dried over \( \text{Na}_2\text{SO}_4 \) and the solvent was removed under reduced pressure. The residue was purified by column chromatography using ethyl acetate as eluent to give 18.5 g (94.0 %) of 69 as red-brown oil.

\(^1\text{H} \text{ NMR (400 MHz, CDCl}_3\text{)} \) \( \delta \) 9.13 (s, H-3, 1H), 8.13 (m, H-5, 1H), 8.07 (m, H-8, 1H), 7.81 (m, H-7, 1H), 7.79 (m, H-6, 1H), 3.62 (q, \( J = 7.1 \text{ Hz, H-11} \)), 3.50 (q, \( J = 7.1 \text{ Hz, H-11'} \)), 1.31 (t, \( J = 7.1 \text{ Hz, H-12}) \), 1.27 (t, \( J = 7.1 \text{ Hz, H-12'}) \).

\(^{13}\text{C} \text{ NMR (100 MHz, CDCl}_3\text{)} \) \( \delta \) 166.3 (9), 148.9 (2), 144.8 (3), 142.1 (4a), 140.3 (8a), 130.8 (6), 130.5 (7), 129.7 (8), 129.2 (5), 43.5 (11), 40.7 (11’), 14.5 (12), 12.8 (12’).

\(^{15}\text{N} \text{ NMR (40 MHz, CDCl}_3\text{)} \) \( \delta \) -52.3 (N-4), -60.0 (N-1), -249.5 (N-10).
**EXPERIMENTAL PART**

6.2.72 5-(Methoxymethyl)-5H-indolo[2,3-b]phenazine-6,13-dione (70)

![Chemical Structure](image)

A lithium 2,2,6,6-tetramethylpiperidine solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of 8 (1.06 g, 5.61 mmol, 1 eq.) and 69 (1.29 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish a dark residue. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) followed by recrystallisation from ethyl acetate to give 145 mg (10.9 %) of 70 as yellow flakes, m.p. 265-267 °C.


**NMR Spectra**

**H NMR (400 MHz, CDCl₃)** δ 8.60 (m, H-1, 1H), 8.47 (m, H-8*/11*, 1H), 8.43 (m, H-8*/11*, 1H), 7.98 (m, H-9/10, 2H), 7.69 (m, H-4, 1H), 7.56 (m, H-3, 1H), 7.48 (m, H-2, 1H), 6.27 (s, H-14, 2H), 3.45 (s, H-15, 3H).

**C NMR (100 MHz, CDCl₃)** δ 178.0 (C-13), 175.5 (C-6), 144.8 (C-6a*/12a*), 144.7 (C-6a*/12a*), 143.3 (C-7a*/11a*), 143.0 (C-7a*/11a*), 140.5 (C-4a), 136.1 (C-5a), 133.4 (C-9*/10*), 133.1 (C-9*/10*), 131.1 (C-8*/11*), 131.0 (C-8*/11*), 129.3 (C-3), 125.8 (C-2), 124.6 (C-1), 124.1 (C-13b), 122.5 (C-13a), 112.3 (C-4), 75.7 (C-14), 56.7 (C-15).

**N NMR (40 MHz, CDCl₃)** δ -50.2 (N-7/12), not found (N-5).
6. 2. 73  5H-Indolo[2,3-b]phenazine-6,13-dione (71)

To an ice-cold solution of 70 (145 mg, 0.42 mmol) in dry CH₂Cl₂ (15 mL) a solution of BBr₃ (1M in CH₂Cl₂, 0.56 mL) was added slowly. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated NaHCO₃ solution (15 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH₂Cl₂. The filter cake was purified by recrystallisation from ethyl acetate to afford 52 mg (41.3 %) of 71 as yellow crystals, m.p. >350 °C.

**HRMS**: m/z calculated for C₁₈H₉N₃NaO₂ ([M+Na]⁺): 322.0587; found: 322.0589.

**¹H NMR (400 MHz, DMSO-ｄ₆)**  δ 13.39 (s, H-5, 1H), 8.35 (m, H-8/11, 2H), 8.30 (m, H-1, 1H), 8.06 (m, H-9/10, 2H), 7.63 (m, H-4, 1H), 7.51 (m, H-3, 1H), 7.42 (m, H-2, 1H).

**¹³C NMR (100 MHz, DMSO-ｄ₆)**  δ 177.3 (C-13), 174.7 (C-6), 146.3 (C-6a*/12a*), 145.5 (C-6a*/12a*), 141.9 (C-7a*/11a*), 141.7 (C-7a*/11a*), 138.9 (C-5a), 138.5 (C-4a), 133.1 (C-9*/10*), 132.9 (C-9*/10*), 130.31 (C-8*/11*), 130.28 (C-8*/11*), 127.9 (C-3), 124.4 (C-2), 124.0 (C-13b), 122.8 (C-1), 119.6 (C-13a), 114.0 (C-4).

**¹⁵N NMR (40 MHz, DMSO-ｄ₆)**  δ not found (N-5/7/12).
6. 2. 74  5-[2-(Dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (72)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of 27 (1.21 g, 5.61 mmol, 1 eq.) and 69 (1.29 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) followed by recrystallisation from ethyl acetate to afford 152 mg (7.3 %) of 72 as yellow crystals, m.p. 248-250 °C.

HRMS: m/z calculated for C_{22}H_{19}N_{4}O_{2} ([M+H]^+): 371.1503; found: 371.1502.

HRMS: m/z calculated for C_{22}H_{18}N_{4}NaO_{2} ([M+Na]^+): 393.1322; found: 393.1323.

\(^{1}\)H NMR (400 MHz, CDCl₃) δ 8.43 (m, H-1, 1H), 8.37 (m, H-8*/11*, 1H), 8.36 (m, H-8*/11*, 1H), 7.91 (m, H-9/10, 2H), 7.42 (m, H-4, 1H), 7.36 (m, H-3, 1H), 7.30 (m, H-2, 1H), 4.85 (m, H-14, 2H), 2.78 (m, H-15, 2H), 2.38 (s, H-17/17', 6H).

\(^{13}\)C NMR (100 MHz, CDCl₃) δ 177.2 (C-13), 175.0 (C-6), 144.9 (C-6a*/12a*), 144.6 (C-6a*/12a*), 143.1 (C-7a*/11a*), 142.8 (C-7a*/11a*), 140.0 (C-4a), 135.7 (C-5a), 133.1 (C-9*/10*), 132.8 (C-9*/10*), 130.9 (C-8*/11*), 130.8 (C-8*/11*), 128.6 (C-3), 125.2 (C-2), 124.5 (C-1), 123.9 (C-13b), 121.2 (C-13a), 111.0 (C-4), 58.3 (C-15), 45.7 (C-17/17'), 43.6 (C-14).

\(^{15}\)N NMR (40 MHz, CDCl₃) δ -50.2 (N-7/12), -235.8 (N-5), -358.8 (N-16).
6. 2. 75 **N,N-Diethylquinoline-2-carboxamide (73)**

To a solution of quinoline-2-carboxylic acid (10.00 g, 57.8 mmol) in dry CH$_2$Cl$_2$ (150 mL) were slowly added oxalyl chloride (6.0 mL, 69.8 mmol) and 3 drops of dry DMF. The mixture was continuously stirred at room temperature until the formed CO$_2$ was completely gone (approximately 1 h). The mixture was ice-cooled, then diethylamine (15.0 mL, 0.14 mol) was added slowly. The mixture was allowed to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated Na$_2$CO$_3$ solution (100 mL). The organic layer was collected and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were washed with water, dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography eluting with ethyl acetate/light petroleum (8:2, v/v) to afford 12.50 g (95.4 %) of 73 as yellow oil.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.23 (d, $J = 8.5$, H-4, 1H), 8.09 (m, H-8, 1H), 7.83 (m, H-5, 1H), 7.73 (m, H-7, 1H), 7.66 (d, $J = 8.5$ Hz, H-3, 1H), 7.57 (m, H-6, 1H), 3.62 (q, $J = 7.1$ Hz, H-11', 2H), 3.44 (q, $J = 7.1$ Hz, H-11, 2H), 1.31 (t, $J = 7.1$ Hz, H-12', 3H), 1.22 (t, $J = 7.1$ Hz, H-12, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.7 (C-9), 154.7 (C-2), 146.6 (C-8a), 136.9 (C-4), 129.8 (C-7), 129.7 (C-8), 127.6 (C-5), 127.2 (C-6), 120.3 (C-3), 43.3 (C-11), 40.3 (C-11'), 14.4 (C-12), 12.9 (C-12').

$^{15}$N NMR (40 MHz, CDCl$_3$) $\delta$ -77.0 (N-1), -251.6 (N-10).
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.6 mL, 21.3 mmol) dropwise (over less than 1 min) to a cold (-70 °C) solution of n-BuLi (2.5 M in hexanes, 8.5 mL, 21.3 mmol) in dry THF (60 mL) under argon. Then the mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to -78 °C and a solution of 73 (1.20 g, 5.26 mmol) in dry THF (30 mL) was added dropwise over 10 min. The mixture was stirred at -78 °C for further 20 min and then a solution of aldehyde 8 (0.40 g, 2.11 mmol) in dry THF (18 mL) was added over 5 min. The flask was then taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL) and extracted with CH₂Cl₂ (3 x 70 mL). The combined organic layers were dried over Na₂SO₄ and the solvent was removed under reduced pressure to give a yellowish residue which was purified by column chromatography eluting with ethyl acetate followed by recrystallisation from ethyl acetate. 0.37 g (21.0 %) of 74 were obtained as yellow flakes, m.p. 285-287 °C.

HRMS: m/z calculated for C₂₁H₁₄N₂O₃ ([M+H]⁺): 343.1077; found: 343.1080.

To a cold solution of 74 (410 mg, 1.20 mmol) in dry CH$_2$Cl$_2$ (120 mL) at 0 °C a solution of BBr$_3$ (1M in CH$_2$Cl$_2$, 1.50 mL) was added slowly. The solution was allowed to reach room temperature and continued stirring for 1 h. The mixture was quenched with saturated NaHCO$_3$ solution (100 mL) and vigorously stirred for 1 h at 60 °C. The cooled mixture was filtrated, the filter cake was washed with water and cold CH$_2$Cl$_2$. Recrystallisation from ethyl acetate afforded 350 mg (98 %) of 75 as yellow crystals, m.p. >350 °C.

**HRMS:** m/z calculated for C$_{19}$H$_{10}$N$_2$NaO$_2$ ([M+Na]$^+$): 321.0634; found: 321.0635.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.28 (br s, H-7, 1H), 9.11 (s, H-13, 1H), 8.33 (m, H-1, 1H), 8.28 (m, H-4, 1H), 8.27 (m, H-11, 1H), 7.98 (m, H-3, 1H), 7.81 (m, H-2, 1H), 7.61 (m, H-8, 1H), 7.48 (m, H-9, 1H), 7.39 (m, H-10, 1H).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 179.0 (C-12), 175.5 (C-6), 148.6 (C-5a), 148.2 (C-4a), 139.0 (C-6a), 138.6 (C-7a), 135.8 (C-13), 132.6 (C-3), 130.3 (C-4), 129.9 (C-1), 129.5 (C-2), 128.5 (C-12a), 128.3 (C-13a), 127.5 (C-9), 124.2 (C-10), 123.9 (C-11a), 122.7 (C-11), 118.3 (C-11b), 113.9 (C-8).

$^{15}$N NMR (40 MHz, DMSO-$d_6$) δ not found (N-5/7).
6.2.78 7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (76)

A solution of 75 (300 mg, 1.01 mmol) in dry DMF (20 mL) was added dropwise to a cooled (-5 to -10 °C) suspension of NaH (140 mg, 3.50 mmol, 60 % in paraffin) in dry DMF (10 mL). After 30 min of stirring 2-chloro-N,N-dimethylethanamine hydrochloride (290 mg, 2.02 mmol) in DMF (15 mL) was added slowly and the reaction mixture was stirred at 75 °C for 5 h. After quenching with water (50 mL) the mixture was extracted with CH₂Cl₂ and the combined organic layers were dried over Na₂SO₄. The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with CH₂Cl₂/methanol (95:5, v/v) affording compound 76 (100 mg, 26.9 %) as yellow flakes, m.p. 240-242 °C.

HRMS: m/z calculated for C₂₃H₂₀N₃O₂ ([M+H]⁺): 370.1550; found: 370.1548.
HRMS: m/z calculated for C₂₃H₁₉N₃NaO₂ ([M+Na]⁺): 392.1369; found: 392.1369.

¹H NMR (500 MHz, CDCl₃) δ 8.99 (s, H-13, 1H), 8.46 (m, H-11, 1H), 8.41 (m, H-4, 1H), 8.03 (m, H-1, 1H), 7.87 (m, H-3, 1H), 7.70 (m, H-2, 1H), 7.52 (m, H-8, 1H), 7.49 (m, H-9, 1H), 7.38 (m, H-10, 1H), 4.90 (m, H-1, 1H), 2.83 (m, H-15, 2H), 2.44 (s, H-17/17', 6H).

¹³C NMR (125 MHz, CDCl₃) δ 179.3 (C-12), 176.4 (C-6), 149.2 (C-4a), 148.6 (C-5a), 139.9 (C-7a), 136.1 (C-13), 136.0 (C-6a), 132.4 (C-3), 131.4 (C-4), 129.5 (C-2), 129.4 (C-1), 128.8 (C-13a), 128.2 (C-9), 127.8 (C-12a), 124.9 (C-10), 124.2 (C-11), 123.8 (C-11a), 120.1 (C-11b), 111.1 (C-8), 58.4 (C-15), 45.7 (C-17, 17'), 43.5 (C-14).

¹⁵N NMR (50 MHz, CDCl₃) δ -69.3 (N-5) -236.9 (N-7), -358.3 (N-16).
6. 2. 79  \(N,N\)-Diethylquinoline-3-carboxamide (77)

To a solution of quinoline-3-carboxylic acid (1.0 g, 5.78 mmol) in dry \(\text{CH}_2\text{Cl}_2\) (30 mL) were slowly added oxalyl chloride (0.65 mL, 6.93 mmol) and 3 drops of dry DMF. The mixture was continuously stirred at room temperature until the formed \(\text{CO}_2\) was completely gone (approximately 1 h). The mixture was ice-cooled, then diethylamine (5.0 mL, 47.9 mmol) was added slowly. The mixture was allowed to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated \(\text{Na}_2\text{CO}_3\) solution (50 mL). The organic layer was collected and the aqueous layer was extracted with \(\text{CH}_2\text{Cl}_2\). The combined organic layers were washed with water, dried over \(\text{Na}_2\text{SO}_4\) and the solvent was removed under reduced pressure. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (8:2, v/v) to obtain 1.05 g (85.0 %) of 77 as yellow oil.

\(1^H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.92 (d, \(J = 2.1\) Hz, H-2, 1H), 8.20 (d, \(J = 2.1\), H-4, 1H), 8.13 (d, \(J = 8.4\) Hz, H-8, 1H), 7.84 (m, H-5, 1H), 7.76 (m, H-7, 1H), 7.59 (m, H-6, 1H), 3.60 (br s, H-11, 2H), 3.32 (br s, H-11', 2H), 1.28 (br s, H-12, 3H), 1.16 (br s, H-12', 3H).

\(13^C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\) 168.6 (C-9), 147.8 (C-2), 134.2 (C-4), 130.5 (C-7), 130.1 (C-8a), 129.2 (C-8), 128.1 (C-5), 127.4 (C-6), 127.1 (C-4a), 43.5 (C-11'), 39.7 (C-11), 14.3 (C-12'), 12.9 (C-12).

\(15^N\) NMR (40 MHz, CDCl\(_3\)) \(\delta\) -74.4 (N-1), not found (N-10).
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.2 mL, 17.89 mmol) dropwise (over less than 1 min) to a cold (-70 °C) solution of n-BuLi (2.5 M in hexane, 7.2 mL, 17.89 mmol) in dry THF (65 mL), under argon, then allowing the mixture to –5 °C to 0 °C over 30 min and further stirring at this temperature for 30 min. The mixture was then recooled to –78 °C and a solution of 77 (1.02 g, 4.47 mmol) and 8 (350 mg, 1.85 mmol) in dry THF (30 mL) was added dropwise over 10 min. After stirring at this temperature for 40 min the flask was then taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure to furnish a brown residue which was purified by column chromatography eluting with ethyl acetate/light petroleum (4:6, v/v) followed by recrystallisation from ethyl acetate to afford 304 mg (47.9 %) of 78 as orange crystals, m.p. 228 °C.

HRMS: m/z calculated for C$_{21}$H$_{14}$N$_2$O$_3$ ([M+H]$^+$): 343.1077; found: 343.1078.
HRMS: m/z calculated for C$_{21}$H$_{14}$N$_2$NaO$_3$ ([M+Na]$^+$): 365.0897; found: 365.0896.

$^1$H NMR (500 MHz, CDCl$_3$) δ 9.67 (m, H-1, 1H), 9.64 (s, H-6, 1H), 8.40 (m, H-12, 1H), 8.12 (m, H-4, 1H), 7.82 (m, H-3, 1H), 7.73 (m, H-2, 1H), 7.57 (m, H-9, 1H), 7.45 (m, H-10, 1H), 7.40 (m, H-11, 1H), 6.08 (s, H-14, 2H), 3.40 (s, H-15, 3H).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 184.6 (C-13), 178.8 (C-7), 152.4 (C-4a), 147.4 (C-6), 139.8 (C-8a), 133.6 (C-13a), 131.1 (C-7a), 131.8 (C-3), 130.1 (C-2), 130.0 (C-4), 128.3 (C-1), 128.1 (C-10), 125.3 (C-11), 124.4 (C-6a), 124.0 (C-12a), 123.7 (C-12), 123.2 (C-13b), 120.9 (C-12b), 112.0 (C-9), 75.4 (C-14), 56.6 (C-15).

$^{15}$N NMR (50 MHz, CDCl$_3$) δ -57.0 (N-5), not found (N-8).
To an ice-cold solution of 78 (124 mg, 0.36 mmol) in dry CH$_2$Cl$_2$ (25 mL) a solution of BBr$_3$ (1M in CH$_2$Cl$_2$, 0.43 mL, 0.43 mmol) was added dropwise. The solution was allowed to reach room temperature and continued stirring for a total of 1 h. The mixture was quenched with saturated NaHCO$_3$ solution (25 mL) and vigorously stirred for 1 h at 60 °C. The cooled mixture was filtrated, the filter cake was washed with water and cold CH$_2$Cl$_2$. Recrystallisation from acetone afforded 75.6 mg (70.0 %) of 79 as orange crystals, m.p. >350 °C.

**HRMS:** m/z calculated for C$_{19}$H$_{11}$N$_3$O$_2$ ([M+H]$^+$): 299.0815; found: 299.0815.

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.09 (s, H-8, 1H), 9.60 (m, H-1, 1H), 9.45 (s, H-6, 1H), 8.15 (m, H-12, 1H), 8.06 (m, H-4, 1H), 7.88 (m, H-3, 1H), 7.78 (m, H-2, 1H), 7.52 (m, H-9, 1H), 7.39 (m, H-10, 1H), 7.32 (m, H-11, 1H).

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 183.8 (C-13), 177.6 (C-7), 151.7 (C-4a), 146.8 (C-6), 138.3 (C-8a), 135.3 (C-7a), 133.9 (C-13a), 131.7 (C-3), 129.8 (C-2), 129.7 (C-4), 127.8 (C-1), 127.1 (C-10), 124.2 (C-11), 124.0 (C-12a), 123.96 (C-6a), 123.0 (C-13b), 122.3 (C-12), 118.5 (C-12b), 113.9 (C-9).

$^{15}$N NMR (40 MHz, DMSO-$d_6$) $\delta$ -53.9 (N-5), -242.4 (N-8).
6.2.82  8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (80)

A solution of 79 (800 mg, 2.68 mmol) in dry DMF (50 mL) was added dropwise to a cooled (-10 to 0 °C) suspension of NaH (430 mg, 10.75 mmol, 60 % in paraffin) in dry DMF (20 mL). After 30 min of stirring 2-chloro-\(\text{N},\text{N}\)-dimethylethanamine hydrochloride (580 mg, 4.03 mmol) in DMF (20 mL) was added slowly and the reaction mixture was stirred at 75 °C for 5 h. After quenching with water (150 mL) the mixture was extracted with ethyl acetate and the combined organic layers were dried over Na\(_2\)SO\(_4\). The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with ethyl acetate/methanol (95:5, v/v) to ethyl acetate/methanol/triethylamine (93:5:2, v/v) affording compound 80 (320 mg, 32.3 %) as red crystals, m.p. 168-172 °C.

HRMS: m/z calculated for C\(_{23}\)H\(_{20}\)N\(_3\)O\(_2\) ([M+H]+): 370.1550; found: 370.1555.
HRMS: m/z calculated for C\(_{23}\)H\(_{19}\)N\(_3\)NaO\(_2\) ([M+Na]+): 392.1369; found: 392.1374.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 9.68 (m, H-1, 1H), 9.61 (s, H-6, 1H), 8.36 (m, H-12, 1H), 8.10 (m, H-4, 1H), 7.80 (m, H-3, 1H), 7.70 (m, H-2, 1H), 7.40 (m, H-9/10, 2H), 7.34 (m, H-11, 1H), 4.74 (m, H-14, 2H), 2.75 (m, H-15, 2H), 2.39 (s, H-17/17’, 6H).

\(^1\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 184.1 (C-13), 178.5 (C-7), 152.3 (C-4a), 147.4 (C-6), 139.5 (C-8a), 133.9 (C-13a), 132.9 (C-7a), 131.7 (C-3), 130.0 (C-4), 129.8 (C-2), 128.4 (C-1), 127.6 (C-10), 124.9 (C-11), 124.4 (C-6a), 124.1 (C-12a), 123.7 (C-12), 123.3 (C-13b), 119.8 (C-12b), 111.0 (C-9), 58.4 (C-15), 45.8 (C-17/17’), 43.4 (C-14).

\(^{15}\)N NMR (50 MHz, CDCl\(_3\)) \(\delta\) -58.1 (N-5), -239.6 (N-8), -358.6 (N-16).
EXPERIMENTAL PART

6.2.83  6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (81)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.15 mL, 18.48 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (-70 °C) solution of n-BuLi (2.5M in hexanes, 7.39 mL, 18.48 mmol, 4 eq.) in dry THF (60 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to -78 °C and a solution of 47 (1.33 g, 4.62 mmol, 1 eq.) in dry THF (20 mL) was added dropwise over 10 min. After stirring the mixture at -75 °C for 30 min a solution of pyridazine-4-carbaldehyde (500 mg, 4.62 mmol, 1 eq.) in dry THF (25 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na₂SO₄ and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) and recrystallisation from ethyl acetate to afford 90 mg (6.1 %) of 81 as red needles, m.p. 204-206 °C.

HRMS: m/z calculated for C₁₈H₁₆N₄NaO₂ ([M+Na]+): 343.1165; found: 343.1166.

¹H NMR (400 MHz, CDCl₃) δ 9.84 (d, J = 1.2 Hz, H-1, 1H), 9.80 (d, J = 1.2 Hz, H-4, 1H), 8.34 (m, H-10, 1H), 7.49 (m, H-7/8, 2H), 7.40 (m, H-9, 1H), 4.79 (m, H-12, 2H), 2.73 (m, H-13, 2H), 2.35 (s, H-15/15', 6H).

¹³C NMR (100 MHz, CDCl₃) δ 179.1 (C-11), 177.1 (C-5), 162.6 (C-1), 146.0 (C-4), 139.7 (C-6a), 133.6 (C-5a), 128.7 (C-8), 125.8 (C-9), 125.6 (C-4a*/11a*), 123.9 (C-10), 123.4 (C-10a), 118.8 (C-10b), 111.4 (C-7), 58.6 (C-13), 45.8 (C-15/15'), 43.8 (C-12).

¹⁵N NMR (40 MHz, CDCl₃) δ 40.2 (N-2), 37.2 (N-3), -235.6 (N-6), -359.4 (N-14).
6.2.84  6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (82)

A lithium 2,2,6,6-tetramethylpiperidine solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.15 mL, 18.48 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of n-BuLi (2.5M in hexanes, 7.39 mL, 18.48 mmol, 4 eq.) in dry THF (60 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of 46 (1.20 g, 4.62 mmol, 1 eq.) in dry THF (40 mL) was added dropwise over 10 min. After stirring the mixture at -75 °C for 30 min a solution of pyridazine-4-carbaldehyde (500 mg, 4.62 mmol, 1 eq.) in dry THF (25 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na₂SO₄ and the solvent was evaporated to furnish a brown residue which was purified by column chromatography eluting with ethyl acetate, followed by ethyl acetate/methanol (9:1, v/v) and recrystallisation from ethyl acetate to afford 55 mg (4.1 %) of 82 as red needles, m.p. 237-239 °C.

HRMS: m/z calculated for C₁₆H₁₂N₃O₃ ([M+H]⁺): 294.0873; found: 294.0874.
HRMS: m/z calculated for C₁₆H₁₁N₃NaO₃ ([M+Na]⁺): 316.0693; found: 316.0695.

1H NMR (400 MHz, CDCl₃) δ 9.91 (d, J = 1.2 Hz, H-1, 1H), 9.86 (d, J = 1.2 Hz, H-4, 1H), 8.42 (m, H-10, 1H), 7.68 (m, H-7, 1H), 7.57 (m, H-8, 1H), 7.48 (m, H-9, 1H), 6.13 (s, H-12, 2H), 3.40 (s, H-13, 3H).

13C NMR (100 MHz, CDCl₃) δ 179.8 (C-11), 177.5 (C-5), 146.5 (C-1), 146.1 (C-4), 140.1 (C-6a), 133.7 (C-5a), 129.3 (C-8), 126.1 (C-9), 125.8 (C-4a*/11a*), 125.2 (C-4a*/11a*), 123.9 (C-10), 123.4 (C-10a), 120.0 (C-10b), 112.3 (C-7), 75.6 (C-12), 56.8 (C-13).

15N NMR (40 MHz, CDCl₃) δ 41.5 (N-2), 38.9 (N-3).
6. 2. 85  
**5H-Pyridazino[4,5-b]carbazole-5,11(6H)-dione (83)**

To an ice-cold solution of 82 (14 mg, 0.048 mmol) in dry CH$_2$Cl$_2$ (5 mL) a solution of BBr$_3$ (1M in CH$_2$Cl$_2$, 57 µL) was added slowly. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated NaHCO$_3$ solution (5 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH$_2$Cl$_2$. The filter residue was purified by column chromatography eluting with ethyl acetate to afford 4 mg (33.6 %) of 83 as orange crystals, m.p. >350 °C.

**HRMS:** m/z calculated for C$_{14}$H$_7$N$_3$NaO$_2$ ([M+Na]$^+$): 272.0430; found: 272.0434.

**$^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.41 (s, 1H), 9.80 (d, $J$ = 1.2 Hz, H-1, 1H), 9.77 (d, $J$ = 1.2 Hz, H-4, 1H), 8.20 (m, H-10, 1H), 7.63 (m, H-7, 1H), 7.51 (m, H-8, 1H), 7.43 (m, H-9, 1H).

**$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 179.5 (C-11), 176.9 (C-5), 146.5 (C-1), 145.7 (C-4), 138.3 (C-6a), 136.6 (C-5a), 127.8 (C-8), 126.4 (C-4a*/11a*), 126.1 (C-4a*/11a*), 124.8 (C-9), 123.4 (C-10a), 122.3 (C-10), 117.3 (C-10b), 114.1 (C-7).

**$^{15}$N NMR (40 MHz, DMSO-$d_6$) not measured.
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REFERENCES

REFERENCES

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### ABBREVIATIONS

8 LIST OF ABBREVIATIONS AND PHYSICAL UNITS

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Å</td>
<td>ångström (a unit of length, 0.1 nm)</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>APCI</td>
<td>atmospheric pressure chemical ionisation</td>
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<td>APT</td>
<td>attached proton test</td>
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<td>aqua bidest.</td>
<td>double-distilled water</td>
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<td>ATCC</td>
<td>American Type Culture Collection</td>
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<td>ATP</td>
<td>adenosine triphosphate</td>
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<td>body mass index</td>
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<td>EGF</td>
<td>epidermal growth factor</td>
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<td>EMA</td>
<td>European Medicines Agency</td>
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<td>eq.</td>
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<td>FGF</td>
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<td>HMBC</td>
<td>heteronuclear multiple bond correlation</td>
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<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
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<td>HRESIMS</td>
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<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
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<td>IGF</td>
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<td>J</td>
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<td>LDA</td>
<td>lithium diisopropylamide</td>
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<td>LHMDS</td>
<td>lithium hexamethyldisilazide (lithium bis(trimethylsilyl)amide)</td>
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<td>reactive oxygen species</td>
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ABBREVIATIONS

rpm  revolutions per minute
RPMI  Roswell Park Memorial Institute
RT  room temperature
s  singlet
sec-BuLi  sec-Butyllithium
SFM  serum free medium
t  triplet
TBAHS  tetrabutylammonium hydrogensulfate
TEA  triethylamine
tert-BuLi  tert-Butyllithium
TGF  transforming growth factor
THF  tetrahydrofuran
TLC  thin layer chromatography
TMP  2,2,6,6-tetramethylpiperidine
TMS  tetramethylsilane
TNF  tumor necrosis factor
TOP  topoisomerase
TRIS  2-amino-2-hydroxymethyl-propane-1,3-diol
v/v  volume to volume ratio
VEGF  vascular endothelial growth factor
WHO  World Health Organisation
XTT  2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide
°C  degree centigrade
δ  chemical shift
9 SPECTROSCOPIC INFORMATION
Furo[3,4-b]pyridine-5,7-dione (PROTON)

3.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.1

Furo[3,4-b]pyridine-5,7-dione (PROTON)

SPECTROSCOPIC INFORMATION

Furil[3,4-b]pyridine-5,7-dione (C13 CF1)

ppm

163 162 161 160 159 158 157 156 155 154 153 152 151 150 149 148 147 146 145 144 143 142 141 140 139 138 137 136 135 134 133 132 131 130 129 128 127 126 125

ppm

0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200
SPECTROSCOPIC INFORMATION

Pure[5,4-b]pyridin-3(2H)-one (PROTON)

Pure[3,4-b]pyridin-3(2H)-one (PROTON)
SPECTROSCOPIC INFORMATION

1-(Phenylsulfonyl)-3H-indole (C13 CPD)

1H NMR (400 MHz, CDCl3) δ 7.90 (s, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.39 - 7.32 (m, 1H), 7.27 (t, J = 7.7 Hz, 1H), 7.23 - 7.17 (m, 2H), 7.11 (t, J = 7.7 Hz, 1H), 6.96 - 6.89 (m, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 8.0 Hz, 1H), 5.96 (s, 2H), 4.60 (s, 2H), 3.80 (s, 3H), 2.83 (s, 3H).
1-(Phenylsulfonyl)-1H-indole (HSQC)

1-(Phenylsulfonyl)-1H-indole (HMBC)
[3-(3-Hydroxymethyl)pyridin-3-yl][1-phenylsulfanyl]-1H-indol-2-yl]methanone (PROTON)
SPECTROSCOPIC INFORMATION

[2-[(3-methyl[1,2]imidazolo[5,4-b]pyridin-3-yl][1-phenylethoxyl]-1H-indol-2-yl]methanone (PROTON)]

[2-[(3-methyl[1,2]imidazolo[5,4-b]pyridin-3-yl][1-phenylethoxyl]-1H-indol-2-yl]methanone (PROTON)]
SPECTROSCOPIC INFORMATION

[2-[(4-Hydroxyphenyl)methyl]pyridin-3-yl][1-(phenylsulfonyl)-1H-indol-2-yl]methanone (HSQC)

[2-[(4-Hydroxyphenyl)methyl]pyridin-3-yl][1-(phenylsulfonyl)-1H-indol-2-yl]methanone (HMBC)
[2-(Hydroxymethyl)pyridin-3-yl]-1H-indol-2-yl]methaneone (COSY)

[2-(Hydroxymethyl)pyridin-3-yl]-1H-indol-2-yl]methaneone (NOESY)
**Generic Display Report**

**Analysis Info**

- **Analysis Name**: D:\Data\MZ_data\Spruetz\HNijhuis\140117\TN093_DI_ESI_HRMS.d
- **Method**: DL_mz_50-1550.m
- **Sample Name**: 0.1 mg/mL in MeOH
- **Comment**:

**Intens. x10^5**

- **393.0909**
- **394.0937**
- **395.0904**
- **413.2663**
- **415.0727**
- **416.0757**
- **417.0725**
- **418.0739**

**MZMethod DI_mz_50-1550.m Operator**

- **Instrument**: maXis HD

---

**Bruker Compass DataAnalysis 4.2**


Page 1 of 1
### Mass Spectrum SmartFormula Report

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### Diagram

![Chemical Structure](image-url)
SPECTROSCOPIC INFORMATION

$\text{Pyridine(3,2,1-a):resin}
\text{e(3,2-b)indol-5(12H)-one (HMBG)}$

$\text{Pyridine(3,2,1-a):resin}
\text{e(3,2-b)indol-5(12H)-one (15N HMBG)}$
6H-Pyrrole[3,2,1-f,g]naptho[2,3-b]indole-5(12H)-one (15N HSOQ)
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spritzer_H\Thomas_N\141117\TN140_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment: m/z 50-1550 m/z

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Set Nebulizer: 0.4 Bar
Scan Begin: 50 m/z
Set End Plate Offset: -500 V
Scan End: 1550 m/z
Set Dry Gas: 4.0 l/min
Set Dry Heater: 200 °C
Set Corona: 0 nA
Set APCI Heater: 0 °C

Intensity

Meas. m/z  # Ion Formula  m/z err [ppm]  mSigma  # mSigma  Score  rdb  e− Conf  N-Rule
251.0811 1 C15H11N2O2 251.0815 1.6  n.a.  1 100.00 11.5 even ok
273.0632 1 C15H10N2NaO2 273.0634 0.9  7.4  1 100.00 11.5 even ok

TN140_DI_ESI_HRMS.d
by: MZ
Page 1 of 1

Brother Compass DataAnalysis 4.2
11/17/2014 2:57:24 PM
1820881.21300

SPECTROSCOPIC INFORMATION
SPECTROSCOPIC INFORMATION

1H-Pyrido[3,2-b][carbazole-5,11(10H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

SIH-Pyrrole[2,3-b]carbazole-5,11(1H)-dione (HMQC)

SIH-Pyrrole[2,3-b]carbazole-5,11(1H)-dione (OS HMQC)
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN036_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: Instrument: maXis HD 1820881.21300

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Scan Begin: 50 m/z
Scan End: 1550 m/z
Focus: Active
Scan End Plate Offset: -500 V
Set Coronal: 0 nA
Set Charging Voltage: 2000 V
Set APCI Heater: 0 °C
Set Dry Gas: 4.0 l/min
Set Dry Heater: 200 °C
Set Nebulizer: 0.4 Bar
Set Divert Valve: Waste

Meas. m/z  # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e- Conf N-Rule
249.0860  1 C15H9N2O2 249.0859  -0.8 11.2 1 100.00 12.5 even ok
271.0481  1 C15H8N2NaO2 271.0478  -1.3 6.2 1 100.00 12.5 even ok

TN036_DI_ESI_HRMS.d
Braker Compass DataAnalysis 4.2 printed: 11/10/2014 11:06:55 AM by: MZ
6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

8-(2-(Dimethylamino)ethyl)-5H-pyrido[1,2-b]carbazole-5,11(6H)-dione (PROTON)

6-(2-(Dimethylamino)ethyl)-5H-pyrido[1,2-b]carbazole-5,11(6H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

6-{2-(Dimethylamino)ethyl}-5H-pyrido[2,3-b]carbazole-5,11(6H)-dione (HMQC)

6-{2-(Dimethylamino)ethyl}-5H-pyrido[2,3-b]carbazole-5,11(6H)-dione (COSY)
SPECTROSCOPIC INFORMATION

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b][carbazole]-5,11-(6H)-diene (1H NMR)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b][carbazole]-5,11-(6H)-diene (COSY)
8-(2-(Dimethylamino)ethyl)-5H-pyrido[3,2-b]carbazole-5,11(1H)-dione (NOESY)
Generic Display Report

Analysis Info

- **Analysis Name**: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN050_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 0.01 mg/mL in MeOH
- **Comment**:

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**MZMethod**: DI_mz_50-1550.m
**Operator**: MZ
**Instrument**: maXis HD

**Acquisition Date**: 11/10/2014 9:57:42 AM
**Instrument**: maXis HD

**Comment**:

- C₁₉H₁₈N₃O₂, m/z 320.1394
- C₁₉H₁₇N₃NaO₂, m/z 342.1213

Bruker Compass DataAnalysis 4.2  printed: 11/10/2014 2:06:36 PM  by: MZ  Page 1 of 1
# Mass Spectrum SmartFormula Report

**Analysis Info**

- **Analysis Name**: D:\Data\MZ_data\Spreitzer_HiThomas_N\141110\TN050_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 0.01 mg/mL in MeOH
- **Comment**:

**Acquisition Parameter**

- **Source Type**: ESI
- **Ion Polarity**: Positive
- **Set Nebulizer**: 0.4 Bar
- **Set Capillary**: 2200 V
- **Set End Plate Offset**: -500 V
- **Set Dry Gas**: 4.0 l/min
- **Set Charging Voltage**: 2000 V
- **Set Divert Valve**: Waste
- **Set APCI Heater**: 0 °C
- **Set Dry Heater**: 200 °C

**Mass Spectrum**

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**TN050_DI_ESI_HRMS.d**

Brother Compass DataAnalysis 4.2  
printed: 11/10/2014 2:06:28 PM  
by: MZ  
Page 1 of 1
1-(3-Methoxypropyl)-1H-indole-3-carboxaldehyde (NOESY)
SPECTROSCOPIC INFORMATION

N,N-Dimethyl-2-pyrindcarboxamide (PROTON)

N,N-Dimethyl-2-pyrindcarboxamide (PROTON)
SPECTROSCOPIC INFORMATION

N,N-Diethyl-2-pyridinecarboxamide (PROTON)

7.74 7.72 7.70 7.68 7.66 7.64 7.62 7.60 7.58 7.56 7.54 7.52 7.50 7.48 7.46 7.44 7.42 7.40 7.38 7.36 7.34 7.32 7.30 7.28 7.26 7.24 7.22 7.20

N,N-Diethyl-2-pyridinecarboxamide (PROTON)

0.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8
SPECTROSCOPIC INFORMATION

N,N-Diethyl-2-pyridincarboxamide (C13 CPD)

N,N-Diethyl-2-pyridincarboxamide (HSQC)
10-(Methoxymethyl)-5H-pyrrole[1,3-b]pyrazolo[3,4-d]pyrimidine (PROTON)
10-(Methoxymethyl)-5H-pyrrole-2,3,5-(carbazole-3,11(1H)-diox [PROTON]

![Spectroscopic Information Diagram]

10-(Methoxymethyl)-5H-pyrrole-2,3,5-(carbazole-3,11(1H)-diox [PROTON]

![Spectroscopic Information Diagram]

ppm
10-(Methoxymethyl)-5H-pyrido[2,3-b][carbazole-3,11(10H)-dione (HSQC)
SPECTROSCOPIC INFORMATION

10-(6-Methoxymethyl)-5H-pyrrole[2,3-b]carbazole 5,11(10H)-dione (COSY)

10-(6-Methoxymethyl)-5H-pyrrole[2,3-b]carbazole 5,11(10H)-dione (1HN HMBC)
Generic Display Report

Analysis Info
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Method: Di_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment:

Acquisition Date: 11/10/2014 10:13:49 AM
Operator: MZ
Instrument: maXis HD

Intens. x10^4

m/z
0.00 0.25 0.50 0.75 1.00 1.25 1.50 1.75 2.00

293.0918
294.0952
295.0978
315.0739
316.0772
317.0797

C₁₇H₁₃N₂O₃, 293.0921

C₁₇H₁₂N₂NaO₃, 315.0740

Bruker Compass DataAnalysis 4.2 printed: 11/10/2014 1:48:06 PM by: MZ Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
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Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: Instrument: maXis HD
Operator: 1820881.21300

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Set Nebulizer: 0.4 Bar
Set Capillary: 2200 V
Set End Plate Offset: -500 V
Set Dry Heater: 200°C
Set Dry Gas: 4.0 l/min
Set Charging Voltage: 2000 V
Set Divert Valve: Waste
Set APCI Heater: 0°C

Mass Spectrum SmartFormula Report

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SPECTROSCOPIC INFORMATION

5H-Pyrrole(2,3-b)carbazole-5,11(10H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

**H-Pyrrole(2,3-b)/carbazole-5,11(10H)-dione (HSQC)**

**H-Pyrrole(2,3-b)/carbazole-5,11(10H)-dione (HMQC)**
SPECTROSCOPIC INFORMATION

Generic Display Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N:141110\TN085_DIL_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment:

Acquisition Date: 11/10/2014 10:59:12 AM
Operator: MZ
Instrument: maXis HD

Intens. x10^4

250.2141 253.2639 255.2794 261.1308 264.2299 268.2749 271.0479 272.0509

C₁₅H₉N₂O₂, 249.0659

250.0690 1+
251.0716 1+

C₁₅H₈N₂NaO₂, 271.0478

273.0535 1+

CuH₂N₃O₄, 249.0659

CuH₂N₃NaO₄, 271.0478

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Bruker Compass DataAnalysis 4.2 printed: 11/10/2014 11:17:16 AM by: MZ Page 1 of 1
### SPECTROSCOPIC INFORMATION

#### Mass Spectrum SmartFormula Report

**Analysis Info**
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN085_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 0.01 mg/mL in MeOH
- **Operator**: MZ
- **Instrument**: maXis HD
- **Comment**: 1820881.21300

**Acquisition Parameter**
- **Source Type**: ESI
- **Scan Begin**: 50 m/z
- **Scan End**: 1550 m/z
- **Ion Polarity**: Positive
- **Set Capillary**: 2000 V
- **Set End Plate Offset**: -500 V
- **Set Dry Gas**: 0.4 Bar
- **Set Nebulizer**: 200 °C
- **Set Charging Voltage**: 4.0 l/min
- **Set Dry Heater**: 0 nA
- **Set Corona**: 20 °C
- **Set APCI Heater**: Waste

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![Mass Spectrum](image_url)
SPECTROSCOPIC INFORMATION

10-[2-(Dimethylamino)ethyl]-2H-pyrazolo[2,3-b]carbazole-5,11(10H)-dione (PROTON)

11.0  10.5  10.0  9.5  9.0  8.5  8.0  7.5  7.0  6.5  6.0  5.5  5.0  4.5  4.0  3.5  3.0  2.5  2.0  1.5  1.0  0.5

ppm

10-[2-(Dimethylamino)ethyl]-2H-pyrazolo[2,3-b]carbazole-5,11(10H)-dione (PROTON)

8.95  8.90  8.85  8.80  8.75  8.70  8.65  8.60  8.55  8.50  8.45  8.40  8.35  8.30

ppm
SPECTROSCOPIC INFORMATION

10-[2-(Dimethylamino)ethyl]-3H-pyrido[2,3-B]carbazole-5,11(10H)-dione (C13 APT)

110-115 ppm

12 ppm

10-[2-(Dimethylamino)ethyl]-3H-pyrido[2,3-B]carbazole-5,11(10H)-dione (C13 APT)
10-[3-(Dimethylamino)ethyl]-5-hydroxy[1,2,3,5,11,11(H,1)h]dione (HSQC)
10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (1HN HMQC)
10-[[3-(methylamino)ethyl]-3H-pyridine][2,3-b]carbazole-5,11(10H)-dione (NOESY)
Generic Display Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141110\TN051_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: 

Acquisition Date: 11/10/2014 10:05:09 AM
Operator: MZ
Instrument: maXis HD

Bruker Compass DataAnalysis 4.2
printed: 11/10/2014 1:54:39 PM
by: MZ
Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
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Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: approx. 0.01 mg/mL in MeOH

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Scan Begin: 50 m/z
Scan End: 1550 m/z
Nebulizer: 0.4 Bar
Capillary: 2000 V
Dry Heater: 200 °C
Charging Voltage: 0 nA
ACPI Heater: 0 °C
Dry Gas: 4.0 l/min
Divert Valve: Waste
Focus: Active

Intens. x10^5

Meas. m/z  # Ion Formula m/z err [ppm] mSigma #mSigma Score rdb e Conf N-Rule
320.1396 1 C19H18N3O2 320.1394 -0.8 9.4 1 100.00 12.5 even ok
342.1214 1 C19H17N3NaO2 342.1213 -0.4 10.3 1 100.00 12.5 even ok

TN051_D1_ESI_HRMS.d
Printed: Bruker Compass DataAnalysis 4.2 11/10/2014 1:54:48 PM by: MZ
N,N-Diethylisourea (PROTON)

N,N-Diethylisourea (PROTON)

SPECTROSCOPIC INFORMATION
N,N-Diethyl-4-aminobenzamide (NOESY)
SPECTROSCOPIC INFORMATION

10-(Methoxymethyl)-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (PROTON)

10-(Methoxymethyl)-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

10-(Methoxymethyl)-5H-pyridin-4,8-b[carbazole-5,11][10H]-dione (HSQC)

10-(Methoxymethyl)-5H-pyridin-4,8-b[carbazole-5,11][10H]-dione (HSQC)

10-(Methoxymethyl)-5H-pyridin-4,8-b[carbazole-5,11][10H]-dione (HSQC)
10-(6-Methoxynaphthalen-1-yl)-pyridine[3,4-b]carbazole-6,11(1H,10H)-dione (10N HMBC)

10-(6-Methoxynaphthalen-1-yl)-pyridine[3,4-b]carbazole-6,11(1H,10H)-dione (COBY)

257
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**Comment:**
- C₁₇H₁₂N₂NaO₃, 315.0740
- 0

**Generic Display Report**

**Analysis Info**
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- **Method:** Di_mz_50-1550.m
- **Sample Name:** approx. 0.01 mg/mL in MeOH
- **Comment:** 315.0736

**Acquisition Date:** 11/10/2014 10:29:37 AM
- **Operator:** MZ
- **Instrument:** maXis HD
Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date: 11/10/2014 10:29:37 AM
Analysis Name: D:\Data\MZ_data\Spreitzer_HiThomas_Ni\141110\TN054_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: 

Instrument: maXis HD
Operator: MZ

Acquisition Parameter

Source Type: ESI
Ion Polarity: Positive
Scan Begin: 50 m/z
Scan End: 1550 m/z
Focus: Active
Set Capillary: 3000 V
Set Plate Offset: -500 V
Set Dry Gas: 200 °C
Set Dry Heater: 0.4 Bar
Set Divert Valve: 4.0 l/min
Set Charging Voltage: Waste
Set Coron: 0 nA
Set APCI Heater: 0 °C

Mass Spectrum SmartFormula Report

TN054_DI_ESI_HRMS.d

by: MZ

Printed: 11/10/2014 1:28:15 PM
Page 1 of 1
SPECTROSCOPIC INFORMATION

SIH-Pyridine[3,4-b]carbazole-5,11(10H)-dione (C13 CPD)

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

SIH-Pyridine[3,4-b]carbazole-5,11(10H)-dione (C13 CPD)

180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 ppm
SPECTROSCOPIC INFORMATION

5H-Pyrrole[3,4-b]carbazole-5,11(1H)-dione (HSQC)

5H-Pyrrole[3,4-b]carbazole-5,11(1H)-dione (HMBC)
SPECTROSCOPIC INFORMATION

Generic Display Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141110\TN087_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment:

Acquisition Date: 11/10/2014 11:15:12 AM
Operator: MZ
Instrument: maXis HD

Intens. x10^5

250.2138
249.0655
251.2170
253.2633
255.2790
258.2786
264.2293
268.2742
264.2293
271.0474
271.0478
272.2942
272.2942
273.0535
273.0535

C₁₅H₉N₂O₂, 249.0659
C₁₅H₈N₂NaO₂, 271.0478

Bruker Compass DataAnalysis 4.2
printed: 11/10/2014 11:21:46 AM
by: MZ
### Analysis Info

**Analysis Name:** D:\Data\MZ_data\Spreitzer_HiThomas_N\141110\TN087_DI_ESI_HRMS.d  
**Method:** DI_mz_50-1550.m  
**Sample Name:** approx. 0.01 mg/mL in MeOH  
**Comment:**

### Acquisition Parameter

- **Source Type:** ESI  
- **Ion Polarity:** Positive  
- **Set Nebulizer:** 0.4 Bar  
- **Focus:** Active  
- **Set Capillary:** 4500 V  
- **Scan Begin:** 50 m/z  
- **Set End Plate Offset:** -500 V  
- **Scan End:** 1550 m/z  
- **Set Dry Gas:** 2000 V  
- **Set Charging Voltage:** 20 V  
- **Set Corona:** 0 nA  
- **Set Dry Heater:** Waste  
- **Set APCI Heater:** 0 °C  
- **Set Divert Valve:** Waste  

### Mass Spectrum SmartFormula Report

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### Diagram

![Diagram of molecule](attachment:image.png)

**TN087_DI_ESI_HRMS.d**  
**Printed:** 11/10/2014 11:22:01 AM  
**By:** MZ  
**Page:** 1 of 1
SPECTROSCOPIC INFORMATION

10-2-(2-Dimethylamino)ethyl)-3H-pyridine-3,4-bis(carbazole-5,11(1H))-dione (PROTON)

16

10-2-(2-Dimethylamino)ethyl)-3H-pyridine-3,4-bis(carbazole-5,11(1H))-dione (C13 APT)
SPECTROSCOPIC INFORMATION

10-[2-(Dimethylamino)ethyl]-2H-pyrido[3,4-b]carbazole-5,11(10H)-dione (C15 APT)

10-[2-(Dimethylamino)ethyl]-3H-pyrido[3,4-b]carbazole-5,11(10H)-dione (2DQC)
SPECTROSCOPIC INFORMATION

10-[2-[Ndimethylamino]ethyl]-3H-pyrido[3,4-b]carbazole-5,11(1H)-dione (HMBG)

10-[2-[Ndimethylamino]ethyl]-3H-pyrido[3,4-b]carbazole-5,11(1H)-dione (15N HMBG)
SPECTROSCOPIC INFORMATION

16-[2-(Dimethylamino)ethyl]-3H-pyrido[4,3-b]carbazole-3,11(10H)-dione (COST)
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<td>MZ</td>
<td>Instrument</td>
<td>maXis HD</td>
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### Graphical Data

![Graphical representation of the data](image)

### Molecular Structure

![Molecular structure of compound 16](image)
SPECTROSCOPIC INFORMATION

8-(Methoxymethyl)-5,11-H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

8-(Methoxymethyl)-5,11-H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 AP'T)

281
SPECTROSCOPIC INFORMATION

6-(Methoxymethyl)-3H-pyrrole[4,3-b]carbazole 5,11(1H)-dione (C13 A17)

6-(Methoxymethyl)-3H-pyrrole[4,3-b]carbazole 5,11(1H)-dione (HMQC)
SPECTROSCOPIC INFORMATION

6-(Methoxymethyl)-3H-pyridine[4,3-b]carbazole-5,11:10'-dione (HMBIC)

6-(Methoxymethyl)-3H-pyridine[4,3-b]carbazole-5,11:10'-dione (15N HMBIC)
Generic Display Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN053_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: 11/10/2014 10:21:31 AM

Acquisition Date: 11/10/2014 10:21:31 AM
Operator: MZ
Instrument: maXis HD

![Graph showing m/z values and intensities for different peaks.]

Bruker Compass DataAnalysis 4.2 printed: 11/10/2014 1:37:56 PM by: MZ Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data_MZ_data\Spreitzer_HiThomas_Ni141110\TN053_Dl_ESI_HRMS.d
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: DI_mz_50-1550.m

Acquisition Parameter

Source Type: ESI
Ion Polarity: Positive
Focus: Active
Scan Begin: 50 m/z
Scan End: 1550 m/z

Scan Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set APCI Heater: 0 °C

Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste

Intens. x10^5

Meas. m/z  #  Ion Formula  m/z  err [ppm]  mSigma  # mSigma  Score  rdb  e Conf  N-Rule
315.0740  1  C17H12N2NaO3  315.0740  0.1  5.2  1  100.00  12.5  even  ok

Mass Spectrum SmartFormula Report

TN053_Dl_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2 printed: 11/10/2014 1:38:05 PM by: MZ
SPECTROSCOPIC INFORMATION

3H-Pyridin-4,5-3H-carbazole-3,11(6H)-dione (1H HMQC)

[Graph showing 3H-Pyridin-4,5-3H-carbazole-3,11(6H)-dione (1H HMQC)]

3H-Pyridin-4,5-3H-carbazole-3,11(6H)-dione (13N HMQC)

[Graph showing 3H-Pyridin-4,5-3H-carbazole-3,11(6H)-dione (13N HMQC)]
Analysis Info

Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141110\TN086_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment:

Acquisition Date: 11/10/2014 11:04:10 AM
Operator: MZ
Instrument: maXis HD

Generic Display Report

Bruker Compass DataAnalysis 4.2
printed: 11/10/2014 11:10:21 AM
by: MZ
Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN086_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: Operator: MZ

Acquisition Parameter

- Source Type: ESI
- Ion Polarity: Positive
- Scan Begin: 50 m/z
- Scan End: 1550 m/z
- Set Dry Heater: 200 °C
- Set Corona: 0 nA
- Set APi Heater: 0 °C
- Set Charging Voltage: 4000 V
- Set Divert Valve: Waste
- Set Dry Gas: 200 °C

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e- Conf N-Rule
249.0856 1 C15H9N2O2 249.0659 0.9 11.9 1 100.00 12.5 even ok
271.0477 1 C15H8N2NaO2 271.0478 0.5 0.3 1 100.00 12.5 even ok

TN086_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2 printed: 11/10/2014 11:10:30 AM by: MZ

Page 1 of 1
SPECTROSCOPIC INFORMATION

6-[1-Dimethylaminoethyl]-3H-pyrido[4,3-b]carbazole-5,11(8H)-dione (PROTON)

1.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

6-[1-Dimethylaminoethyl]-3H-pyrido[4,3-b]carbazole-5,11(8H)-dione (PROTON)
6-[2-(Dimethylamino)ethyl]-2H-pyrido[4,3-b]carbazole-5,11(6H)-dione (DSQC)

6-[2-(Dimethylamino)ethyl]-2H-pyrido[4,3-b]carbazole-5,11(6H)-dione (DDMRC)
SPECTROSCOPIC INFORMATION

6-[3-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-3,11(10H)-dione (15N-EMBC)

N,N-Diethyl-2-pyrinocarbazole (NOEST)
**Analysis Info**

**Analysis Name**
D:\Data\_MZ_data\Spreitzer\Thomas_NI141104\TN061_DI_ESI_HRMS.d

**Method**
DI_mz_50-1550.m

**Sample Name**
approx. 10 ug/mL in MeOH

**Comment**

**Intens. x10^5**

0.0 0.5 1.0 1.5

316.2244 318.2403 320.1394 324.3372 326.2432 328.2607 330.2765 332.2921 334.2350 336.2509 342.1213

**Intens. x10^5**

0.0 0.5 1.0 1.5

316.2244 318.2403 320.1394 324.3372 326.2432 328.2607 330.2765 332.2921 334.2350 336.2509 342.1213

**Intens. x10^5**

0.0 0.5 1.0 1.5

316.2244 318.2403 320.1394 324.3372 326.2432 328.2607 330.2765 332.2921 334.2350 336.2509 342.1213

**Generic Display Report**

**Acquisition Date**

**Operator**
MZ

**Instrument**
maXis HD

**Bruker Compass DataAnalysis 4.2**


by: MZ

Page 1 of 1
# Mass Spectrum SmartFormula Report

## Analysis Info

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## Sample Name
approx. 10 ug/mL in MeOH

## Comment

## Method
DI_mz_50-1550.m

## Instrument
maXis HD 1820881.21300

## Acquisition Parameter

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## Mass Spectrum

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**TN061_Di_ESI_HRMS.d**

**Bruker Compass DataAnalysis 4.2**

printed: 11/4/2014 11:40:17 AM by: MZ Page 1 of 1
Spectroscopic Information

IR Pyrrole (6,5-H) carboxylic acid (1H) N-oxide

Spectroscopic data for IR Pyrrole (6,5-H) carboxylic acid (1H) N-oxide is shown in the image. The spectrum displays various peaks at specific ppm values, corresponding to different chemical shifts. The peaks are labeled with numbers that likely correspond to specific atomic or functional groups within the molecule. The structure is illustrated with carbon atoms labeled 1 to 11, and functional groups like the carboxylic acid and N-oxide are indicated.

The IR spectrum shows the characteristic peaks for the IR Pyrrole (6,5-H) carboxylic acid (1H) N-oxide, providing insights into the molecular structure and chemical bonding within the compound.
SPECTROSCOPIC INFORMATION

S.Si: Pyridine(4,5-3)-carbazole-5,11(6H)-dione 2-oxide (15N HMBC)

S.Si: Pyridine(4,5-3)-carbazole-5,11(6H)-dione 2-oxide (15N HSQC)
Generic Display Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\150112\TN157\DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:10 in MeOH
Comment: 265.0607

Acquisition Date: 1/12/2015 5:16:08 PM
Operator: MZ
Instrument: maXis HD

Bruker Compass DataAnalysis 4.2
printed: 1/12/2015 5:19:43 PM
by: MZ
Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN157_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:10 in MeOH
Comment: 

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste

Scan Begin: 50 m/z
Set End Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set APCI Heater: 0 °C

Intens. x10^6

Meas. m/z | # | Ion Formula | m/z | err [ppm] | mSigma | # mSigma | Score | rdb | e¯ | Conf | N-Rule
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287.0427 | 1 | C15H8N2NaO3 | 287.0427 | 0.0 | 12.2 | 1 | 100.00 | 12.5 | even | ok

TN157_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2

printed: 1/12/2015 5:19:50 PM by: MZ
SPECTROSCOPIC INFORMATION

6-[2-(Dimethylamino)ethyl]-5H-pyrrole[4,3-b](carbazole-5,11(6H)-dione 2-oxide (DMSO)

6-[2-(Dimethylamino)ethyl]-5H-pyrrole[4,3-b](carbazole-5,11(6H)-dione 2-oxide (DMSO)
Mass Spectrum SmartFormula Report

Analysis Info
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Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_Ni\141104\TN158_DI_ESI_HRMS.d
Method: DI.mz_50-1550.m
Sample Name: approx. 10 ug/mL in MeOH
Comment: Instrument: maXis HD 1820881.21300
Operator: MZ

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N,N-Diethyl-2-pyrazinocarboxamide (C13 CPD)

N,N-Diethyl-2-pyrazinocarboxamide (HSQC)
N,N-Diethyl-2-pyrazinylurea (NOESY)
6-(Methoxymethyl)-3H-pyrazine[2,3-b][carbazole-9,11(1H)]-dione (PROTON)

9.2 9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 3.7 3.6 3.5 3.4 3.3 ppm

13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
6-(Methoxymethyl)-5H-pyrimido[2,3-h]benzolo-5,11(11H)-dione (HSQC)
SPECTROSCOPIC INFORMATION

Generic Display Report

Analysis Info

Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_Ni141110\TN056_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment:

Acquisition Date: 11/10/2014 10:37:07 AM
Operator: MZ
Instrument: maXis HD

Intens. x10^2

C_{16}H_{11}N_{3}NaO_{3}, 316.0693

Bruker Compass DataAnalysis 4.2 printed: 11/10/2014 1:21:31 PM by: MZ Page 1 of 1
Generic Display Report

Analysis Info

Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141110\TN072_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH

Analysis Info

Acquisition Date: 11/10/2014 10:50:32 AM
Operator: MZ
Instrument: maXis HD

Comment

SPECTROSCOPIC INFORMATION

Bruker Compass DataAnalysis 4.2  printed: 11/10/2014 11:29:30 AM  by: MZ  Page 1 of 1
### Analysis Info

- **Acquisition Date**: 11/10/2014 10:50:32 AM
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN072_D\ESI_HRMS.d
- **Method**: Dl_mz_50-1550.m
- **Sample Name**: approx. 0.01 mg/mL in MeOH
- **Instrument**: maXis HD 1820
- **Comment**: 88.1213

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SPECTROSCOPIC INFORMATION

6-[1-(Dimethylamino)ethyl]-5H-pyrano[2,3-b]carbazole-5,11(1H)-dione (PROTON)

13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

6-[1-(Dimethylamino)ethyl]-5H-pyrano[2,3-b]carbazole-5,11(1H)-dione (PROTON)

9.00 8.98 8.96 8.94 8.92 8.90 8.88 8.86 8.86 8.82 8.80 8.78 8.76 8.74 8.72 8.70 8.68 8.66 8.64 8.62 8.60 8.58 8.56 8.54 8.52 8.50 8.48 8.46 8.44 8.42 8.40 8.38 8.36 8.34 8.32 8.30 8.28 8.26 8.24 8.22 8.20 8.18 8.16 8.14 8.12 8.10 8.08 8.06 8.04 8.02 8.00 ppm
6-[(2-Dimethylamino)ethyl]-2H-pyrroline[2,3-b]carbazole-5,11(6H)-dione (HMQC)
SPECTROSCOPIC INFORMATION

6-[2-((Dimethylamino)ethyl)-5H-pyrazino][2,3-b]carbazole-5,11(11H)-dione (25N KMBG)

6-[2-((Dimethylamino)ethyl)-5H-pyrazino][2,3-b]carbazole-5,11(11H)-dione (C2007)

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6-[2-(Dimethylamino)ethyl]-3H-pyran-2,3(2H)-dione (NOESY)
Analysis Info

Acquisition Date: 11/4/2014 10:42:25 AM
Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_N\141104\TN091_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 10 ug/mL in MeOH
Operator: MZ
Instrument: maXis HD

Comment:

321.1346 1+
C₁₈H₁₇N₄O₂, 321.1346

343.1165 1+
C₁₈H₁₆N₄NaO₂, 343.1165

Generic Display Report

Bruker Compass DataAnalysis 4.2  printed: 11/4/2014 11:54:09 AM   by: MZ  Page 1 of 1
Mass Spectrum SmartFormula Report

**Analysis Info**

- **Analysis Name**: D:\Data\MZ_data\Speitzer\Thomas_N\141104\TN091_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 10 ug/mL in MeOH
- **Instrument**: maXis HD
- **Operator**: MZ

**Acquisition Parameter**

- **Source Type**: ESI
- **Ion Polarity**: Positive
- **Scan Begin**: 50 m/z
- **Scan End**: 1550 m/z
- **Set Nebulizer**: 0.4 Bar
- **Set Capillary**: 1800 V
- **Set End Plate Offset**: -500 V
- **Set Dry Gas**: 4.0 l/min
- **Set Dry Heater**: 200 °C
- **Set Charging Voltage**: 2000 V
- **Set Divert Valve**: Waste
- **Set Corona**: 0 nA
- **Set APCI Heater**: 0 °C

**Mass Spectrum**

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**Comment**

TN091_DI_ESI_HRMS.d

1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (C13 CPD)

![Spectroscopic Information Graph]

1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (11Hz/2C)

![Spectroscopic Information Graph]

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SPECTROSCOPIC INFORMATION

1-[2-(Dimethylamino)ethyl]-1H-indole-3-carboxaldehyde (1HIMBC)

1-[2-(Dimethylamino)ethyl]-1H-indole-3-carboxaldehyde (15N IMBC)
SPECTROSCOPIC INFORMATION

Generic Display Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spreezter_H\Thomas_N\150112\TN097_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --&gt; 1:100 in MeOH
Comment: 

Acquisition Date: 1/12/2015 2:19:59 PM
Operator: MZ
Instrument: maXis HD

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Mass Spectrum SmartFormula Report

Analysis Info
Acquisition Date 1/12/2015 2:19:59 PM
Analysis Name D:\Data\MZ_data\Spreitzer_HiThomas_Ni\150112\TN097_DI_ESI_HRMS.d
Method DI_mz_50-1550.m
Sample Name in MeOH --> 1:100 in MeOH
Comment

Acquisition Parameter
Source Type ESI Ion Polarity Positive Set Nebulizer 0.4 Bar
Focus Active Set Capillary 2500 V Set Dry Heater 200 °C
Scan Begin 50 m/z Set End Plate Offset -500 V Set Dry Gas 4.0 l/min
Scan End 1550 m/z Set Charging Voltage 2000 V Set Divert Valve Waste
Set Corona 0 nA Set APCI Heater 0 °C

+MS, 0.5-2.6min #1-147

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e Conf N-Rule
217.1339 1 C13H17N2O 217.1335 -1.8 12.1 1 100.00 6.5 even ok
239.1160 1 C13H16N2NaO 239.1155 -2.0 10.9 1 100.00 6.5 even ok

TN097_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2 printed: 1/12/2015 2:24:38 PM by: MZ Page 1 of 1
SPECTROSCOPIC INFORMATION

1-(3-Dimethylamino)propyl]-1H-Indole-3-carboxaldehyde (PROTON)

1-(3-Dimethylamino)propyl]-1H-Indole-3-carboxaldehyde (C13 APT)
SPECTROSCOPIC INFORMATION

1-[3-(Dimethylamino)propyl]-5H-indole-3-carboxaldehyde (C13-AF7)

190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 ppm

1-[3-(Dimethylamino)propyl]-5H-indole-3-carboxaldehyde (HMQC)
SPECTROSCOPIC INFORMATION

1-H-[3-(Dimethylamino)propyl]-1H-indole-3-carboxaldehyde (HMQC)

1-H-[3-(Dimethylamino)propyl]-1H-indole-3-carboxaldehyde (1H HMQC)
8-(3-(Dimethylamino)pyrrol-5-yl)-4H-pyrido[3,4-b]carbazole-5,11(6H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

6-[(3-[(dimethylamino)propyl]-3H-pyridin-4-yl)b-carbazole-5,11(11H)-dione (HSSQC)
6-[3-(Dimethylamino)propyl]-2H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

6-3-(Dimethylamino)propyl-5H-pyrido[4,3-b]carbazole 5,11(6H) dione (NOESY)
Generic Display Report

Analysis Info
- Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_N\141104\TN118_D1_ESI_HRMS.d
- Method: DI_mz_50-1550.m
- Sample Name: approx. 10 ug/mL in MeOH
- Comment

Acquisition Date: 11/4/2014 12:18:40 PM
- Operator: MZ
- Instrument: maXis HD

[m/z vs. Intensity graph with peaks labeled]

Bruker Compass DataAnalysis 4.2
by: MZ
Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spraitzer\Thomas_N\141104\TN118_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 10 ug/mL in MeOH
Comment:

Acquisition Date: 11/4/2014 12:18:40 PM
Operator: MZ
Instrument: maXis HD

Instrument Parameters:
- Scan Begin: 50 m/z
- Scan End: 1550 m/z
- Set Charging Voltage: 2000 V
- Source Type: Active
- Set Capillary: 3000 V
- Set End Plate Offset: -500 V
- Set Dry Gas: 4.0 l/min
- Set Dry Heater: 200 °C
- Set AP CI Heater: 0 °C
- Set Divert Valve: Waste
- Set Nebulizer: 0.4 Bar
- Set Corona: 0 nA

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e− Conf N-Rule
334.1549 1 C20H20N3O2 334.1550 0.3 21.8 1 100.00 12.5 even ok

TN118_DI_ESI_HRMS.d
SPECTROSCOPIC INFORMATION

6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-3,11(6H)-dione (PROTON)

![NMR spectrum of 6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-3,11(6H)-dione (PROTON)]

6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-3,11(6H)-dione (PROTON)

![NMR spectrum of 6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-3,11(6H)-dione (PROTON)]

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SPECTROSCOPIC INFORMATION

6-[3-(Dimethylamino)propyl]-5H-pyrazolo[2,3-b]carbazole-5,11(6H)-diones (C13 API)

180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 ppm

6-[(3-Dimethylamino)propyl]-5H-pyrazolo[2,3-b]carbazole-5,11(6H)-diones (HMQC)
6-[3-(Dimethylamino)propyl]-5H-pyrano[2,3-b]carbazole-5,11(6H)-dione (HMBC)
### SPECTROSCOPIC INFORMATION

**Analysis Info**

- **Analysis Name**: D:\Data\_MZ\data\Spreitzer\Thomas_N141104\TN116_SI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 10 ug/mL in MeOH

**Comment**

- 335.1506
- 336.1535
- 337.2544
- 357.1326
- 358.1355
- 359.1382

- C₁₉H₁₉N₄O₂, 335.1503
- C₁₉H₁₈N₄NaO₂, 357.1322

**Intens. x10⁵**

- 335.1506
- 336.1535
- 337.2544
- 357.1326
- 358.1355
- 359.1382

**Generic Display Report**

- Bruker Compass DataAnalysis 4.2
- by: MZ

---

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Mass Spectrum SmartFormula Report

Analysis Info
- Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_N\141104\TN116_DI_ESI_HRMS.d
- Method: DI mz_50-1550.m
- Sample Name: approx. 10 ug/mL in MeOH
- Comment: approx. 10 ug/mL in MeOH

Acquisition Information
- Instrument: maXis HD 1820881.21300

Acquisition Parameter
- Source Type: ESI
- Ion Polarity: Positive
- Set Nebulizer: 0.4 Bar
- Set Dry Heater: 200 °C
- Set Dry Gas: 4.0 l/min
- Set Charging Voltage: 0 nA
- Set Corona: 142.1592
- Set APCI Heater: 0 °C
- Set Divert Valve: Waste

Mass Spectrum SmartFormula Report

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30
SPECTROSCOPIC INFORMATION

1-[2-(Morpholin-4-yl)ethyl]-1H-indole-3-carboxaldehyde (HMB-C)

1-[2-(Morpholin-4-yl)ethyl]-1H-indole-3-carboxaldehyde (13N HMB-C)
8-2-(4-Morpholinyl)[ethyl]-5H-pyrido[4,3-b]carbazole-5,11(11H)-dione (C13 APT)

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8-2-(4-Morpholinyl)[ethyl]-5H-pyrido[4,3-b]carbazole-5,11(11H)-dione (C13 APT)
SPECTROSCOPIC INFORMATION

6-[6-Morpholinylethyl]-3H-pyrido[4,3-b]carbazole-5,11(1H,11H)-dione (HISQC)

6-[6-Morpholinylethyl]-3H-pyrido[4,3-b]carbazole-5,11(1H,11H)-dione (HMBC)
**Analysis Info**

- **Analysis Name:** D:\Data\_MZ_data\Spreitzer\Thomas_N\141104\TN108_DI_ESI_HRMS.d
- **Method:** DI_mz_50-1550.m
- **Sample Name:** approx. 10 ug/mL in MeOH
- **Comment:** +MS, 1.5-3.8min #86-219

**Generic Display Report**

- **Acquisition Date:** 11/4/2014 10:56:41 AM
- **Operator:** MZ
- **Instrument:** maXis HD

**Intens.**

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**Chemical Structures**

- **C₂₁H₂₀N₃O₃, 362.1499**
- **C₂₁H₁₉N₃NaO₃, 384.1319**
**Analysis Info**

**Analysis Name:** D:\Data\MZ_data\Spreitzer\Thomas_Ni\141104\TN108_DI_ESI_HRMS.d

**Method:** DI_mz_50-1550.m

**Sample Name:** approx. 10 ug/mL in MeOH

**Comment:**

**Acquisition Parameter**

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**Mass Spectrum SmartFormula Report**

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**TN108_DI_ESI_HRMS.d**

**Bruker Compass DataAnalysis 4.2**

**Printed:** 11/4/2014 11:59:09 AM **By:** MZ
SPECTROSCOPIC INFORMATION

6-[1H-Morpholino(ethyl)-5H-pyrazine/(2,3-b)carbazole-5,11(H)-dione (PROTON)

13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

6-[1H-Morpholino(ethyl)-5H-pyrazine/(2,3-b)carbazole-5,11(H)-dione (PROTON)

9.0 8.9 8.5 8.4 7.6 7.5 7.4 7.3 7.2 7.1 5.0 4.9 4.8 3.7 3.6 3.5 2.9 2.8 2.7 2.6 2.5
**Generic Display Report**

**Analysis Info**
- **Analysis Name**: D:\Data\MZ_data\Spreitzer\Thomas_N:\141104\TN107 DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 10 ug/mL in MeOH
- **Operator**: MZ
- **Instrument**: maXis HD

**Comment**
+MS, 0.0-1.7min #1-95

---

**Data Display**

- **m/z** values: 363.1450, 364.1480, 365.1508, 366.2253, 369.3473, 373.1269, 383.1112, 385.1271, 386.1301, 387.1063
- **Intens.** values: 1+, 0.5, 1.0, 1.5, 2.0, 2.5, 5x10

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**Bruker Compass DataAnalysis 4.2**

- **printed**: 11/4/2014 11:56:42 AM
- **by**: MZ

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*Page 1 of 1*
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_Ni\141104\TN107_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 10 ug/mL in MeOH
Comment: TN107_DI_ESI_HRMS.d

Acquisition Parameter

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<td>Set Divert Valve</td>
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<td></td>
<td>Set Corona</td>
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<td>Set APCI Heater</td>
<td>0 °C</td>
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Meas. m/z  # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e− Conf N-Rule
363.1450 1 C20H19N4O3 363.1452 0.5 19.7 1 100.00 13.5 even ok
385.1272 1 C20H18N4NaO3 385.1271 -0.2 11.0 1 100.00 13.5 even ok

+MS, 0.0-1.7 min #1-55

TN107_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2
1-(2-[(3-Piperidinyl)ethyl]-1H-indole-3-carboxaldehyde (C13 APT)

34

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

185 140 135 130 125 120 115 110 80 75 60 55 50 45 25 ppm
### Generic Display Report

**Analysis Info**

- **Analysis Name**: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\150112\TN109_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: in MeOH -> 1:500 in MeOH
- **Comment**:

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<th>0.0-1.5min #1-86</th>
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<tbody>
<tr>
<td>257.1652</td>
<td>+MS, 0.0-1.5min #1-86</td>
</tr>
<tr>
<td>279.1469</td>
<td>+MS, 0.0-1.5min #1-86</td>
</tr>
<tr>
<td>257.1648</td>
<td>C₁₆H₂₁N₂O, 257.1648</td>
</tr>
<tr>
<td>279.1468</td>
<td>C₁₆H₂₀N₂NaO, 279.1468</td>
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</tbody>
</table>

**Intens. x10^5**

- **m/z**: 257.1652
- **m/z**: 279.1469
- **CuH₁₂N₂O₂, 257.1648**
- **CuH₁₂N₂NaO₂, 279.1468**

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Bruker Compass DataAnalysis 4.2  
printed: 1/12/2015 3:49:57 PM  
by: MZ  
Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_HiThomas_Ni150112\TN109_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:500 in MeOH
Comment:

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Scan Begin: 50 m/z
Scan End: 1550 m/z
Set Capillary: 1500 V
Set End Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste
Set APCI Heater: 0 °C

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e Conf N-Rule
257.1652 1 C16H21N2O 257.1648 -1.3 10.0 1 100.00 7.5 even ok
279.1469 1 C16H20N2NaO 279.1468 -0.5 9.2 1 100.00 7.5 even ok

TN109_DI_ESI_HRMS.d
Bruker Compass DataAnalysis 4.2 printed: 1/12/2015 3:50:04 PM by: MZ Page 1 of 1
6-[L-Piperidin-1-yl]-5H-pyrrole[4,3-b]carbazole 5,11(11H)-dione (HMQC)
**Mass Spectrum SmartFormula Report**

**Analysis Info**

**Analysis Name**: D:\Data\_MZ_data\Spreitzer\Thomas_N\141104\TN111_Di_ESI_HRMS.d  
**Acquisition Date**: 11/4/2014 11:15:02 AM  
**Method**: Di_mz_50-1550.m  
**Operator**: MZ  
**Sample Name**: approx. 10 ug/mL in MeOH  
**Instrument**: maXis HD  
**Comment**: 1820881.21300

---

**Acquisition Parameter**

- **Source Type**: ESI  
- **Focus**: Active  
- **Scan Begin**: 50 m/z  
- **Scan End**: 1550 m/z  
- **Ion Polarity**: Positive  
- **Set Capillary**: 3000 V  
- **Set End Plate Offset**: -500 V  
- **Set Dry Gas**: 200 °C  
- **Set Charging Voltage**: 2000 V  
- **Set Dry Heater**: 4.0 l/min  
- **Set Corona**: 0 nA  
- **Set APCI Heater**: Waste  
- **Set Nebulizer**: Waste

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**Measurements**

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<th>mSigma</th>
<th># mSigma</th>
<th>Score</th>
<th>rdb</th>
<th>e°</th>
<th>Conf</th>
<th>N-Rule</th>
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<td>even</td>
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</tbody>
</table>

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**Chemical Structure**

![Chemical Structure](attachment:image.png)
8-[(2-[(1-Piperidino[ethyl]-5H-pyrazino)2,3-b]carbazolic-5,11(str)-dione (C13 APT)

---

6-[(1-Piperidino[ethyl]-5H-pyrazino)2,3-b]carbazolic-5,11(str)-dione (3HQC)
SPECTROSCOPIC INFORMATION

6-[2-(3-Piperidinyl)ethyl]-5H-pyrazino[1,2-b]pyrazino[6,11H,11H]-dione (1HNMR)

6 [2-(3-Piperidinyl)ethyl]-5H-pyrazino[1,2-b]pyrazino[6,11H,11H]-dione (13CNMR)

390
Mass Spectrum SmartFormula Report

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<td>Sample Name</td>
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**Acquisition Parameter**

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<tr>
<td>Set APIC Heater</td>
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<tr>
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<tr>
<td>Set APIC Heater</td>
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<td>Scan Begin</td>
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<tr>
<td>Scan End</td>
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<td>2000 V</td>
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<tr>
<td>Set Corona</td>
<td>0 nA</td>
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<tr>
<td>Set APIC Heater</td>
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**Mass Spectrum**

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<tr>
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<th>Ion Formula</th>
<th>m/z</th>
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<th>mSigma</th>
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<td>13.5</td>
<td>even</td>
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<td>383.1479</td>
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<td>C21H20N4NaO2</td>
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<td>11.5</td>
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<td>100.00</td>
<td>13.5</td>
<td>even</td>
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</table>

**Graphic Representation**

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SPECTROSCOPIC INFORMATION

1-(2-[(1-Pyrrolidinyl)ethyl]-1H-indole-3-carboxaldehyde (PROTON)

[Diagram of 1-(2-[(1-Pyrrolidinyl)ethyl]-1H-indole-3-carboxaldehyde (PROTON)]

13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

1-(2-[(1-Pyrrolidinyl)ethyl]-1H-indole-3-carboxaldehyde (PROTON)

[Diagram of 1-(2-[(1-Pyrrolidinyl)ethyl]-1H-indole-3-carboxaldehyde (PROTON)]

10.0 9.9 9.8 9.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 4.4 4.3 4.2 3.0 2.9 2.8 2.7 2.6 2.5 2.4 1.8 1.7 1.5 ppm
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_HiThomas_N\141117\TN112_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment:

Acquisition Date: 11/17/2014 2:18:00 PM
Acquisition Name: D:\Data\MZ_data\Spreitzer_HiThomas_N\141117\TN112_DI_ESI_HRMS.d
Operator: MZ
Instrument: maxIs HD 1820881.21300

 Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Target: ~500 V
Set Charging Voltage: 2000 V
Set Divert Valve: Waste
Set Corona: 2 nA
Set APCI Heater: 100 °C

Mass Spectrum SmartFormula Report

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<tr>
<th>Meas. m/z</th>
<th># Ion Formula</th>
<th>m/z</th>
<th>err [ppm]</th>
<th>mSigma</th>
<th># mSigma</th>
<th>Score</th>
<th>rdb</th>
<th>e</th>
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<td>265.1311</td>
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TN112_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2
printed: 11/17/2014 2:23:17 PM
by: MZ
Page 1 of 1
SPECTROSCOPIC INFORMATION


[Diagram of a molecule with spectroscopic data]


[Diagram of another molecule with spectroscopic data]
6-[2-(1-Pyrrolidinylethyl)-3H-pyridine-4,5-b]carbazole-5,11(1H)-dione (HSQC)

6-[2-(1-Pyrrolidinylethyl)-3H-pyridine-4,5-b]carbazole-5,11(1H)-dione (HMBC)
6-[2-(1-Pyrrolidinyl)ethenyl]-3H-pyrido[1,2-b]pyrazine-3,11(10H)-dione (13N-HMSQ2)
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data\MZ_data\Spritzer\Thomas_N\141104\TN113_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 10 ug/mL in MeOH
Comment: 

Acquisition Parameter

Source Type: ESI
Ion Polarity: Positive
Scan Begin: 50 m/z
Scan End: 1550 m/z
Set Corona: 0 nA
Set Dry Gas: 4.0 l/min
Set APCI Heater: 0 °C
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Divert Valve: Waste
Set Charging Voltage: 2000 V
Set End Plate Offset: -500 V

Mass Spectrum

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e− Conf N-Rule
346.1546 1 C21H20N3O2 346.1550 1.1 9.3 1 100.00 13.5 even ok
368.1363 1 C21H19N3NaO2 368.1369 1.7 6.3 1 100.00 13.5 even ok

TN113_DI_ESI_HRMS.d
Brother Compass DataAnalysis 4.2 printed: 11/4/2014 12:03:38 PM by: MZ Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_Ni141104\TN114_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 10 ug/mL in MeOH
Comment:

Acquisition Date: 11/4/2014 11:31:08 AM
Analysis Parameter:

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<td>4.0 l/min</td>
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<td>Set Corona</td>
<td>0 nA</td>
<td>Set APCI Heater</td>
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</table>

Mass Spectrum SmartFormula Report

Meas. m/z 347.1504 1 C20H19N4O2
m/z 347.1503 -0.5 8.5 1 100.00 13.5 even ok
mSigma 369.1325 1 C20H18N4NaO2
mSigma 369.1322 -0.8 8.4 1 100.00 13.5 even ok
Score rdb e Conf N-Rule
347.1504 1 C20H19N4O2 347.1503 -0.5 8.5 1 100.00 13.5 even ok
369.1325 1 C20H18N4NaO2 369.1322 -0.8 8.4 1 100.00 13.5 even ok

411
SPECTROSCOPIC INFORMATION

3-(2-Hydroxyethyl)-1,3-oxazolidin-2-one (1H NMR)

3-(2-Hydroxyethyl)-1,3-oxazolidin-2-one (15N 1H NMR)
SPECTROSCOPIC INFORMATION

3-(2-Chloroethyl)-1,2-oxazolidin-2-one (C13 A97)

---

3-(2-Chloroethyl)-1,2-oxazolidin-2-one (H10Q2)

---
6-(2-oxo-1,3-oxazolidin-3-yl)pyridine(3,3'-carbazole,5,11(6H) diene) [HSQC]

6-(2-oxo-1,3-oxazolidin-3-yl)pyridine(3,3'-carbazole,5,11(6H) diene) [HMBC]

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6-[2-Oxo-1,3-oxazolidin-5-s(yethyl)]-3H-pyridol(4,3-b)carbazole-3,11(6H)-dien (1H-MBRC)

6-[2-Oxo-1,3-oxazolidin-5-s(yethyl)]-3H-pyridol(4,3-b)carbazole-3,11(6H)-dien (CD3OD)
SPECTROSCOPIC INFORMATION

Analysis Info
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN132_Di_ESI_HRMS.d
- **Method**: Di_mz_50-1550.m
- **Sample Name**: approx. 0.01 mg/mL in MeOH
- **Comment**: 1+MS, 0.0-1.8min #1-103

Analysis of compounds with m/z values: 362.1135, 363.1166, 367.3682, 369.2974, 374.3027, 382.4405, 384.0955, 385.0986, 386.1012, 385.0986, 386.1012.

Chemical structures:
- CuH2N2O2, m/z 362.1135
- 42, m/z 384.0955

Generic Display Report

Bruker Compass DataAnalysis 4.2
printed: 11/10/2014 1:05:42 PM
by: MZ
Page 1 of 1
## Mass Spectrum SmartFormula Report

**Analysis Info**

- **Analysis Name**: D:\Data\MZ_data\Spreitzer_HiThomas_N141110\TN132_DI_ESI_HRMS.d
- **Method**: D\_mz\_50-1550.m
- **Sample Name**: approx. 0.01 mg/mL in MeOH
- **Comment**: Instrument: maXis HD 1820881.21300

**Comment**

### Acquisition Parameter

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<td>Set Corona</td>
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### Mass Spectrum

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<th># Ion Formula</th>
<th>m/z</th>
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<th>mSigma</th>
<th># mSigma</th>
<th>Score</th>
<th>rdb</th>
<th>e⁻</th>
<th>Conf</th>
<th>N-Rule</th>
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<td>384.0955</td>
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**TN132_DI_ESI_HRMS.d**

**Printed by**: MZ DataAnalysis 4.2 11/10/2014 1:05:53 PM
SPECTROSCOPIC INFORMATION

SPECTROSCOPIC INFORMATION


![Spectroscopic Graph]

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\_MZ_data\Spreitzer\Thomas_N\141104\TN152_DI_ESI_HRMS.d
Method DI_mz_50-1550.m
Sample Name approx. 10 ug/mL in MeOH
Comment

Acquisition Parameter

Source Type ESI
Ion Polarity Positive
Focus Active
Scan Begin 50 m/z
Scan End 1550 m/z
Set Nebulizer 0.4 Bar
Set Dry Heater 200 °C
Set Dry Gas 4.0 l/min
Set Capillary -500 V
Set Divert Valve Waste
Set End Plate Offset 2000 V
Set Charging Voltage 0 nA
Set APCI Heater 0 °C
Scan Begin 50 m/z
Scan End 1550 m/z
Set Corona 0 nA
Set APCI Heater 0 °C

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<th>m/z</th>
<th>err [ppm]</th>
<th>mSigma</th>
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<th>Score</th>
<th>rdb</th>
<th>e− Conf</th>
<th>N-Rule</th>
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![Mass Spectrum SmartFormula Report](image.png)
SPECTROSCOPIC INFORMATION

6-[2-(2-Oxo-1,3-oxoazin-3-yl)ethyl]-1H-pyrazolo[1,2-b]carbazole-5,11(8H)-dione (PROTON)

6-[2-(2-Oxo-1,3-oxoazin-3-yl)ethyl]-1H-pyrazolo[1,2-b]carbazole-5,11(8H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

8-(2-[(3'-O-sulfo-3,5'-cyclic)-3'-O-ethyl-3H-pyrrole(2,3-b)]carbonyl-3,4,11(13)H]-dione (C13 APT)

---

6-(2-[(3'-O-sulfo-3,5'-cyclic)-3'-O-ethyl-3H-pyrrole(2,3-b)]carbonyl-3,4,11(13)H]-dione (C13 APT)
6-[2-(2-oxo-1,3-oxazolidin-3-yl)ethyl]pyrazine[2,3-b]carbazole-5,11(1H)-diene (DQQC)
6-[2-[2-(2-Morpholin-3-yl)ethyl]-5H-pyrazine[2,3-b]carbazole-5,11(6H)-dione [15N HMQC]
SPECTROSCOPIC INFORMATION

Generic Display Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN164_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment:

Acquisition Date: 11/10/2014 2:05:44 PM
Operator: MZ
Instrument: maXis HD

Bruker Compass DataAnalysis 4.2 printed: 11/10/2014 2:09:48 PM by: MZ Page 1 of 1
**Mass Spectrum SmartFormula Report**

**Analysis Info**
- **Analysis Name**: D:\Data\MZ_data\Sprizter_Hi\Thomas_N\141110\TN164_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 0.01 mg/mL in MeOH
- **Comment**: MZ

**Acquisition Parameter**
- **Focus**: Active
- **Scan Begin**: 50 m/z
- **Scan End**: 1550 m/z
- **Nebulizer**: 0.4 Bar
- **Source Type**: Set Capillary
- **Set End Plate Offset**: -500 V
- **Set Charging Voltage**: 2000 V
- **Set Corona**: 0 nA
- **Set Dry Gas**: 4.0 l/min
- **Set Dry Heater**: 200 °C
- **Set APCI Heater**: 0 °C
- **Set Divert Valve**: Waste

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**Comment**: By: MZ | Page 1 of 1
SPECTROSCOPIC INFORMATION

N,N-Diethyl-1H-indole-3-carboxamide (C13 CPD)

[Diagram of N,N-Diethyl-1H-indole-3-carboxamide]

N,N-Diethyl-1H-indole-3-carboxamide (HSQC)

[Diagram of HSQC spectrum for N,N-Diethyl-1H-indole-3-carboxamide]
SPECTROSCOPIC INFORMATION

N,N-Dimethyl-1-(methylamino)-1H-indole-3-carboxamide (15N HMQC)

N,N-Dimethyl-1-(methylamino)-1H-indole-3-carboxamide (NOESY)
1-(2-[(dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (PROTON)

SPECTROSCOPIC INFORMATION

1-(2-[(dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (1H NMR)

47

ppm

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
1-[2-(Dimethylamino)ethyl] N,N-diethyl-1H-indole-3-carboxamide (C13 APT)

1-[2-(Dimethylamino)ethyl] N,N-diethyl-1H-indole-3-carboxamide (HMQC)
1-[2-((Dimethylamino)ethyl)-N,N-dicarbonyl-1H-indole-3-carboxamide (3HN HMBC)
### Mass Spectrum SmartFormula Report

**Analysis Info**

- **Analysis Name**: D:\Data\_MZ_data\Spreitzer_HiThomas_Ni\150112\TN078_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: in MeOH \(\rightarrow\) 1:200 in MeOH
- **Operator**: MZ
- **Instrument**: maXis HD
- **Acquisition Date**: 1/12/2015 2:03:54 PM
- **Comment**: acquisition

**Acquisition Parameter**

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<th>Set Dry Gas</th>
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<th>Scan End</th>
<th>Set Charging Voltage</th>
<th>0 °C</th>
<th>Set APCI Heater</th>
<th>0 ° C</th>
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<tbody>
<tr>
<td>1550 m/z</td>
<td>0 ° C</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

**Intens.**

- **m/z**: 55, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550

**Intens. x10**

- **Intens.**: 0.0, 0.5, 1.0, 1.5, 2.0

**Meas. m/z**

- **m/z**: 310.1892, 575.4069, 884.5878

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<th>err [ppm]</th>
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**Formula**

- C17H25N3O

**Score**

- 100.00

**rdb**

- 6.5

**e-Conf**

- even

**N-Rule**

- ck

---

*TN078_DI_ESI_HRMS.d*

*Bruker Compass DataAnalysis 4.2*  
*Printed: 1/12/2015 2:13:19 PM*  
*By: MZ*  
*Page 1 of 1*
6-Methylamino[5H-pyrimido][3,4-b][carbazolo][1,11-b(11H)]-dioxa (ESQC)

6-Methylamino[5H-pyrimido][3,4-b][carbazolo][1,11-b(11H)]-dioxa (ESQC)
6-(Methoxymethyl)-5H-pyrindone/5,6-b(carbazole-5,11)diene (15N ammoC)
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141117\TN176_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment:

Acquisition Parameter
Source Type: ESI
Scan Begin: 50 m/z
Scan End: 1550 m/z
Focus: Active
Set Capillary: 0.4 Bar
Set End Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e^ Conf N-Rule
294.0873 1 C16H12N3O3 294.0873 0.2 13.5 1 100.00 12.5 even ok
316.0696 1 C16H11N3NaO3 316.0693 -1.0 9.2 1 100.00 12.5 even ok
SPECTROSCOPIC INFORMATION

SIH-Pyrimido[1,6-b]carbazole-5,11(1H) dione (HSQC)

SIH-Pyrimido[1,6-b]carbazole-5,11(1H) dione (HMQC)

464
Generic Display Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\150112\TN181_Di_ESI_HRMS.d
Method: Di_mz_50-1550.m
Sample Name: in MeOH
Comment: +MS, 0.0-1.8min #1-100

250.2140
248.1983
254.1362
250.0611
1+
250.0611

258.2745
261.1596
264.2296
1+
272.0430

268.2748
276.2298
278.2454

272.0433

276.2298

272.0430

Intens. x10^5

0.0
0.2
0.4
0.6
0.8
1.0

1x10^5
2x10^5
3x10^5
4x10^5

250
255
260
265
270
275
m/z

CuH4N3O2, 250.0611

CuH4N3NaO2, 272.0430

Bruker Compass DataAnalysis 4.2  printed: 1/12/2015 1:36:59 PM  by: MZ
Mass Spectrum SmartFormula Report

**Analysis Info**

- **Analysis Name**: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN181_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: in MeOH
- **Comment**: 

**Acquisition Parameter**

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<td>Set End Plate Offset</td>
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<tr>
<td></td>
<td></td>
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<td>2000 V</td>
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<td>Set Corona</td>
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**Scan Details**

- **Scan Begin**: 50 m/z
- **Scan End**: 1550 m/z

**Intens. x106**

- 304.2616
- 585.5330
- 235.2382
- 102.1280

**Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e− Conf N-Rule**

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<tr>
<td>272.0433</td>
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<td>100.00</td>
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</table>

**Diagram**

![Diagram](image-url)
8-[(1-Dimethylamino)ethyl]-2H-pyrido[3,4-b]carbazole-3,11(4H)-dione (PROTON)


8-[(1-Dimethylamino)ethyl]-2H-pyrido[3,4-b]carbazole-3,11(4H)-dione (PROTON)

4.90 4.85 4.80 4.75 4.70 4.65 4.60 4.55 2.80 2.75 2.70 2.65 2.60 2.55 2.50 2.45 2.40 2.35 2.30 ppm
SPECTROSCOPIC INFORMATION

6-[2-((Dimethylamino)ethyl)-5H-pyrrolidin-6,6-b]-carbazole-5,11(6H)-dione (C13 APT)

6-[2-((Dimethylamino)ethyl)-5H-pyrrolidin-6,6-b]-carbazole-5,11(6H)-dione (C13 APT)
SPECTROSCOPIC INFORMATION

6-(2-(Dimethylamino)ethyl)-2H-pyrrole[3,4-b]carbazole-3,11(6H)-dione (C15 APT)

178 176 174 172 170 168 166 164 162 160 158 156 154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110 108

6-(2-(Dimethylamino)ethyl)-2H-pyrrole[3,4-b]carbazole-3,11(6H)-dione (HSQC)
SPECTROSCOPIC INFORMATION

6-[2-(Dimethylamino)ethyl]-5H-pyrrolo[3,6-b]carbazole-5,11(6H)-dione (DMSO)

[Graphical representation of the spectroscopic data]

6-[2-(Dimethylamino)ethyl]-5H-pyrrolo[3,6-b]carbazole-5,11(6H)-dione (DMSO)
Generic Display Report

Analysis Info

Analysis Name: D:\Data\_MZ_data\Sprieter_H\Thomas_N\150112\TN177_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH -> 1:500 in MeOH
Comment: 

Acquisition Date: 1/12/2015 5:23:11 PM
Operator: MZ
Instrument: maXis HD

Bruker Compass DataAnalysis 4.2 printed: 1/12/2015 5:26:22 PM by: MZ Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN177_Di_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:500 in MeOH
Comment:

Acquisition Parameter
Focus: ESI
Scan Begin: 50 m/z
Scan End: 1550 m/z
Source Type: Active
Ion Polarity: Positive
Set Capillary: 1500 V
Set End Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set APCI Heater: 0 °C
Set Nebulizer: 0.4 Bar
Set Dry Gas: 4.0 l/min
Set Dry Heater: 200 °C
Set Divert Valve: Waste

Intens. x10^5

Meas. m/z  # Ion Formula  m/z err [ppm]  mSigma  # mSigma  Score  rdb  e-  Conf  N-Rule
321.1350 1 C18H17N4O2  321.1346  -0.6  5.6  1  100.00 12.5  even  ok
343.1167 1 C18H16N4NaO2  343.1165  0.6  5.6  1  100.00 12.5  even  ok

TN177_Di_ESI_HRMS.d
 Bruker Compass DataAnalysis 4.2  printed: 1/12/2015 5:26:30 PM  by: MZ  Page 1 of 1
SPECTROSCOPIC INFORMATION

Zethyl 3-[(3-pyridinyl)methyl]-1H-indole-2-carboxylate (HMBC)

\[ \text{Chemical Structure} \]

Zethyl 3-[(3-pyridinyl)methyl]-1H-indole-2-carboxylate (HMBC)
Mass Spectrum SmartFormula Report

### Analysis Info
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141117\TN160_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: 0.1 mg/mL in MeOH
- **Comment**

### Acquisition Parameter

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304.2613
585.5322
809.7828
930.7211

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TN160_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2

printed: 11/17/2014 5:33:53 PM
by: MZ
Page 1 of 1
SPECTROSCOPIC INFORMATION

Ethyl 5-(5-pyrimido[4,5-b]pyridin-1-yl)indole-2-carboxylate (PROTON)

N
H
O
O
N
2'2'
3'3'
4'4'
5'
O
11
2
4
7
6
10
11
ppm

Ethyl 5-(5-pyrimido[4,5-b]pyridin-1-yl)indole-2-carboxylate (PROTON)

ppm
Ethyl 3-((pyrimidin-2-yl)carboxy)-1H-indole-2-carboxylate (C13 APT)

SPECTROSCOPIC INFORMATION

485
SPECTROSCOPIC INFORMATION

Zileutol 5-(5-pyrimidinylcarboxyl)-1H-indole-2-carboxylate (HSQC)

Zileutol 5-(5-pyrimidinylcarboxyl)-1H-indole-2-carboxylate (HMBC)

Zileutol 5-(5-pyrimidinylcarboxyl)-1H-indole-2-carboxylate (COSY)
Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (15N HMBC)

Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (15N HSQC)
Generic Display Report

Acquisition Date: 11/17/2014 5:19:59 PM
Analysis Name: D:\Data\_MZ_data\Sprayter_H\Thomas_N_141117\TN159_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment: 296.3061 297.3093 300.2297 302.2455 303.2487 304.2613 305.2644 306.2727 308.1648 311.2557 313.3319 316.3209 318.0851 319.0880 320.0904

Intens. x10^6

m/z

 Bruker Compass DataAnalysis 4.2 printed: 11/17/2014 5:28:12 PM by: MZ Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141117\TN159_DI_ESI_HRMS.d
Method: D1_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment:

Acquisition Parameter
Source Type: ESI
Focus: Active
Scan Begin: 50 m/z
Scan End: 1550 m/z
Scan Begin m/z: 304.2613
Scan End m/z: 585.5332

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e¯ Conf N-Rule
318.0851 1 C16H14N3NaO3 318.0849 -0.5 11.8 1 100.00 11.5 even ok

Mass Spectrum SmartFormula Report
by: MZ
11/17/2014 5:28:04 PM

Bruker Compass DataAnalysis 4.2
printed: 11/17/2014 5:28:04 PM
by: MZ
Page 1 of 1
SPECTROSCOPIC INFORMATION

5H-Pyrrolidine-4,5,6,7-tetrahydro (HSQC)

5H-Pyrrolidine-4,5,6,7-tetrahydro (HMBC)
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spreitzer_HiThomas_Ni141117\TN162_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment:

Acquisition Date: 11/17/2014 5:41:25 PM
Analysis Name: D:\Data\_MZ_data\Spreitzer_HiThomas_Ni141117\TN162_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Operator: MZ
Instrument: maXis HD

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Capillary: 4000 V
Set Dry Gas: 4.0 l/min
Set End Plate Offset: -500 V
Set Divert Valve: Waste
Set Charging Voltage: 2000 V
Set Coron: 0 nA
Set APCI Heater: 0 °C
Scan Begin: 50 m/z
Set End Plate Offset: -500 V
Scan End: 1550 m/z
Set Coron: 0 nA

Meas. m/z  # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e- Conf N-Rule
250.0612 1 C14H8N3O2 250.0611 -0.4 11.0 1 100.00 12.5 even ok
272.0432 1 C14H7N3NaO2 272.0430 -0.7 10.8 1 100.00 12.5 even ok

TN162_DI_ESI_HRMS.d
Printed: 11/17/2014 5:49:51 PM
by: MZ
Zethyl 1-[2-(dimethylamino)ethyl]-3-[5-oxynicotinoyl]-1H-indole-2-carboxylate (HMBIC)
**Mass Spectrum SmartFormula Report**

**Analysis Info**
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_HiThomas_N141117\TN175_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: 0.1 mg/mL in MeOH
- **Comment**: 

**Acquisition Parameter**
- **Source Type**: ESI
- **Set Capillary**: 2000 V
- **Set Dry Gas**: 4.0 l/min
- **Set Charging Voltage**: 2000 V
- **Set Corona**: 0 nA
- **Set APCI Heater**: 0 °C
- **Set Nebulizer**: 0.4 Bar
- **Set Dry Heater**: 200 °C
- **Set End Plate Offset**: -500 V
- **Set Divert Valve**: Waste

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<th>#</th>
<th>Ion Formula</th>
<th>m/z</th>
<th>err [ppm]</th>
<th>mSigma</th>
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<th>Score</th>
<th>rdb</th>
<th>e¯</th>
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<td>11.5</td>
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<td>367.1770</td>
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![Mass Spectrum](image.png)
Ethyl 1-(2-(dimethylamino)ethyl)-1H-indole-2-carboxylate (PROTON)
SPECTROSCOPIC INFORMATION

Ethyl 1-[N-(dimethylamino)ethyl]-1H-indole-2-carboxylate (C15 APT)

[Chemical structure image]

---

Ethyl 1-[N-(dimethylamino)ethyl]-1H-indole-2-carboxylate (C15 APT)

[Chemical structure image]
Etethyl 1-(2-dimethylamino)ethyl-1H-indole-2-carboxylate (S15 AP7)

Etethyl 1-(2-dimethylamino)ethyl-1H-indole-2-carboxylate (D1SQC)
SPECTROSCOPIC INFORMATION

Ethyl 1-[2-(dimethylamino)ethyl]-1H-tetole-2-carboxylate (HMQC)

[Diagram of the spectroscopic analysis]

Ethyl 1-[2-(dimethylamino)ethyl]-1H-tetole-2-carboxylate (13C HMQC)

[Diagram of the spectroscopic analysis]

Ethyl 1-[2-(dimethylamino)ethyl]-1H-tetole-2-carboxylate (1H HMQC)

[Diagram of the spectroscopic analysis]
SPECTROSCOPIC INFORMATION

10-[2-(Dimethylamino)ethyl]-112-pyrimido[4,5-b]carbazole-3,11(1H)-dione (PROTON)

10-[2-(Dimethylamino)ethyl]-112-pyrimido[4,5-b]carbazole-3,11(1H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

10-[(2-Dimethylamino)ethyl]-5H-pyrrolo[4,3-b]carbazole-3,11(1H)-dione (C13 APT)

180 175 170 165 160 155 150 145 140 135 130 125 120 115 110

10-[(2-Dimethylamino)ethyl]-5H-pyrrolo[4,3-b]carbazole-3,11(1H)-dione (HSQC)
10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]xanthenes 5,11(1H)-dione (ESN EMB)
**Analysis Info**

**Acquisition Date**: 11/4/2014 12:45:21 PM

**Analysis Name**: D:\Data\_MZ_data\Spiretze\Thomas_N\141104\TN163_DI_ESI_HRMS.d

**Method**: DI_mz_50-1550.m

**Sample Name**: approx. 10 ug/mL in MeOH

**Comment**: +MS, 0.0-2.2min #1-127

---

**Generic Display Report**

**Operator**: MZ

**Instrument**: maXis HD
Mass Spectrum SmartFormula Report

**Analysis Info**

**Analysis Name**: D:\Data\_MZ_data\Spritzer\Thomas_N\141104\TN163_DI_ESI_HRMS.d  
**Method**: DI_mz_50-1550.m  
**Sample Name**: approx. 10 ug/mL in MeOH  
**Comment**:  

**Acquisition Date**: 11/4/2014 12:45:21 PM  
**Operator**: MZ  
**Instrument**: maXiS HD 1820881.21300

**Acquisition Parameter**

- **Source Type**: ESI
- **Ion Polarity**: Positive
- **Scan Begin**: 50 m/z
- **Scan End**: 1550 m/z
- **Set Nebulizer**: 0.4 Bar
- **Set Dry Heater**: 200 °C
- **Set Capillary**: 1800 V
- **Set End Plate Offset**: -500 V
- **Set Dry Gas**: 4.0 l/min
- **Set Charging Voltage**: 2000 V
- **Set Divert Valve**: Waste
- **Set Corona**: 0 nA
- **Scan Begin**: 0 °C
- **Set APCI Heater**: 0 °C

**Mass Spectrum**

- Measured m/z: 321.1349, 343.1170, 663.2445, 983.3705
- Ion Formula: C18H17N4O2, C18H16N4NaO2
- Error [ppm]: -0.8, -1.5
- Sigma m/s: 3.3, 6.4
- Score: 100.00
- rtb: 12.5
- Confidence: even
- Rule: ok

**Chemical Structure**

![Chemical Structure Diagram]
SPECTROSCOPIC INFORMATION

Fura(3,4-b)(quinolin-1(2H)-one (C13 APT)

ppm

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

Fura(3,4-b)(quinolin-1(2H)-one (C13 APT)

ppm

72 170 168 166 164 162 154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 80 78 76 74 72 70 68

517
SPECTROSCOPIC INFORMATION

Pure[6,4-8quinoline-1(3H)-one (HMQC)

Pure[6,4-8quinoline-1(3H)-one (HMBC)
SPECTROSCOPIC INFORMATION

6H-indole[2',3':6,7']benz[1,4]benzodiazepin-3,4,6-trione (PROTON)

6H-indole[2',3':6,7']benz[1,4]benzodiazepin-3,4,6-trione (PROTON)
**SPECTROSCOPIC INFORMATION**

483: Nucleos[2',3',6',7']tetrahydro[1,8-bipyrimidine-3,4-biquinoline]-33(12H)-one (C13 APT)

![Spectroscopic Image](image)

483: Nucleos[2',3',6',7']tetrahydro[1,8-bipyrimidine-3,4-biquinoline]-33(12H)-one (HSQC)
SPECTROSCOPIC INFORMATION

6H-indole[2',3':6,7]carbazole[3,6-b]quinolin-13(12H)-one (HMBG)

6H-indole[2',3':6,7]carbazole[3,6-b]quinolin-13(12H)-one (15N HMBG)

ppm

523
6H-furano[2',3':6,7]carbazolo[3,4-b]pyrazolo[1,5-d][1,2,4]triazin-15(12H)-on (15N HSQC)

6H-furano[2',3':6,7]carbazolo[3,4-b]pyrazolo[1,5-d][1,2,4]triazin-15(12H)-on (COSY)

524
Mass Spectrum SmartFormula Report

Analysis Info
Acquisition Date 11/17/2014 6:10:10 PM
Analysis Name D:\Data\_MZ_data\Speitzer_HiTThomas_Ni\141117\TN171_DI_ESI_HRMS.d
Method DI_mz_50-1550.m
Sample Name 0.1 mg/mL in MeOH
Comment

Acquisition Parameter
Source Type ESI
Ion Polarity Positive
Scan Begin 50 m/z
Scan End 1550 m/z
Set Capillary 4000 V
Set End Plate Offset -500 V
Set Dry Gas 4.0 l/min
Set Dry Heater 200 °C
Set Charging Voltage 2000 V
Set Divert Valve Waste
Set Corona 0 nA
Set APCI Heater 0 °C

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb eConf N-Rule
301.0974 1 C19H13N2O2 301.0972 -0.7 15.1 1 100.00 14.5 even ok
323.0794 1 C19H12N2NaO2 323.0791 -0.9 27.6 1 100.00 14.5 even ok

TN171_DI_ESI_HRMS.d
Bruker Compass DataAnalysis 4.2 printed: 11/17/2014 6:16:24 PM by: MZ
SPECTROSCOPIC INFORMATION

69H-indole[2,3-b]pyridazine-6,12(1H)-dione (C15 APT)

ppm

180 178 176 174 172 170 168 166 164 162 160 158 156 154 152 150 148 146 144 142 140 138 136 134 132 130

69H-indole[2,3-b]pyridazine-6,12(1H)-dione (C15 APT)

ppm

31 130 129 128 127 126 125 124 123 122 121 120 119 118 117 116 115 114
Generic Display Report

Analysis Info
- Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141117\TN172_DI_ESI_HRMS.d
- Method: DI_mz_50-1550.m
- Sample Name: 0.1 mg/mL in MeOH
- Comment: +MS, 0.0-1.7min #1-96

Acquisition Date: 11/17/2014 6:15:51 PM
Operator: MZ
Instrument: maXis HD

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Mass Spectrum SmartFormula Report

### Analysis Info
- **Acquisition Date**: 11/17/2014 6:15:51 PM
- **Analysis Name**: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141117\TN172_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: 0.1 mg/mL in MeOH
- **Operator**: MZ
- **Instrument**: maXis HD
- **Serial Number**: 1820881.21300

### Acquisition Parameter

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<td>Set Dry Gas</td>
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<td>Set APCI Heater</td>
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<td>Set Corona</td>
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### Mass Spectrum

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<th>Ion Formula</th>
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<th>rdb</th>
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![Chemical Structure](image-url)

TN172_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2 printed: 11/17/2014 6:19:09 PM by: MZ
SPECTROSCOPIC INFORMATION

11-[2-(N,N-Dimethylamino)ethyl]-6H-indole[2,3-b]acridine-6,12(11H)-dione (C33 APT)

ppm

60

11-[2-(N,N-Dimethylamino)ethyl]-6H-indole[2,3-b]acridine-6,12(11H)-dione (C33 APT)
SPECTROSCOPIC INFORMATION

$11\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]\text{-4H-indole}(2,3,4)$-cyclohexene-6,12(11H)-dione [HSQC]

$11\text{-}[2\text{-}(\text{dimethylamino})\text{ethyl}]\text{-4H-indole}(2,3,4)$-cyclohexene-6,12(11H)-dione [HMBC]
11-[2-(methylamino)ethyl]-6H-indolo[2,3-b]carbazole-6,12(11H)-dione (11N IND8C)

11-[2-(methylamino)ethyl]-6H-indolo[2,3-b]carbazole-6,12(11H)-dione (CO87)
Generic Display Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_N141104\TN173_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 10 ug/mL in MeOH
Comment:

Acquisition Date: 11/4/2014 1:25:35 PM
Operator: MZ
Instrument: maXis HD

Bruker Compass DataAnalysis 4.2
printed: 11/4/2014 1:33:44 PM
by: MZ
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Mass Spectrum SmartFormula Report

Analysis Info
Acquisition Date 11/4/2014 1:25:35 PM

Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_N\141104\TN173_DI_ESI_HRMS.d
Method: D1_mz_50-1550.m
Operator: MZ
Instrument: maXis HD 1820881.21300
Sample Name: approx. 10 ug/mL in MeOH
Comment:

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<tr>
<td>Set Corona</td>
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Scan Parameter

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Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e− Conf N-Rule
370.1545 1 C22H20N3O2 370.1550 1.4 21.6 1 100.00 15.5 even ok
392.1368 1 C23H19N3NaO2 392.1369 0.4 10.4 1 100.00 15.5 even ok

TN173_DI_ESI_HRMS.d

SPECTROSCOPIC INFORMATION

N,N'-Dioxy-4-quinolinoxalamide (HMQC)

N,N'-Dioxy-4-quinolinoxalamide (HMBC)
SPECTROSCOPIC INFORMATION

N,N-Diethyl-4-quinolinicarbamide (1H-NMR)

N,N-Diethyl-4-quinolinicarbamide (GOF)
13-(Methoxymethyl)-7H-indole[3,2-\text{\textit{d}}]/phenanthroline-7,13(12H)-dione (PROTON)

SPECTROSCOPIC INFORMATION
12-(Methoxymethyl)-7H-indole/3,2-1[phenanthridine-7,13(12H)-dione (BSQG)

12-(Methoxymethyl)-7H-indole/3,2-1[phenanthridine-7,13(12H)-dione (EMEC)
SPECTROSCOPIC INFORMATION

12-(Methoxymethyl)-7H-isole[3,2-c]phenanthridine-7,13(12H)-dione (1sN HMBG)

12-(Methoxymethyl)-7H-isole[3,2-c]phenanthridine-7,13(12H)-dione (COSY)
Analysis Info
- Analysis Name: D:\Data\MZ_data\Spaetzer_H\Thomas_N\141117\TN161_DI_ESI_HRMS.d
- Method: DI_mz_50-1550.m
- Sample Name: 0.1 mg/mL in MeOH
- Comment:

Acquisition Date: 11/17/2014 5:33:29 PM
- Operator: MZ
- Instrument: maXis HD

Generic Display Report

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Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141117\TN161_DI_ESI_HRMS.d
Method: DI.mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment: 

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Focus: Active
Scan Begin: 50 m/z
Scan End: 1550 m/z
Set Capillary: 3500 V
Set End Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste
Set APCI Heater: 0 °C

Meas. m/z | # | Ion Formula | m/z | err [ppm] | mSigma | # | mSigma | Score | rdb | e- | Conf | N-Rule | MZ 0.0-1.5min #1-85
---------|---|------------|-----|----------|--------|---|--------|-------|-----|----|------|--------|-------------------
343.1081 | 1 | C21H15N2O3 | 343.1077 | -1.0 | 9.0 | 1 | 100.00 | 15.5 | even | ok |
365.0901 | 1 | C21H14N2NaO3 | 365.0897 | -1.1 | 19.1 | 1 | 100.00 | 15.5 | even | ok |
7H-indole[3,2-\text{a}](\text{phenoaxanthin} \text{-7,13(12H)} \text{-dione (35Q)C})

7H-indole[3,2-\text{a}](\text{phenoaxanthin} \text{-7,13(12H)} \text{-dione (35MC)C})
SPECTROSCOPIC INFORMATION

7H-Isoindo[3,2-d]phenanthridine-7,15(12H)-dione (11N HMBC)

7H-Isoindo[3,2-d]phenanthridine 7,15(12H)-dione (COSY)
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141117\TN165_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment:

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Scan Begin: 50 m/z
Scan End: 1550 m/z
Focus: Active
Set Capillary: 4000 V
Set End Plate Offset: -500 V
Set Dry Gas: 4.0 l/min
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set APCI Heater: 0 °C
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Divert Valve: Waste

Mass Spectrum SmartFormula Report

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<th>m/z</th>
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<th>#</th>
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<th>Score</th>
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<th>ε−</th>
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TN165_DI_ESI_HRMS.d

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SPECTROSCOPIC INFORMATION


Diagram showing the 1H NMR spectrum with peaks at various ppm values and chemical shifts.


Diagram showing the 1H NMR spectrum with peaks at various ppm values and chemical shifts.
SPECTROSCOPIC INFORMATION

7H-indole[3,2-b]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)

7H-indole[3,2-b]phenanthridine-7,13(12H)-dione 5-oxide (C13 APT)

565
7H-indole[3,2-d]phenanthridine-7,13(12H)-dione 5-oxide (C13 APT)

7H-indole[3,2-d]phenanthridine-7,13(12H)-dione 5-oxide (HSQC)
SPECTROSCOPIC INFORMATION

7H-Indole[3,2-j]phenanthridine-7,13-[12H]-dione 5-oxide (13N HSQC)

7H-Indole[3,2-j]phenanthridine-7,13-[12H]-dione 5-oxide (COSY)
SPECTROSCOPIC INFORMATION
**Analysis Info**
- **Analysis Name**: D:\Data\_MZ_data\Sprizter_H\Thomas_N\141117\TN166_Di_ESI_HRMS.d
- **Method**: Di_mz_50-1550.m
- **Sample Name**: 0.1 mg/mL in MeOH
- **Comment**: 0.0-2.8min #1-160

**Generic Display Report**

![Spectroscopic Information Graph]

**Bruker Compass DataAnalysis 4.2**
- **Acquisition Date**: 11/17/2014 6:00:27 PM
- **Operator**: MZ
- **Instrument**: maXis HD

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**Page 1 of 1**
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spritzer_H\Thomas_N\141117\TN166_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment: MZ

Acquisition Parameter
Source Type: ESI
Focus: Active
Scan Begin: 50 m/z
Scan End: 1550 m/z
Ion Polarity: Positive
Set Capillary: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste
Set APCI Heater: 0 °C

Intens. x10^5

Meas. m/z  # Ion Formula m/z err [ppm] mSigma  # mSigma Score rdb  e^- Conf N-Rule
315.0767 1 C4H7N14O4 315.0769 0.7  n.a.    1 100.00 8.5 even  ok
2 C19H11N2O3 315.0764 -0.8 400.7  2 0.00 15.5 even  ok
337.0585 1 C19H10N2NaO3 337.0584 0.3  5.5  1 100.00 15.5 even  ok
SPECTROSCOPIC INFORMATION

12-(2-(Dimethylamino)ethyl)-7H-indole-3,6,11,12-tetrahydro-7,11(12H)-dione (PROTON)

12-(2-(Dimethylamino)ethyl)-7H-indole-3,6,11,12-tetrahydro-7,11(12H)-dione (PROTON)

12-(2-(Dimethylamino)ethyl)-7H-indole-3,6,11,12-tetrahydro-7,11(12H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

12-[2-(Dimethylamino)ethyl]-7H-indole-1,3(1H,13H)-dione (C13 APT)

12-[2-(Dimethylamino)ethyl]-7H-indole-1,3(1H,13H)-dione (HMQC)
SPECTROSCOPIC INFORMATION

12-[2-(Dimethylamino)ethyl]-7H-indole[3,2-b]phemanthridine-7,13(12H)-dione (11H)BCC

13-[2-(Dimethylamino)ethyl]-7H-indole[3,2-b]phemanthridine-7,13(12H)-dione (12H)BCC

13-[2-(Dimethylamino)ethyl]-7H-indole[3,2-b]phemanthridine-7,13(12H)-dione (11N)BCC
12-[3-(Dimethylamino)ethyl]-7H-indolo[3,2-a]phenanthridine-7,13(12H)-dione (15N HMQC)

13-[3-(Dimethylamino)ethyl]-7H-indolo[3,2-a]phenanthridine-7,13(12H)-dione (COV)
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data\MZ_datal\Spritzer\Thomas_Ni\141104\TN167_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 10 ug/mL in MeOH
Comment: MaXis HD 1820881.21300

Acquisition Parameter

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![Mass Spectrum](attachment:image)

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TN167_DI_ESI_HRMS.d

SPECTROSCOPIC INFORMATION

12-(2-(Dimethylamino)ethyl)-7H-indole[3,2-a](1H)-benzo[b]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)

12-(2-(Dimethylamino)ethyl)-7H-indole[3,2-a](1H)-benzo[b]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)
SPECTROSCOPIC INFORMATION

12-(2-Dimethylamino)ethyl-7H-indole-3,4-phenanthridine-7,13(12H)-dione 5-oxide (C13 APT)

13-(2-Dimethylamino)ethyl-7H-indole-3,4-phenanthridine-7,13(12H)-dione 5-oxide (C13 APT)

ppm

582
SPECTROSCOPIC INFORMATION

12-[2-(Dimethylamino)ethyl]-7H-indole/3,2-1(phenanthridine/7,13(1H)-dione 5-oxide (15N EMBC)

12-[2-(Dimethylamino)ethyl]-7H-indole/3,2-1(phenanthridine/7,13(1H)-dione 5-oxide (COXY)
Generic Display Report

Analysis Info

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Bruker Compass DataAnalysis 4.2  printed: 11/4/2014 1:26:53 PM  by: MZ  Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_N\141104\TN168_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 10 ug/mL in MeOH
Comment: MZOperator

Acquisition Date: 11/4/2014 1:21:03 PM
Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_N\141104\TN168_DI_ESI_HRMS.d
Operator: MZ
Instrument: maXis HD 1820881.21300

Acquisition Parameter

Source Type: ESI
Scan Begin: 50 m/z
Scan End: 1550 m/z
Scan End Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set APCI Heater: 0 °C
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e⁻ Conf N-Rule
386.1491 1 C23H20N3O3 386.1499 2.0 1.3 1 100.00 15.5 even ok
408.1311 1 C23H19N3NaO3 408.1319 1.9 8.3 1 100.00 15.5 even ok

66

TN168_DI_ESI_HRMS.d
Bruker Compass DataAnalysis 4.2
Page 1 of 1
SPECTROSCOPIC INFORMATION

1-(2-Quinolinyl)-1,2,3,4-butanetetrol (PROTON)

ppm

13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.

1-(2-Quinolinyl)-1,2,3,4-butanetetrol (PROTON)

ppm

9.2 9.1 9.0 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 3.8 3.7 3.6 3.5 3.4
SPECTROSCOPIC INFORMATION

1-(2-Quinolyl)-1,2,3,4-butanetetrol (C13 CP)
SPECTROSCOPIC INFORMATION

1-(2-Quinolineyl)-1,2,3,4-butanetetrol (C13 CPD)

1-(2-Quinolineyl)-1,2,3,4-butanetetrol (HSQC)
SPECTROSCOPIC INFORMATION

1-(2-Quinolineyl)-1,2,3,4-butanetetrol (1HMC)

1-(2-Quinolineyl)-1,2,3,4-butanetetrol (15N 1HMC)
2-Quinolinocarboxylic acid (PROTON)

14.5 14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

2-Quinolinocarboxylic acid (PROTON)
SPECTROSCOPIC INFORMATION

2-Quinoliniccarboxylic acid (HMBC)

2-Quinoliniccarboxylic acid (13C-HMBC)
SPECTROSCOPIC INFORMATION

N,N-Diethyl-2-quinolinonecarboxamide (PROTON)

N,N-Diethyl-2-quinolinonecarboxamide (Cl3 AP7)

ppm

ppm
SPECTROSCOPIC INFORMATION

N,N-Diethyl-2-quinazolinecarboxamide (C13 APT)

N,N-Diethyl-2-quinazolinecarboxamide (DSQC)
SPECTROSCOPIC INFORMATION

5-(Methoxymethyl)-5H-indole(2,3-5)phenazine-4,13-dione (18N HMQC)

5-(Methoxymethyl)-5H-indole(2,3-5)phenazine-4,13-dione (COSY)
**SPECTROSCOPIC INFORMATION**

**Generic Display Report**

**Analysis Info**

- **Analysis Name**: D:\Data\_MZ\data\Spreitzer_H\Thomas_N\141110\TN137_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 0.01 mg/mL in MeOH
- **Comment**:

  - 366.0849
  - 367.0879
  - 368.0909
  - 369.2974

  +MS, 0.0-1.8min #1-104

**Comment**:

- **Operator**: MZ
- **Instrument**: maXis HD

**Bruker Compass DataAnalysis 4.2**

- **Printed**: 11/10/2014 1:23:41 PM
- **By**: MZ

Page 1 of 1
Mass Spectrum SmartFormula Report

**Analysis Info**
- Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN137_DI_ESI_HRMS.d
- Method: DI_mz_50-1550.m
- Sample Name: approx. 0.01 mg/mL in MeOH
- Instrument: maxis HD
- Operator: MZ
- Acquisition Date: 11/10/2014 1:19:19 PM
- Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN137_DI_ESI_HRMS.d

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**Mass Spectrum**

- Meas. m/z: 366.0849, 366.0849
- # Ion Formula: C20H13N3NaO3
- err [ppm]: 0.1, 9.8
- mSigma: 1, 100.00
- # mSigma: 1, 15.5
- Score: even
- N-Rule: ok

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**Diagram**

- Compound representation
- Molecular weight: 70
Generic Display Report

Analysis Info
- Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141110\TN138_DI_ESI_HRMS.d
- Method: DI_mz_50-1550.m
- Sample Name: approx. 0.01 mg/mL in MeOH
- Comment: +MS, 0.0-2.3min #1-129

Analysis Name: C$_{18}$H$_9$N$_3$NaO$_2$, 322.0587

Bruker Compass DataAnalysis 4.2 printed: 11/10/2014 1:32:17 PM by: MZ Page 1 of 1
### Mass Spectrum SmartFormula Report

**Analysis Info**
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_HiThomas_N\141110\TN138_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 0.01 mg/mL in MeOH
- **Comment**: MZOperator

**Acquisition Parameter**
- **Source Type**: ESI
- **Scan Begin**: 50 m/z
- **Scan End**: 1550 m/z
- **Ion Polarity**: Positive
- **Set Capillary**: 3300 V
- **Set End Plate Offset**: -500 V
- **Set Charging Voltage**: 2000 V
- **Set Corona**: 0 nA
- **Set Nebulizer**: 0.4 Bar
- **Set Dry Heater**: 200 °C
- **Set Dry Gas**: 4.0 l/min
- **Set Divert Valve**: Waste
- **Set APCI Heater**: 0 °C

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![Mass Spectrum](image)

**TN138_DI_ESI_HRMS.d**

**Printer**: Bruker Compass DataAnalysis 4.2

**Printed**: 11/10/2014 1:32:25 PM

**By**: MZ
SPECTROSCOPIC INFORMATION

5-[1-(Dimethylamino)ethyl]-5H-indole[2,3-b]pyrazine-6,13-dione (PROTON)

13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

5-[1-(Dimethylamino)ethyl]-5H-indole[2,3-b]pyrazine-6,13-dione (PROTON)

N 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

N
SPECTROSCOPIC INFORMATION

5-[2-(Dimethylamino)ethyl]-3H-indole[2,3-b]phthanthrene-6,13-dione (HQC)

5-[2-(Dimethylamino)ethyl]-3H-indole[2,3-b]phthanthrene-6,13-dione (HMBG)
SPECTROSCOPIC INFORMATION

5-[2-[(Dimethylamino)ethyl]-thieno]-2,3-benzoazole-6,13-dione

5-[(Dimethylamino)ethyl]-2,3-benzoazole-6,13-dione (CD3CN)

5-[(Dimethylamino)ethyl]-2,3-benzoazole-6,13-dione (CD3OD)
Analysis Info

Acquisition Date: 11/4/2014 12:25:53 PM
Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_N\141104\TN139_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Operator: MZ
Sample Name: approx. 10 ug/mL in MeOH
Instrument: maXis HD
Comment: 

Generic Display Report

Bruker Compass DataAnalysis 4.2
by: MZ
Page 1 of 1
Mass Spectrum SmartFormula Report

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TN139_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2 printed: 11/4/2014 12:32:27 PM by: MZ Page 1 of 1
SPECTROSCOPIC INFORMATION

N,N-Diethyl-2-quinolinesulfonamide (PROTON)

N,N-Diethyl-2-quinolinesulfonamide (PROTON)
N,N-Diethyl-2-quinolino-carbamide (C13 APT)

SPECTROSCOPIC INFORMATION
N,N-Diethyl-2-quinolincarbamamide (1HN HSQC)

N,N-Diethyl-2-quinolincarbamamide (COSY)
SPECTROSCOPIC INFORMATION

7-(Methoxymethyl)-6H-indole[2,3-b]carbazole-6,12(1H)-diene (PROTON)

12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

7-(Methoxymethyl)-6H-indole[2,3-b]carbazole-6,12(1H)-diene (PROTON)
SPECTROSCOPIC INFORMATION

7-(Methoxymethyl)-5H-indolo[1,2-b]carbazole-6,12(7H)-dione (C13 APT)

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

17.39 17.69
17.39 17.69
1.49 1.55
1.49 1.55

7-(Methoxymethyl)-5H-indolo[1,2-b]carbazole-6,12(7H)-dione (C13 APT)

180 178 176 174 172 170 168 166 164 162 160 158 156 154 152 150 148 144 142 140 138 136 134 132
ppm

6a 13 3 4
6a 5a 7a
6 12
7-(Methoxymethyl)-6H-indole|3,2-b|azepin-6,12(7H)-diene (C13 APT)

7-(Methoxymethyl)-6H-indole|3,2-b|azepin-6,12(7H)-diene (HSQC)
7-(3-Methylthio)-4-(1H)isoquinoline (HMQC)
SPECTROSCOPIC INFORMATION

7-(Methoxymethyl)-8H-indole[3,2-b]carbazole-6,12(7H)-dien-8a (15N HMQC)

7-(Methoxymethyl)-8H-indole[3,2-b]carbazole-6,12(7H)-dien (COSY)
### Analysis Info

**Analysis Name:** D:\Data\MZ_data\Spreitzer_HiThomas_N\141110\TN145_DI_ESI_HRMS.d  
**Method:** DI_mz_50-1550.m  
**Sample Name:** approx. 0.01 mg/mL in MeOH  
**Comment:**

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### Mass Spectrum SmartFormula Report

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<td>365.0900</td>
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**TN145_DI_ESI_HRMS.d**

**Broker Compass DataAnalysis 4.2**  
**printed:** 11/10/2014 1:51:00 PM  
**by:** MZ  
**Page 1 of 1**
SPECTROSCOPIC INFORMATION

6H-indole(3,2-b)carbazole-4,12(7H)-dione (C13 CPD)

[Graphical representation of the molecule with ppm values]

6H-indole(3,2-b)carbazole-4,12(7H)-dione (C13 CPD)

[Graphical representation of the molecule with ppm values]
SPECTROSCOPIC INFORMATION

6H-indole[3,2-b]carbazole-8,12(1H,3H)-dione (COST)

[Diagram of the molecule and NMR spectrum]
Generic Display Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN136_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: 

Acquisition Date: 11/10/2014 1:11:29 PM
Operator: MZ
Instrument: maXis HD

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[C19H10N2NaO2, 321.0634] 0.0
[C19H10N2NaO2, 322.0666] 1+
[C19H10N2NaO2, 323.0694] 1+
[C19H10N2NaO2, 324.3372] 1+

---

[C6H10N2O, 320.2558]
[C6H10N2O, 321.0635]
[C6H10N2O, 322.0666]
[C6H10N2O, 323.0695]
[C6H10N2O, 324.3372]

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Bruker Compass DataAnalysis 4.2 printed: 11/10/2014 1:15:25 PM by: MZ
Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN136_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: approx. 0.01 mg/mL in MeOH
Comment: approx. 0.01 mg/mL in MeOH

Acquisition Date: 11/10/2014 1:11:29 PM
Acquisition Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141110\TN136_DI_ESI_HRMS.d
Operator: MZ
Instrument: maXis HD

Comment

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Scan Begin: 50 m/z
Scan End: 1550 m/z
Set Capillary: 2500 V
Set End Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste
Set APCI Heater: 0 °C

Mass Spectrum

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e- Conf N-Rule
321.0635 1 C19H10N2NaO2 321.0634 -0.3 12.0 1 100.00 15.5 even ok

Diagram
7-[(1-Dimethylaminomethyl)-1H-indole-3,2-b]-acridine-4,12(7H) dione (PROTON)

76
7-(2-(Dimethylamino)ethyl)-6H-Indole(3,2-b)carbazole-4,12(1H)-dione (PROTON)

7-(2-(Dimethylamino)ethyl)-6H-Indole(3,2-b)carbazole-4,12(1H)-dione (APT)
SPECTROSCOPIC INFORMATION

7-[2-(Dimethylamino)ethyl]-4H-(nucleoside)-1(2,3,5)-dehydronucleoside (C13 APT)

7-[2-(Dimethylamino)ethyl]-4H-(nucleoside)-1(2,3,5)-dehydronucleoside (HSQC)
7-[2-(Dimethylamino)ethyl]-6H-indole-3,12(1H)-dione (HMBB)
SPECTROSCOPIC INFORMATION

7- [2-(Dimethylamino)ethyl]-6H-indole[3,2-b]acridine-4,12(7H)-dione (COSY)

7- [2-(Dimethylamino)ethyl]-6H-indole[3,2-b]acridine-4,12(7H)-dione (NOE)

644
Generic Display Report

Analysis Info
- Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_N\141104\XXQ3_Di_ESI_HRMS.d
- Method: DI_mz_50-1550.m
- Sample Name: approx. 10 ug/mL in MeOH
- Comment:

Acquisition Date: 11/4/2014 10:34:54 AM
- Operator: MZ
- Instrument: maXis HD

Bruker Compass DataAnalysis 4.2
printed: 11/4/2014 11:50:02 AM by: MZ Page 1 of 1
Analysis Name: D:\Data\MZ_data\Spreitzer\Thomas_Ni\141104\XXQ3_DI_ESI_HRMS.d
Method: DI mz 50-1550
Sample Name: approx. 10 ug/mL in MeOH
Comment: 11/4/2014 10:34:54 AM

Acquisition Parameter

Source Type: ESI  Ion Polarity: Positive  Set Nebulizer: 0.4 Bar
Focus: Active  Set Capillary: 1800 V  Set Dry Heater: 200 °C
Scan Begin: 50 m/z  Set End Plate Offset: -500 V  Set Dry Gas: 4.0 l/min
Scan End: 1550 m/z  Set Charging Voltage: 2000 V  Set Divert Valve: Waste
Set Corona: 0 nA  Set APCI Heater: 0 °C

Mass Spectrum SmartFormula Report

XXQ3_DI_ESI_HRMS.d

by: MZ  Page 1 of 1

Bruker Compass DataAnalysis 4.2  printed: 11/4/2014 11:50:11 AM  by: MZ
SPECTROSCOPIC INFORMATION

8-(Methoxymethyl)-7H-indole[2,3-](phenanthridine-7,13(HH))-dione (PROTON)

8-(Methoxymethyl)-7H-indole[2,3-](phenanthridine-7,13(HH))-dione (C13 APT)
8-(Methoxymethyl)-7H-indole[2,3-e]phenanthridine-7,13(14H)-dione (HMQC)
SPECTROSCOPIC INFORMATION

8-(Methoxymethyl)-7H-indole[2,3-\{phenanthridine-7,13(8H)-dione (13N HMB)
8-(Methoxymethyl)-7H-indole[2,3-\(\beta\)]phenanthridine-7,13(4H)-dione (NOESY)

8-(Methoxymethyl)-7H-indole[2,3-\(\beta\)]phenanthridine-7,13(4H)-dione (COSY)
### Analysis Info

- **Analysis Name:** D:\Data\MZ_data\Spreitzer_H\Thomas_N\141117\AK018_DI_ESI_HRMS.d
- **Method:** DI.mz_50-1550.m
- **Sample Name:** 0.1 mg/mL in MeOH
- **Method:** maXis HD
- **Instrument:** MZ
- **Operator:**
- **Comment:**

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### Mass Spectrum SmartFormula Report

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![Chemical Structure](attachment:image.png)

**AK018_DI_ESI_HRMS.d**

**Printed:** 11/17/2014 6:53:05 PM by: MZ
SPECTROSCOPIC INFORMATION

7H-indole[2,3-\(\text{f}\)]phenanthridine-7,13(1H)-dione (C15 CPD)

7H-indole[2,3-\(\text{f}\)]phenanthridine-7,13(1H)-dione (HMQC)
SPECTROSCOPIC INFORMATION

7H-indole[2,3-\text{\textit{b}}]:7H-phenanthridine-7,13(11H)-dione (1H-MBC)

7H-indole[2,3-\text{\textit{b}}]:7H-phenanthridine-7,13(11H)-dione (15N H-MBC)
SPECTROSCOPIC INFORMATION

7H-indole[2,3-c]phenanthridine-7,13(8H)-dione (1H HSQC)

7H-indole[2,3-c]phenanthridine-7,13(8H)-dione (COSY)
Mass Spectrum SmartFormula Report

**Analysis Info**

- Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141117\TN149_DI_ESI_HRMS.d
- Method: DI_mz_50-1550.m
- Sample Name: 0.1 mg/mL in MeOH
- Comment

**Acquisition Parameter**

- Source Type: ESI
- Ion Polarity: Positive
- Set Nebulizer: 0.4 Bar
- Set Capillary: 3000 V
- Set Dry Heater: 200 °C
- Scan Begin: 50 m/z
- Scan End: 1550 m/z
- Set Dry Gas: 4.0 l/min
- Set APCI Heater: 0 °C
- Set Charging Voltage: 0 nA
- Set Dod Heater: Set Capillary: 3000 V
- Focus: Active
- Set End Plate Offset: -500 V
- Set Divert Valve: Waste

**Mass Spectrum SmartFormula Report**

- Meas. m/z: 299.0815
- Ion Formula: C19H11N2O2
- err [ppm]: -0.1
- mSigma: 1.8
- # mSigma: 1
- Score: 100.00
- conf: 15.5
- N-Rule: even

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**Chemical Structure**

[Chemical structure image]
SPECTROSCOPIC INFORMATION

8-[(3-Methylamino)ethyl]-7H-isoindole, 1,3'-[phenanthridine-7,13(HH)-dione (PROTON)]

8-[(3-Methylamino)ethyl]-7H-isoindole, 1,3'-[phenanthridine-7,13(HH)-dione (C13 APT)]
8-[2-(Dimethylamino)ethyl]-7H-indole-2,3-(6H,11H)-dione (C13 APT)
SPECTROSCOPIC INFORMATION

8-[1-(Dimethylamino)ethyl]-7H-indolo[2,3-b]phenoxazirine-7,13(8H)-dione (1H-MBC)

8-[1-(Dimethylamino)ethyl]-7H-indolo[2,3-b]phenoxazirine-7,13(8H)-dione (15N H-MBC)
SPECTROSCOPIC INFORMATION

8-[[3-(Dimethylamino)ethyl]-7H-indole]-2,3-(phenanthridine-7,13)bis(diene) (NOESY)

8-[[3-(Dimethylamino)ethyl]-7H-indole]-2,3-(phenanthridine-7,13)bis(diene) (COSY)
### Generic Display Report

**Analysis Info**

- **Analysis Name**: D:\Data\MZ_data\Spreitzer\Thomas_Ni141104\AK024_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 10 ug/mL in MeOH

**Comment**

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**Acquisition Date**: 11/4/2014 9:58:40 AM

**Operator**: MZ

**Instrument**: maXis HD

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Bruker Compass DataAnalysis 4.2  
by: MZ  
Page 1 of 1
**SPECTROSCOPIC INFORMATION**

**Mass Spectrum SmartFormula Report**

**Analysis Info**
- **Acquisition Date**: 11/4/2014 9:58:40 AM
- **Analysis Name**: D:\Data\MZ_data\Spreitzer\Thomas_N\141104\AK024_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: approx. 10 ug/mL in MeOH
- **Comment**: 1820881.21300

**Acquisition Parameter**
- **Source Type**: ESI
- **Scan Begin**: 50 m/z
- **Scan End**: 1550 m/z
- **Set Corona**: 0 nA

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**Comment**

AK024_DI_ESI_HRMS.d

**Bruker Compass DataAnalysis 4.2**

**Printed**: 11/4/2014 11:34:03 AM by: MZ
6-[1-(Dimethylamino)ethyl]-5H-pyrrole-4,3-(1H,3H)-dione (PROTON)

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6-[1-(Dimethylamino)ethyl]-5H-pyrrole-4,3-(1H,3H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,3-b]carbazole-3,11(10H)-dione (C13 APT)

147 146 145 144 143 142 141 140 139 138 137 136 135 134 133 132 131 130 129 128 127 126 125 124 123 122 121 120 119 118 117 116 115 114 113 112 111 11

ppm

6-2-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,3-b]carbazole-3,11(10H)-dione (HMQC)
SPECTROSCOPIC INFORMATION

6-(1-[(N,N-diethylamino)ethyl]-2H-pyridine-4,5,6,7-tetrahydro-1,10H-dione 1HMBC)
6-[2-((Dimethylamino)ethyl)-5H-pyrido[4,3-b]carbazole-3,11(6H)-dione (1N HMBC)
**Analysis Info**

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**Generic Display Report**

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**Chemical Structures**

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Bruker Compass DataAnalysis 4.2  
printed: 1/12/2015 2:48:06 PM  
by: MZ  
Page 1 of 1
**Mass Spectrum SmartFormula Report**

**Analysis Info**
- **Acquisition Date**: 1/12/2015 2:44:59 PM
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN178_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: in MeOH → 1:100 in MeOH
- **Comment**: Instrument: maXis HD 182088,1.21300

**Acquisition Parameter**

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**Mass Spectrum**

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<th>N-Rule</th>
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<td>8.4</td>
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<td>12.5</td>
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<td>12.5</td>
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TN178_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2

printed: 1/12/2015 2:48:11 PM by: MZ
SPECTROSCOPIC INFORMATION

6-(Methoxymethyl)-3H-pyridin-4,5-b[1,2-b]benzoxacine-5,11(11H)-dione (PROTON)

6-(Methoxymethyl)-3H-pyridin-4,5-b[1,2-b]benzoxacine-5,11(11H)-dione (PROTON)
SPECTROSCOPIC INFORMATION

8-(Methoxymethyl)-5H-pyrazino[4,5-f]carbazole-5,11(16H)-dione (PROTON)

8.64 8.63 8.60 8.58 8.56 8.54 8.52 8.50 8.48 8.46 8.44 8.43 8.40 8.38 8.36 8.34 8.3 ppm

82

8-(Methoxymethyl)-5H-pyrazino[4,5-f]carbazole-5,11(16H)-dione (PROTON)

7.76 7.74 7.72 7.70 7.68 7.66 7.64 7.62 7.60 7.58 7.56 7.54 7.52 7.50 7.48 7.46 7.44 7.42 7.40 7.38 7.36 7.34 7.32 7.30 7.28 7.26 7.24 7.2 ppm

685
6-(Methoxymethyl)-3H-pyrrolizine[4,5-b]carbazole-5,11(6H)-dione (3DQC)

6-(Methoxymethyl)-3H-pyrrolizine[4,5-b]carbazole-5,11(6H)-dione (3MBC)
SPECTROSCOPIC INFORMATION

6-(6-Methyl-2H-pyridin-3-yl)-5H-[1,3]benzoxolo[5,11]dioxo 13N HMBG

6-(6-Methyl-2H-pyridin-3-yl)-5H-[1,3]benzoxolo[5,11]dioxo 13N HMBG

688
### Mass Spectrum SmartFormula Report

**Analysis Info**
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_HiThomas_Ni150112\TN179_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: in MeOH → 1:10 in MeOH
- **Comment**:

**Acquisition Parameter**
- **Source Type**: ESI
- **Ion Polarity**: Positive
- **Set Nebulizer**: 0.4 Bar
- **Set Dry Heater**: 200 °C
- **Set Dry Gas**: 4.0 l/min
- **Set Capillary**: 2500 V
- **Set End Plate Offset**: -500 V
- **Set Charging Voltage**: 2000 V
- **Set Divert Valve**: Waste
- **Set Corona**: 0 nA
- **Set APCI Heater**: 0 °C

<table>
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<tr>
<th>Meas. m/z</th>
<th>#</th>
<th>Ion Formula</th>
<th>m/z err [ppm]</th>
<th>mSigma</th>
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**Comment**

TN179_DI_ESI_HRMS.d

**Printed**: 1/12/2015 2:20:51 PM by: MZ

Page 1 of 1
SPECTROSCOPIC INFORMATION

Generic Display Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\150112\TN180_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH
Comment: +MS, 0.0-2.1min #1-118

Acquisition Info
Acquisition Date: 1/12/2015 1:43:34 PM
Operator: MZ
Instrument: maXis HD

Chemical Structure

Bruker Compass DataAnalysis 4.2
printed: 1/12/2015 1:57:24 PM
by: MZ
Page 1 of 1
### Mass Spectrum SmartFormula Report

**Analysis Info**
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_HiThomas_N\150112\TN180_Di_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: in MeOH
- **Comment**

**Acquisition Date** 1/12/2015 1:43:34 PM  
**Analysis Parameter**

| Source Type | Ion Polarity | Positive | Set Nebulizer | Method MZOperator
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</thead>
<tbody>
<tr>
<td>ESI</td>
<td>-</td>
<td>-</td>
<td>0.4 Bar</td>
<td>maXis HD 1820881.21300</td>
</tr>
</tbody>
</table>

**Scan Begin** 50 m/z  
**Scan End** 1550 m/z  
**Set Capillary** 2200 V  
**Set End Plate Offset** -500 V  
**Set Dry Gas** 4.0 l/min  
**Set Dry Heater** 200 °C  
**Set Charging Voltage** 2000 V  
**Set Divert Valve** Waste  
**Set Corona** 0 nA  
**Set APCI Heater** 0 °C

<table>
<thead>
<tr>
<th>Meas. m/z</th>
<th># Ion Formula</th>
<th>m/z</th>
<th>err [ppm]</th>
<th>mSigma</th>
<th># mSigma</th>
<th>Score</th>
<th>rdb</th>
<th>e¯</th>
<th>Conf</th>
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<td>10.7</td>
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<td>ok</td>
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</tbody>
</table>

**Mass Spectrum**

The mass spectrum shows peaks at m/z 272.0434, 235.2386, 140.1050, 421.3772, and 585.5334.

**Chemical Structure**

![Chemical Structure Diagram](image_url)

**Information**

- **Analysis Name**: TN180_Di_ESI_HRMS.d  
- **Operator**: MZ  
- **Instrument**: maXis HD 1820881.21300  
- **Acquisition Date**: 1/12/2015 1:57:29 PM  
- **Printed by**: MZ  
- **Page**: 1 of 1
SPECTROSCOPIC INFORMATION

**N,N-Diethylpyrimidin-5-carboxamide (C13 CPD)**

**N,N-Diethylpyrimidin-5-carboxamide (DMSQC)**
$N,N$-Dithiopyrimidin-5-carboxamide (NOESY)
3,6-Dimethoxy-2-nitrobenzaldehyde (C13 CPD)

3,6-Dimethoxy-2-nitrobenzaldehyde (HSQC)
SPECTROSCOPIC INFORMATION

3,6-Dimethoxy-2-nitrobenzaldehyde (DMBC)

3,6-Dimethoxy-2-nitrobenzaldehyde (NOGST)
SPECTROSCOPIC INFORMATION

N,N'-[3,6-Dimethoxy-2-nitrophenoxy)methyl]formamide (1HQC)

N,N'-[3,6-Dimethoxy-2-nitrophenoxy)methyl]formamide (1HQC)
5,8-Dimethoxyquinazoline (1H NMR)

5,8-Dimethoxyquinazoline (NOESY)
SPECTROSCOPIC INFORMATION

5.8-Quinolinocellene (PROTON)

5.8-Quinolinocellene (PROTON)

711
SPECTROSCOPIC INFORMATION

5,8-Quinolinedione (C13 CPQ)

5,8-Quinolinedione (H5QG)
Mass Spectrum SmartFormula Report

**Analysis Info**
- **Acquisition Date**: 11/17/2014 2:03:35 PM
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_HiThomas_N\141117\TN082_DI_ESI_HRMS.d
- **Method**: DI_mz_50-1550.m
- **Sample Name**: 0.1 mg/mL in MeOH
- **Comment**

**Acquisition Parameter**
- **Source Type**: ESI
- **Ion Polarity**: Positive
- **Focus**: Active
- **Scan Begin**: 50 m/z
- **Scan End**: 1550 m/z
- **Set End Plate Offset**: -500 V
- **Set Dry Gas**: 200 °C
- **Set Charging Voltage**: 2000 V
- **Set Corona**: 0 nA
- **Set Nebulizer**: 0.4 Bar
- **Set Dry Heater**: 4.0 l/min
- **Set Divert Valve**: Waste
- **Set APCI Heater**: 0 °C

**Meas. m/z** | **Ion Formula** | **m/z** | **err [ppm]** | **mSigma** | **Score** | **rdb** | **e⁻ Conf** | **N-Rule**
--- | --- | --- | --- | --- | --- | --- | --- | ---
161.0345 | C8H5N2O2 | 161.0346 | 0.2 | n.a. | 1 | 100.00 | 7.5 | even | ok
183.0166 | C8H4N2NaO2 | 183.0165 | -0.6 | 5.1 | 1 | 100.00 | 7.5 | even | ok

**Comment**

Bruker Compass DataAnalysis 4.2 printed: 11/17/2014 2:11:42 PM by: MZ
8-Anilinoquinolone-5,8-dione (PROTON)

8-Anilinoquinolone-5,8-dione (13 C PD)
SPECTROSCOPIC INFORMATION

6-Azilinoquinolines-5,8-dione (C15 CPD)

6-Azilinoquinolines-5,8-dione (HSQC)
**Generic Display Report**

**Analysis Info**
- **Analysis Name:** D:\Data\_MZ_data\Spreitzer_H\Thomas_N\150112\TN104_DI_ESI_HRMS.d
- **Method:** DI_mz_50-1550.m
- **Sample Name:** in MeOH --> 1:500 in MeOH
- **Comment:**

**Acquisition Date:** 1/12/2015 2:30:26 PM

**Operator:** MZ

**Instrument:** maXis H

---

**MZMethod DI_mz_50-1550.m**

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<td>252.0765</td>
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<tr>
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</tr>
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<td>261.1306</td>
<td>3</td>
</tr>
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<tr>
<td>274.0587</td>
<td>6</td>
</tr>
<tr>
<td>276.2295</td>
<td>7</td>
</tr>
</tbody>
</table>

**Comment:** +MS, 0.5-1.6min #29-88

---

**Comment:**

- **C_{14}H_{10}N_{3}O_{2}, 252.0768**
- **C_{14}H_{9}N_{3}NaO_{2}, 274.0587**

---

**Generic Display Report**

**Bruker Compass DataAnalysis 4.2**

**Printed:** 1/12/2015 2:34:35 PM

**By:** MZ

**Page:** 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN104_Di_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:500 in MeOH
Comment:

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Scan Begin: 50 m/z
Scan End: 1550 m/z
Set Capillary: 2200 V
Set End Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste
Set APCI Heater: 0 °C

Mass Spectrum

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e Conf N-Rule
252.0765 1 C14H10N3O2 252.0768 1.0 7.4 1 100.00 11.5 even ok
274.0587 1 C14H9N3NaO2 274.0587 -0.2 10.6 1 100.00 11.5 even ok
848.4565

TN104_Di_ESI_HRMS.d
Bruker Compass DataAnalysis 4.2 printed: 1/12/2015 2:34:41 PM by: MZ
SPECTROSCOPIC INFORMATION

6-Brbenz-5,8-dimethoxyquinazoline (HMQC)

6-Brbenz-5,8-dimethoxyquinazoline (HSQC)
6-Bromo-5,8-dimethoxyquinoline (15N HMBC)

6-Bromo-5,8-dimethoxyquinoline (NOESY)
Generic Display Report

Analysis Info

Acquisition Date: 11/17/2014 2:23:02 PM
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141117\TN117_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment:

![Graph with m/z values and peaks]

**Comment:**

270.9902 276.2300
278.2455 281.2953
284.3063 288.2899
290.9743 292.9723
296.3063

+MS, 0.0-1.6min #1-92

0.00 0.25 0.50 0.75 1.00 1.25 1.50 1.75 2.00
5x10^4

0.00 0.25 0.50 0.75 1.00 1.25 1.50 1.75 2.00
5x10^4

C_{10}H_{9}BrN_{2}NaO_{2}, 290.9740
C_{10}H_{10}BrN_{2}O_{2}, 268.9920

Bruker Compass DataAnalysis 4.2 printed: 11/17/2014 2:32:00 PM by: MZ Page 1 of 1
**Analysis Info**

- Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141117\TN117_DI_ESI_HRMS.d
- Method: DI_mz_50-1550.m
- Sample Name: 0.1 mg/mL in MeOH
- Comment

**Acquisition Parameter**

- Source Type: ESI
- Ion Polarity: Positive
- Scan Begin: 50 m/z
- Scan End: 1550 m/z
- Set Charging Voltage: 2000 V
- Set Divert Valve: Waste
- Set Corona: 0 nA
- Set APCI Heater: 0 °C
- Set Dry Gas: 4.0 l/min
- Set Dry Heater: 200 °C
- Set Nebulizer: 0.4 Bar
- Set End Plate Offset: -500 V
- Set Focus: 200 °C

**Mass Spectrum SmartFormula Report**

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<th>Ion Formula</th>
<th>m/z</th>
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<th>Score</th>
<th>rdb</th>
<th>e⁻</th>
<th>Conf</th>
<th>N-Rule</th>
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</table>

**Br**

- Br

**N**

- 93
2-Aniline-6-methyl-1,4-benzoquinone (PROTON)

94a

9.0  8.5  8.0  7.5  7.0  6.5  6.0  5.5  5.0  4.5  4.0  3.5  3.0  2.5  2.0  1.5  1.0  0.5

ppm

2-Aniline-6-methyl-1,4-benzoquinone (PROTON)

7.5  7.0  6.5  6.0  5.5  5.0  4.5  4.0  3.5  3.0  2.5  2.0  1.5  1.0  0.5

ppm
2-Acyliso-8-methyl-1,4-benzquinone (PROTON)

2-Acyliso-8-methyl-1,4-benzquinone (C13 APT)

94a
2-Anilino-8-methyl-1,4-benzoquinone (15N HSQC)

2-Anilino-8-methyl-1,4-benzoquinone (NOESY)
Analysis Info

Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\150112\TN123_Di_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:500 in MeOH
Comment:

Acquisition Date: 1/12/2015 3:49:48 PM
Operator: MZ
Instrument: maXis HD
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN123_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:500 in MeOH
Comment:

Acquisition Date: 1/12/2015 3:49:48 PM
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN123_DI_ESI_HRMS.d
Operator: MZ
Instrument: maXis HD

Acquisition Parameter
Source Type: ESI
Scan Begin: 50 m/z
Scan End: 1550 m/z
Ion Polarity: Positive
Set Capillary: 3000 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste
Set APCI Heater: 0 °C

Mass Spectrum SmartFormula Report

236.0684
449.1471
685.4353
879.5792

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e Conf N-Rule
214.0862 1 C13H12NO2 214.0863 0.1 7.6 1 100.00 8.5 even ok
236.0684 1 C13H11NNaO2 236.0682 -0.9 0.8 1 100.00 8.5 even ok

TN123_DI_ESI_HRMS.d
Printed: 1/12/2015 3:55:33 PM
by: MZ
Page 1 of 1
2-Acetox-5-methyl-1,4-benzoxazine (PROTON)

2-Acetox-5-methyl-1,4-benzoxazine (C13 APT)
2-Nitro-5-methyl-1,4-hexaquinone (3HQC)

94b

2-Nitro-5-methyl-1,4-hexaquinone (3HMBC)
2-Anilino-5-methyl-1,4-benzquinone (15N HSQC)

2-Anilino-5-methyl-1,4-benzquinone (NOESY)
SPECTROSCOPIC INFORMATION

Generic Display Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Speirizer_H\Thomas_N\150112\TN124_Dl_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:500 in MeOH
Comment: +MS, 0.0-2.2min #1-124

Acquisition Date: 1/12/2015 4:05:56 PM
Operator: MZ
Instrument: maXis HD

Bruker Compass DataAnalysis 4.2  printed: 1/12/2015 4:09:25 PM  by:  MZ  Page 1 of 1
### Mass Spectrum SmartFormula Report

**Analysis Info**

**Analysis Name**: D:\Data\_MZ_data\Spreitzer_HiThomas_N\150112\TN124_DI_ESI_HRMS.d  
**Method**: DI_mz_50-1550.m  
**Sample Name**: in MeOH -> 1:500 in MeOH  
**Comment**:  

**Acquisition Parameter**

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<tr>
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<td>Set Corona</td>
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<td>Set APCI Heater</td>
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**Mass Spectrum**

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<th>m/z</th>
<th>err [ppm]</th>
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<th>Score</th>
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![Chemical Structure](attachment:94b.png)

**TN124_DI_ESI_HRMS.d**  
**Bruker Compass DataAnalysis 4.2**  
**printed**: 1/12/2015 4:09:31 PM  
**by**: MZ  
**Page 1 of 1**
SPECTROSCOPIC INFORMATION

2-Methyl-3H-carbazole-1,4(9H)-dione (PROTON)

2-Methyl-3H-carbazole-1,4(9H)-dione (1H NMR)

95a
### Spectroscopic Information

**2-Methyl-1H-carbazole-3,4(9H)-dione (C13 CPD)**

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**2-Methyl-1H-carbazole-3,4(9H)-dione (HMQC)**

- **95a**

---

743
Generic Display Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\150114\TN126_DI_APB\HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 100 ug/mL in MeOH
Comment:

Acquisition Date: 1/14/2015 10:42:38 AM
Operator: MZ
Instrument: maXis HD

Generic Display Report

Bruker Compass DataAnalysis 4.2  printed: 1/14/2015 3:00:51 PM  by: MZ  Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150114\TN126_DI_APCI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 100 ug/mL in MeOH
Comment: 

Acquisition Parameter

Source Type: APCI
Ion Polarity: Positive
Set Nebulizer: 1.6 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 3.0 l/min
Set Divert Valve: Waste
Set Charging Voltage: 3000 nA
Set APCI Heater: 470 °C

Scan Begin: 50 m/z
Set End Plate Offset: -500 V
Scan End: 1550 m/z
Set Capillary: 1600 V
Set Corona: 2000 V
Focus: Active

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score db e Conf N-Rule
212.0709 1 C13H10NO2 212.0706 -1.5 2.7 1 100.00 9.5 even ok

95a

TN126_DI_APCI_HRMS.d

Bruker Compass DataAnalysis 4.2 printed: 1/14/2015 3:01:02 PM by: MZ
3-Methyl-1H-carbazole-1,4(2H)-dione (PROTON)

13.0 12.9 12.8 12.7 12.6 12.5 12.4 12.3 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 2.3 2.2 2.1 2.0 1.9
SPECTROSCOPIC INFORMATION

3-Methyl-11S-carboxy-14H15H3-Dirac (15N HSQC)
### Analysis Info

- **Analysis Name**: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN125_DI_ESI_HRMS.d
- **Method**: DL_mz_50-1550.m
- **Sample Name**: in MeOH
- **Comment**:

### Acquisition Parameter

- **Source Type**: ESI
- **Ion Polarity**: Positive
- **Set Nebulizer**: 0.4 Bar
- **Scan Begin**: 50 m/z
- **Set End Plate Offset**: -500 V
- **Set Dry Gas**: 4.0 l/min
- **Scan End**: 1550 m/z
- **Set Charging Voltage**: 2000 V
- **Set Corona**: 0 nA
- **Set APCI Heater**: 0 °C
- **Set Dry Heater**: 200 °C
- **Set Capillary**: 3000 V
- **Set Divert Valve**: Waste

### Mass Spectrum SmartFormula Report

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SPECTROSCOPIC INFORMATION

9-(1-Dimethylaminocarbonyl)-2-methyl-3H-imidazo[1,2-b]pyridine (PROTON)

96a

9-(1-Dimethylaminocarbonyl)-2-methyl-3H-imidazo[1,2-b]pyridine (PROTON)

96a
SPECTROSCOPIC INFORMATION

9-[2-(Dimethylamino)ethyl]-2-methyl-13H-carbazole-1,4(9H)-dione (PROTON)

96a

9-[2-(Dimethylamino)ethyl]-2-methyl-13H-carbazole-1,4(9H)-dione (C13 APT)

96a
9-(2-(Dimethylamino)ethyl)-2-methyl-1H-carbazole-1,4(9H)-dione (C13 APT)

96a

9-(2-(Dimethylamino)ethyl)-2-methyl-1H-carbazole-1,4(9H)-dione [HSQC]
9 [2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(2H)-dione (1H-BB)

96a

9 [2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(2H)-dione (13N H-MBC)
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\150112\TN129_DIELSI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH → 1:500 in MeOH
Comment: 

Acquisition Date: 1/12/2015 4:14:56 PM
Instrument: maXis HD
Operator: 

Acquisition Parameter

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<td>Set APCI Heater 0 °C</td>
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Intens.
x10^5

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e¯ Conf N-Rule
283.1446 1 C17H19N2O2 283.1441 -1.9 7.0 1 100.0 9.5 even ok
305.1264 1 C17H18N2NaO2 305.1260 -1.3 4.7 1 100.0 9.5 even ok

96a

TN129_DIELSI_HRMS.d

Bruker Compass DataAnalysis 4.2 printed: 1/12/2015 4:20:17 PM by: MZ
SPECTROSCOPIC INFORMATION

9-(2-Hydroxymethyl)-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

96b

9-(2-Hydroxymethyl)-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

96b
9-[2-(Dimethylamino)ethyl]-2-methyl-13-carbazole-1,4(9H)-dione (PROTON)

96b

9-[2-(Dimethylamino)ethyl]-2-methyl-13-carbazole-1,4(9H)-dione (C13 APT)
9-(2-(Dimethylamino)ethyl)-3-methyl-1H-carbazole-1,4(9H)-dione [C35 APT]

96b

9-(2-(Dimethylamino)ethyl)-3-methyl-1H-carbazole-1,4(9H)-dione [HMQC]
9-[2-((Dimethylamino)ethyl)-methyl]-carbazole-1,4(9H)-dione (HMBC)
Generic Display Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\150112\TN128_Di_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH -> 1:500 in MeOH
Comment:

Acquisition Date: 1/12/2015 3:55:11 PM
Operator: MZ
Instrument: maXis HD

[Graph showing mass spectrometry data with peaks at m/z values and corresponding intensities]

Bruker Compass DataAnalysis 4.2  printed: 1/12/2015 4:02:56 PM  by: MZ  Page 1 of 1
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data\MZ_data\Spreitzer_Hi\Thomas_N\150112\TN128_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:500 in MeOH
Comment:

Acquisition Parameter

Source Type: ESI
Ion Polarity: Positive
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Charging Voltage: 283.1444
Set Corona: 0 nA
Set APCI Heater: 0 °C
Set Capillary: 2800 V
Set End Plate Offset: -500 V
Set Divert Valve: Waste
Set Charging Voltage: 2000 V
Set Charging Voltage: 0 °C
Set Charging Voltage: 1550 m/z
Set Charging Voltage: 50 m/z

Meas. m/z  #  Ion Formula  m/z  err [ppm]  mSigma  #  mSigma  Score  rdb  e  Conf  N-Rule
283.1444  1  C17H19N2O2  283.1441  -1.2  8.1  1  100.00  9.5  even  ok
305.1260  1  C17H18N2NaO2  305.1260  -0.0  13.6  1  100.00  9.5  even  ok

TN128_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2
 printed: 1/12/2015 4:03:10 PM  by: MZ  Page 1 of 1
3-Amino-8-(2-(dimethylaminomethyl)-2-methyl-1H-carbazole-1,4(6H)-dione (PROTON)

97a
SPECTROSCOPIC INFORMATION

3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (C13 APT)

3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (HSQC)
3-Amino-8-[2-(dimethylamino)ethyl]-1,3-indolizinedione (IDMC)

3-Amino-8-[2-(dimethylamino)ethyl]-2-methyl-1,3-indolizinedione (IDMC)
Generic Display Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN131_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH --> 1:500 in MeOH
Comment: +MS, 0-2.0min #1-112

Acquisition Date: 1/12/2015 4:43:36 PM
Operator: MZ
Instrument: maXis HD

Bruker Compass DataAnalysis 4.2  printed: 1/12/2015 4:46:38 PM  by: MZ  Page 1 of 1

C_{17}H_{20}N_{3}O_{2}, 298.1550
Mass Spectrum SmartFormula Report

**Analysis Info**
- **Analysis Name**: D:\Data\MZ_data\Spreitzer_HiThomas_Ni150112\TN131_Di_ESI_HRMS.d
- **Method**: Di_mz_50-1550.m
- **Sample Name**: in MeOH --> 1:500 in MeOH
- **Operator**: MZ
- **Instrument**: maXis HD 1820881.21300

**Acquisition Parameter**
- **Source Type**: ESI
- **Ion Polarity**: Positive
- **Set Nebulizer**: 0.4 Bar
- **Set Capillary**: 1200 V
- **Set Dry Heater**: 200 °C
- **Set Dry Gas**: 4.0 l/min
- **Set End Plate Offset**: -500 V
- **Set Divert Valve**: Waste
- **Set Charging Voltage**: 2000 V
- **Set Corona**: 0 nA
- **Set APCI Heater**: 0 °C

**Mass Spectrum**

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![Formula Image](image)

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TN131_Di_ESI_HRMS.d

Printed by: MZ

Page 1 of 1
SPECTROSCOPIC INFORMATION

5,11-Bis(2-[dimethylamino]ethyl) 11,11a-dihydropyrido[3,2-b]carbazole-6,12(5H,6H)-dione (C15 AFT)

12.6 10b,14a 11a,15a 6a,17a

180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 ppm

98

5,11-Bis(2-[dimethylamino]ethyl) 11,11a-dihydropyrido[3,2-b]carbazole-6,12(5H,6H)-dione (3DQCs)
SPECTROSCOPIC INFORMATION

5,11-Bis(1-[(dimethylamino)ethyl]-11,11a-dihydroindolo[3,2-b]carbazole-6,13(5H,11H)-dione (ESIMBC)
Generic Display Report

Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\150112\TN182_DI_ESI_HRMS.d
Method: DI mz_50-1550.m
Sample Name: in MeOH
Comment: 429.2284 437.1933 443.3340 447.2925 451.2103 459.4880

MZMethod DI_mz_50-1550.m Operator in MeOH Instrument maXis HD

Bruker Compass DataAnalysis 4.2 printed: 1/12/2015 1:27:54 PM by: MZ
Mass Spectrum SmartFormula Report

Analysis Info
Analysis Name: D:\Data\MZ_data\Spreitzer_HiThomas_Ni\150112\TN182_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: in MeOH
Comment:

Acquisition Parameter
Source Type: ESI
Ion Polarity: Positive
Scan Begin: 50 m/z
Scan End: 1550 m/z
Set Capillary: 1500 V
Set End Plate Offset: -500 V
Set Charging Voltage: 2000 V
Set Corona: 0 nA
Set Nebulizer: 0.4 Bar
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Waste
Set APCI Heater: 0 °C

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e^− Conf N-Rule
429.2284 1 C26H29N4O2 429.2285 0.2 11.1 1 100.00 14.5 even ok
451.2103 1 C26H28N4NaO2 451.2104 0.3 15.6 1 100.00 14.5 even ok

TN182_DI_ESI_HRMS.d
Bruker Compass DataAnalysis 4.2 printed: 1/12/2015 1:28:02 PM by: MZ Page 1 of 1
SPECTROSCOPIC INFORMATION

6-[(2-Chloroethyl)pyridyl][4,5,6,7]-carbazole-3,11(10H)-dione (1HMBI)

6-[(2-Chloroethyl)pyridyl] [4,5,6,7]-carbazole-3,11(10H)-dione (1HMBI)
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name: D:\Data\MZ_data\Spreitzer_H\Thomas_N\141117\TN122_DI_ESI_HRMS.d
Method: DI_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment: maXis HD 1820881.21300

Acquisition Parameter

Source Type: ESI
Ion Polarity: Positive
Set Nebulizer: 0.4 Bar
Set Capillary: 4000 V
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set APCI Heater: 0 °C
Set Corona: 0 nA
Set Charging Voltage: 304.2614
Set Divert Valve: Waste

Scan Begin: 50 m/z
Set End Plate Offset: -500 V
Scan End: 1550 m/z
Set Changing Voltage: 2000 V
Set Coron: 0 nA
Scan End: 0 °C

Intens. x10^5

Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e Conf N-Rule
311.0580 1 C17H12CN2O2 311.0582 0.5 3.9 1 100.00 12.5 even ok
333.0399 1 C17H12CN2NaO2 333.0401 0.7 32.3 1 100.00 12.5 even ok
333.0393 2 C12H12Cl2N6Na 333.0393 -1.9 144.8 2 0.91 8.5 even ok

TN122_DI_ESI_HRMS.d
Bruker Compass DataAnalysis 4.2
printed: 11/17/2014 2:50:11 PM
by: MZ
Page 1 of 1
SPECTROSCOPIC INFORMATION

6-Vinyl-1H-pyridine[4,3-b]carbazole-5,11(1H)-dione (PROTON)

13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

6-Vinyl-1H-pyridine[4,3-b]carbazole-5,11(1H)-dione (PROTON)

ppm

9.5 9.4 9.3 9.2 9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0

ppm
6-Vinyl-3H-pyrrole[4,3-5]carbazole-5,11(6H)-diones (2D HMQC)

6-Vinyl-3H-pyrrole[4,3-5]carbazole-5,11(6H)-dione (1D HMBC)
Generic Display Report

Analysis Info
Analysis Name: D:\Data\_MZ_data\Spritzer_H\Thomas_N\141117\TN120_Dl_ESI_HRMS.d
Method: Dl_mz_50-1550.m
Sample Name: 0.1 mg/mL in MeOH
Comment: 275.0820

Analysis Date: 11/17/2014 2:31:53 PM
Operator: MZ
Instrument: maXis HD

Intens. x10^6
275.0820
277.2333
279.2488
280.2622
281.2953
282.2795
283.107
284.3063
285.2932
286.2892
287.3058
287.3107
289.2456
290.2456
292.2611
295.3109
297.0634

C₁₇H₁₁N₂O₂, 275.0815
1+
276.0853
279.2488
280.2622
281.2953
282.2795
283.107
284.3063
285.2932
286.2892
287.3058
287.3107
289.2456
290.2456
292.2611
295.3109
297.0634

C₁₇H₁₀N₂NaO₂, 297.0634
1+
298.0666
290.2456
292.2611
295.3109
297.0637

Bruker Compass DataAnalysis 4.2 printed: 11/17/2014 2:36:05 PM by: MZ Page 1 of 1
Mass Spectrum SmartFormula Report

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**Analysis Info**

- **Analysis Name**: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141117\TN120_Di_ESI_HRMS.d
- **Method**: DI_TRACE_50-1550.m
- **Sample Name**: 0.1 mg/mL in MeOH
- **Instrument**: maXis HD 1820881.21300

**Acquisition Parameter**

- **Set Nebulizer**: 0.4 Bar
- **Set Dry Heater**: 200 °C
- **Set Dry Gas**: 4.0 l/min
- **Set Charging Voltage**: 4.000 V
- **Set Divert Valve**: Waste

**Meas. m/z**  | **Ion Formula** | **m/z** | **err [ppm]** | **mSigma** | **# mSigma** | **Score** | **rdb** | **Conf** | **N-Rule**
--- | --- | --- | --- | --- | --- | --- | --- | --- | ---
275.0820 | C17H11N2O2 | 275.0815 | -1.8 | 13.5 | 1 | 100.00 | 13.5 | even | ok
297.0637 | C17H10N2NaO2 | 297.0634 | -0.8 | 12.7 | 1 | 100.00 | 13.5 | even | ok

**N-Rule**

- **ok**

**Comment**

- **Analysis Date**: 11/17/2014 2:31:53 PM
- **Analysis Name**: D:\Data\_MZ_data\Spreitzer_H\Thomas_N\141117\TN120_Di_ESI_HRMS.d
- **Method**: DI_TRACE_50-1550.m
- **Sample Name**: 0.1 mg/mL in MeOH
- **Instrument**: maXis HD 1820881.21300

---

**TN120_Di_ESI_HRMS.d**

**Bruker Compass DataAnalysis 4.2**

**printed**: 11/17/2014 2:36:15 PM **by**: MZ **Page 1 of 1**
10 ZUSAMMENFASSUNG


Als Ergebnis der vorliegenden Arbeit wurden drei zentrale Fragen beantwortet:

1. Welchen Einfluss auf die zytotoxische Aktivität hat die Variation des Pyridin-Systems durch andere \( \pi \)-Elektronenmangelstrukturen (Pyridin-, Pyrimidin-, Pyrazin- und Pyridazin-Isomere).
2. Kann durch Erweiterung dieses \( \pi \)-Elektronenmangels um eine weitere Benzoanellierung, die zu Calothrixin-Isomeren führt, die Wirkung zusätzlich erhöht werden? Hintergrund dieses Vorhabens war die bekannte hohe wachstumshemmende Aktivität der Calothrixine, die bekannten Naturstoffe aus *Calothrix cyanobacteria* darstellen.
3. Durch welche Arten von synergistisch wirksamen Seitenketten mit basischen funktionellen Gruppen kann eine weitere Optimierung erzielt werden?

Mit der vorliegenden Arbeit ist es berechtigt, zu erwarten, dass mit all den bisher hergestellten Verbindungen nun eine Substanzbibliothek in einer Größe und Diversität vorliegt, die in der Folge auch ein Rational Drug Design für die weitere Entwicklung sinnvoll erscheinen lassen.
ABSTRACT

With over 14 million new cases of cancers worldwide (2012) and over 8.2 million cancer related deaths, there is a strong interest in the development of new drugs fighting this disease. Despite recent advances in chemotherapy, the goal to develop agents with a broad range of application, high selectivity and low toxicity is still unachieved.

In this thesis new cytotoxic compounds were developed based on previous structures containing a tetracyclic carbazole skeleton of the ellipticine quinone type (1) with cytotoxic properties. Tetracyclic analogues of this compound endowed with a basic \(N,N\)-dimethylethanamine side chain were synthesised. Subsequently, the tetracyclic ring system was expanded by a benzene ring leading to the pentacyclic calothrixin skeleton, which is known to possess cytotoxic properties. Subsequently, calothrixin isomers (2a, 2b, 2c) with the same side chain were synthesised. In the following, the compounds were subjected to viability assays in which derivatives of the tetracyclic series demonstrated the highest cytotoxic potential. Subsequent side chain modifications and further biological tests were performed.

As a result, three main questions were answered:

1. Does the substitution of the pyridine system by other \(\pi\)-electron deficient heterocycles (pyrazine, pyrimidine, pyridazine) enhance the antiproliferative activity of the compounds?
2. Does the expansion of the \(\pi\)-electron deficient system by a further benzo-annulation, which leads to calothrixin isomers, enhance the cytotoxic properties? The rationale for this modification is the documented cytotoxic properties of the calothrixins, which are natural compounds of Calothrix cyanobacteria.
3. Can the antiproliferative activity be enhanced by linking different basic substituents to the skeleton?

With the compounds synthesised, our library was expanded sufficiently to attempt further developments via a rational drug design approach.
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Education

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Conferences

2013 Oral Presentation at the 3rd Meeting of the Paul Ehrlich Medchem
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