SYNTHESSES OF MARINE NATURAL PRODUCTS AND THEIR ANALOGS AS POTENTIAL ANTICANCER AGENTS

by

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A DISSERTATION

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DWAYAJA H. NADKARNI

CHEMISTRY

ABSTRACT

Cancer is an abnormal and uncontrolled growth of cells and is one of the most common causes of millions of deaths worldwide. Currently used chemotherapeutic drugs have severe side effects. Hence, there is a need to develop new cancer therapeutic drugs that can offer higher survival rates and fewer limitations. About 60% of the known anticancer drugs are either natural products or are derived from natural products. Development of marine natural products into drugs has received increasing attention as a part of humanity’s quest for newer sources of cancer drugs. Marine natural products are isolated only in small quantities from natural sources, precluding their thorough biological evaluations. One of the better ways of producing marine natural products in larger quantities is through chemical synthesis. This dissertation focuses on the synthesis of some of the heterocyclic quinonoid marine natural products, their analogs and their biological evaluation.

The dissertation starts with an introduction that details the challenges in cancer therapy, the available chemotherapeutic drugs and the role of natural products, particularly, marine natural products in cancer drug discovery. Described in the Chapter 1 of this dissertation is the synthesis of analogs of a marine natural product, makaluvamines. Several of the final compounds were evaluated against a number of cancer cell lines in vitro. The most active compounds were evaluated in vivo in mouse
xenograft models of a number of cancers. Chapter 2 describes the development of a new synthetic methodology for another related class of marine alkaloids containing a bispyrroloquinone ring system. This research focuses on the development of a CAN mediated oxidative cyclization reaction as a facile methodology for the incorporation of a pyrrole ring onto a 6-aminoindole-4,7-quinones. This dissertation culminates with the total synthesis of four marine alkaloids, zyzzyanones A-D in Chapter 3. The synthetic strategy is an extension of the methodology developed in the second chapter. The key step in these syntheses is an oxidative cyclization of 6-aminoindole-4,7-quinones with acetals in the presence of Mn(OAc)_3 as the oxidative cyclization reagent.

Keywords: Cancer, marine natural products, alkaloids, makaluvamine, zyzzyanones, bispyrroloquinone, oxidative cyclization, CAN, Mn(OAc)_3
DEDICATION

This dissertation is dedicated to Shri Swami Samarth, my mother (Late) Mrs. Shanta H. Nadkarni and my father Mr. Hemanand V. Nadkarni.
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<td>ADR-RES</td>
<td>Adriamycin resistant</td>
</tr>
<tr>
<td>AR</td>
<td>Androgen receptor</td>
</tr>
<tr>
<td>ATM</td>
<td>Ataxia telangiectasia mutated</td>
</tr>
<tr>
<td>ATR</td>
<td>ATM and Rad3-related</td>
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<td>A2780</td>
<td>Human ovarian cancer cell line</td>
</tr>
<tr>
<td>Bax</td>
<td>Bcl-2 associated X-protein</td>
</tr>
<tr>
<td>BOC</td>
<td>tert-Butoxycarbonyl</td>
</tr>
<tr>
<td>(BOC)₂O</td>
<td>Di-tert-butyl dicarbonate</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Breast cell lymphoma - 2</td>
</tr>
<tr>
<td>BT</td>
<td>Breast tumor</td>
</tr>
<tr>
<td>CAN</td>
<td>Cerric ammonium nitrate</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>Deuterated chloroform</td>
</tr>
<tr>
<td>CD₃OD</td>
<td>Deuterated methanol</td>
</tr>
<tr>
<td>Cdk</td>
<td>Cyclin dependent kinase</td>
</tr>
<tr>
<td>Chk</td>
<td>Serine / threonine protein kinase</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>CH₃CN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>CH₃COOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese hamster ovary</td>
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<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<td>DNA</td>
<td>Deoxyribose nucleic acid</td>
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<tr>
<td>E2F1</td>
<td>transcription factor</td>
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<td>ER</td>
<td>Estrogen receptor</td>
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<td>EtOAc</td>
<td>Ethyl acetate</td>
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<td>Et$_3$N</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>HCT</td>
<td>Human colon tumor</td>
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<td>HCOOEt</td>
<td>Ethyl formate</td>
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<td>HCOONH$_4$</td>
<td>Ammonium formate</td>
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<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
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<td>HS</td>
<td>Harvey H-ras</td>
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<tr>
<td>IC$_{50}$</td>
<td>50 % proliferation inhibition</td>
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<tr>
<td>IMR</td>
<td>Human primary fibroblasts</td>
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<tr>
<td>IOSE144</td>
<td>Immortalized non-tumorigenic human ovarian surface epithelial cells</td>
</tr>
<tr>
<td>KH</td>
<td>Potassium hydride</td>
</tr>
<tr>
<td>LAH</td>
<td>Lithium aluminum hydride</td>
</tr>
<tr>
<td>LnCAP</td>
<td>Androgen-sensitive human prostate adenocarcinoma</td>
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<tr>
<td>MCF-7</td>
<td>Michigan cancer foundation -7</td>
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<tr>
<td>MDA-MB</td>
<td>M.D. Anderson, metastatic breast</td>
</tr>
<tr>
<td>MDM2</td>
<td>Murine double minute 2</td>
</tr>
<tr>
<td>MDR</td>
<td>Multi-drug resistant</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>Mn(OAc)$_3$</td>
<td>Manganese acetate</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
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<td>------------</td>
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<td>NaOMe</td>
<td>Sodium methoxide</td>
</tr>
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<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<td>OVCAR-3</td>
<td>Human ovarian cancer</td>
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<tr>
<td>PARP</td>
<td>Poly-(ADP-Ribose) polymerase</td>
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<tr>
<td>PC3</td>
<td>Classical prostatic cancer cell line</td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate specific antigen</td>
</tr>
<tr>
<td>Rf</td>
<td>Retardation factor</td>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>T-47D</td>
<td>Human ductal breast epithelial tumor cell line (whole cell lysate)</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutyl ammonium fluoride</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>TRAMP</td>
<td>Transgenic adenocarcinoma mouse prostate</td>
</tr>
<tr>
<td>wt</td>
<td>Wild type</td>
</tr>
</tbody>
</table>
INTRODUCTION

Background

Cancer is one of the leading causes of death worldwide. According to global statistics on cancer published by GLOBOCAN in 2011, cancer accounted for 7.6 million deaths worldwide.\(^1\) Deaths from cancer are projected to continue rising with an estimated 12 million deaths in 2030.\(^2\) About 70 % of cancer associated deaths occurred in middle and low-income countries. In US, cancer deaths of about 569,490 per year account for 1 in 4 deaths and are the second most common cause of fatality as of 2011.\(^3\) The most frequent types of cancer in men are lung, stomach, liver, colorectal, oesophagus and prostate. Among women the most common cancers are breast, lung, stomach, colorectal and cervical.\(^2\)

Cancer is a self propelling mechanism of abnormal growth of cells and is a disease caused by a number of mutations in the human genetic make-up. This has been supported by the literature reports of a number of oncogenes which gain efficiency in their functioning, simultaneously with the non-functionality of tumor suppressor genes. The genetic alterations are caused over a period of time by complicated mechanisms leading to a stepwise transformation of mass of normal cells into malignant tumors. There are more than 100 distinct types and subtypes of tumors found in different organs of the body. These varied forms of malignant cancers are a result of six major acquired capabilities of the cancer cells during the tumor development.\(^4\) Hanahan et al\(^4\) have summarized these acquired capabilities as “1) self-sufficiency in growth signals 2)
insensitivity to growth inhibitory signals 3) evasion of programmed cell death (apoptosis) 4) limitless replicative potential 5) sustained angiogenesis and 6) tissue invasion and metastasis.\textsuperscript{4} Conventional treatment for cancer is a combination of surgery (removal of the localized tumor), chemotherapy (administration of chemical agents to curb the growth of cancer cells) and radiation therapy.

The cancerous cells are very similar to normal cells. Hence, chemicals that are toxic to cancer cells are non-selective and can be toxic to fast growing normal cells in the body like those in the intestine and bone marrow. As a result of the toxic side effects and the varied types and subtypes of cancer, humanity has not yet been able to find effective remedies for cancer as it has for some of the other life-threatening diseases. Further, resistance to existing conventional drugs is a severe problem where the malignant cancer cells which were initially suppressed by a specific drug may develop a resistance mechanism to counter the therapeutic effect of the drug. For this reason cancer chemotherapy is usually a combination therapy comprising of several drugs administered over a span of time.

**Currently used therapeutic agents:** Cancer chemotherapeutic agents are classified mainly based on their structural classes, their mechanisms of action and on the types of cancer they function on.\textsuperscript{5-7} Some of the current therapeutic agents available for the treatment of cancer follow.

**Anti-metabolites:** Anti-metabolites can be used in cancer therapy as they impede DNA synthesis and therefore hinder cell division and the growth of tumors.\textsuperscript{8} The effect
of hindrance of cell division is felt more by cancer cells in comparison to normal cells as these divide faster than normal cells. Anti-metabolites act as pseudo building blocks similar to purine or a pyrimidine, thus preventing the incorporation of the actual building blocks in DNA and hence arresting the S-phase of the cell cycle.\(^8\) They also affect RNA synthesis. A few examples of anti-metabolites used in cancer therapy are Thioguanine, Gemcitabine, 5-Fluorouracil\(^9\) (Figure 1).

\[\text{Thioguanine} \quad \text{Gemcitabine} \quad \text{5-Fluorouracil}\]

**Figure 1**: Examples of anti-metabolite chemotherapeutic agents.

**DNA alkylating agents**: These agents affect all phases of the cell cycle by causing crosslinks in the N-7 guanine residues, strand breaks and abnormal base pairing in DNA thus inhibiting cell division and eventually resulting in apoptosis.\(^10\) A few examples of DNA alkylating anticancer agents are cis-platin, mechloretamine, chlorambucil (Figure 2).

\[\text{Cisplatin} \quad \text{Mechlorethamine} \quad \text{Chlorambucil}\]

**Figure 2**: Examples of DNA alkylating agents
**Anthracyclines**: Anthracyclines are a class of anticancer drugs isolated from the bacteria, *Streptomycites pugetus*.

They are the most effective among the anticancer drugs as they function through mechanisms which include inhibition of DNA or RNA synthesis by intercalation between base pairs, damage of DNA caused by reactive species like free radicals and inhibition of Topoisomerase II, thus preventing untangling of supercoiled DNA preventing replication and transcription. Two examples of anthracyclon chemotherapeutic agents are daunomycin, doxorubicin (Figure 3). These are used in the treatment of a wide range of leukemia, lymphomas, uterine, breast and ovarian cancers.

![Daunomycin and Doxorubicin](image)

**Figure 3**: Examples of Anthracycline chemotherapeutic agents.

**Antimitotic (tubulin binding) agents**: Tubulin binding agents stop the cell cycle at the G2/M phase resulting in cell death. 

Mechanistically, during mitosis the sister chromatids are separated by proteins called kinetochores. Any interference with the kinetochores prevents further cell division by arresting mitosis leading to apoptosis. The examples of this class of drugs are vincristine and taxol (Figure 4).
**Figure 4**: Examples of tubulin binding chemotherapeutic agents

**Topoisomerase inhibitors**: Topoisomerases are enzymes that are associated with resolving the tangling of supercoiled DNA strands caused during DNA replication, transcription and various other stages of normal cellular functioning. These enzymes are of two types, I and II. They differ in their structures, mechanisms and cellular functions. Type I enzyme exists as a monomer and causes transient single strand breaks in duplex DNA, facilitating a change in the linking number of circular DNA by one. Type II enzyme is dimeric and cause cuts in both strands of duplex DNA to cause an opening through which a second DNA can be passed, resulting in linking number changes in steps of two. These enzymes are targets for some of the cancer drugs that are currently in clinical use. Examples of topoisomerase I inhibitors are camptothecins such as topotecan and irinotecan (Figure 5). Examples of topoisomerase II inhibitors are m-AMSA and etoposide (Figure 6).
Anticancer antibiotics: These are cancer drugs which slow down the immune system and are used in combination therapy to prevent metabolism of the drug molecules with which it is combined in therapy. Examples of anticancer antibiotic drugs are mitomycin C and amoxicillin (Figure 7).
**Anti-hormonal therapy:** Cancers like breast cancers are dependent on hormones like estrogen for their growth. Anti-hormonal therapy involves use of molecules to block the steroid or hormone responsible for the growth of the cancer cells.\(^\text{17}\) Examples of this class of drugs are tamoxifen and letrozole (Figure 8).

![Tamoxifen](image1)
![Letrozole](image2)

**Figure 8:** Tamoxifen (anti-estrogen therapy), letrozole (aromatase inhibitor)

**Small molecule inhibitors (targeted therapies):** Targeted approach to cancer therapy is a relatively new field. One way of materializing this approach is to investigate relationship of cancer cells with particular genes e.g. relationship of CML with BCR-ABL which resulted in gleevec.\(^\text{18}\) The targeted approach in cancer research also led to the development of tyrosine kinase inhibitors like tarceva\(^\text{19}\) (Figure 9).

![Tarceva](image3)
![Gleevec](image4)

**Figure 9:** Examples of targeted therapeutic agents.
Other novel approaches towards development of small molecule anticancer agents involve histone-deacetylase (HDAC’s) inhibitors, which function by affecting the transcription of genes. e.g. Vorinostat from Aton Pharma\textsuperscript{20} and telomerase inhibitor which affect telomere maintenance required for tumor progression. e.g. BIBR1532 from Boehringer Ingelheim.\textsuperscript{21}

**Monoclonal antibodies:** Several monoclonal antibodies approved in chemotherapy involve binding of radioactive nuclei to the antibody so as to use the targeting capacity of antibodies. e.g. Rituximab targets CD-20 antigen.\textsuperscript{22} Another monoclonal antibody which functions by angiogenesis inhibition is herceptin.\textsuperscript{23}

**Nature as a source of anticancer drugs:** More than 60\% of the clinically used cancer drugs are derived from natural products.\textsuperscript{24} The application of natural products in drug discovery can be attributed to both the uniqueness of their chemical structures and a possibility of facile adaptability of the natural products to human body because of their existence in biological ecosystems.\textsuperscript{25} Natural products can hence be viewed as nature’s library of privileged structures endowed with the ability to interact with specific biological targets.\textsuperscript{26}

**Plant based natural products as anticancer agents:** Several of clinically used cancer drugs are obtained from natural sources like terrestrial trees (Camptothecin) and plants (Vinca alkaloids). Following is a brief description of these two drugs:
Camptothecin and its analogs: Camptothecin was isolated in 1958 by Wall and Wani from the bark of the *Camptotheca acuminata* (Happy Tree) found in China. The structure of camptothecin was first elucidated in 1966, which showed that it had a pentacyclic quinoline ring system with a lactone containing a stereocenter. The progress of this natural product itself as a cancer drug was hampered because of problems associated with its poor water solubility and severe toxicity. However, the core structure provided ample scope for derivatization and discovery of synthetic analogs of camptothecin. The interest in camptothecin and development of its analogs was further accelerated by the discovery of its novel mechanism of action. Camptothecin inhibits topoisomerase I and there by causes the stabilization of the ternary complex of DNA-drug-Topo-I. Following decades saw an increase in the number of camptothecin analogs synthesized. Two semisynthetic analogs with improved water solubility; topotecan for treatment of metastatic colorectal carcinoma and irinotecan for ovarian and small-cell lung cancer were approved by FDA and marketed by Pfizer and GlaxoSmithKline as camptosar and hycomtin respectively. Till date several other synthetic analogs have been developed like belotecan, lurtotecan, gimatecan, exatecan, to name just a few. Many of these analogs are in different phases of clinical trials. Several interesting approaches towards improving the pharmacokinetic profiles and therapeutic efficacy of these analogs have been investigated. The efforts towards developing new camptothecin analogs into effective drugs have been summarized in recently published reviews.
**Figure 10**: Most recent camptothecin analogs.

**Vinca alkaloids**: Vinca alkaloids were serendipitously discovered from flowering plants *Catharanthus Roseus* (Madagascar periwinkle). More than 70 alkaloids were isolated from the sap of this plant species in 1950’s of which vincristine and vinblastine were found to exhibit cytotoxic action.\(^{14}\) Vincristine was approved by FDA and is currently used in treatment of hodgkins and non-hodgkins lymphoma, advanced testicular carcinoma and kaposi sarcoma while vinblastine is approved for small cell lung cancer, ewing sarcoma, melanoma and hodgkins and non-hodgkins lymphoma.\(^{31}\) Primary mechanism of action of these drugs were found to be microtubule destabilization. Further development of this family of compounds led to semisynthetic derivatives such as vindesine, vinorelbine and vinflunine. Vindesine is not approved by FDA, however the drug is approved in Europe for treatment of breast cancer, colorectal cancer, non-small cell lung cancer and renal cancer.\(^{31}\) Vinorelbine was found to be clinically effective against advanced non small cell lung cancer and advanced breast cancer and was approved by FDA in 1994.\(^{32}\)
Vinflunine is the most recent difluorinated semi synthetic derivative of vinca alkaloids. Vinflunine has been clinically evaluated in the treatment of advanced solid tumors, TCCU, metastatic breast cancer, advanced non small cell lung carcinoma and malignant pleural mesothelioma. A number of clinical trials are in progress for the vinca alkaloid analogs, the most recent one being phase I trial as a combination therapy with pemetrexed against advanced solid tumors which in spite of not showing efficacy did establish the effectiveness of the drug in stabilizing the disease.

**Marine Natural Products:** Another newly discovered resource of such potent anticancer compounds is the ocean. These compounds are called marine natural products. Nitrogen containing marine natural products are called marine alkaloids. Oceans account for a major proportion of earth’s surface and are home to a wide range of marine invertebrate species. Marine natural products are isolated from the invertebrate animals such sponges, molluscs, ascidians, corals, algae, and others. The increased competition among these species for nutrition and space; results in chemical adaptations in these
organisms in the form of secondary metabolites. Interestingly, cyanobacteria and other species of bacteria which are a source of nutrition for the invertebrates are the original source of these molecules. The secondary metabolites play an important role in defense, predation and reproduction of these organisms. These secondary metabolites are marine natural products. They belong to a variety of structural classes such as alkaloids, terpenoids, peptides, sugars, steroids etc. 

**Clinically used marine natural product based drugs:** The interest in development of drugs from marine sources began in 1951 with the discovery of arabino and ribo pentosyl nucleosides from marine sponges *Cryptotheca crypta* in Florida by Werner and Bergmann. The chemical derivatives of these nucleosides, cytarabine and vidarabine were developed for clinical use eventually against solid tumors (Figure 12). The importance of marine natural products in drug discovery suffered a decline in the 1990’s due to the lack of interest by pharmaceutical giants. However, the interest in research and development of marine natural products as drugs or lead molecules has been revived in the past decade with the discovery of new marine natural product based drugs such as ET-743 (trabectidin or yondelis, marketed by Pharmamar) (Figure 12).

![Vidarabine](image1.png) ![Cytarabine](image2.png) ![ET-743](image3.png)

*Figure 12:* Marine derived anticancer agents in clinical use.
Ecteinascidin-743: This drug was approved by the European Union for treatment of refractory soft tissue sarcoma. In early 1969, this compound was obtained from extracts of Caribbean tunicate *Ecteinascidia turbinata* and was shown to possess antitumor activity against L1210 leukemia cells (IC$_{50}$, 0.5 ng/ml). The structure was published in 1990 by Rinehart and Wright. Further *in vivo* studies of this compound in mouse models, demonstrated impressive antitumor efficacy on P388 lymphoma, B16 melanoma, M5076 ovarian sarcoma, Lewis and LX-1 human lung carcinoma and MX-1 tumors. It is produced in large scale using a semisynthetic route reported by Cuevas *et al.* Detailed mechanistic studies have been reported by Incalci *et al* which suggest that it is a DNA binding agent that subsequently causes apoptosis. Phase I and II clinical trials demonstrated the efficacy of this drug against solid tumors like breast and renal carcinomas and soft tissue sarcomas such as osteosarcomas and liposarcomas. The studies also demonstrated synergistic effects of ET-743 in combination therapy with drugs such as doxorubicin, taxol etc.

Eribulin mesylate: Another marine derived cancer drug approved by the FDA very recently in November 2010 is Eribulin mesylate for the treatment of metastatic breast cancer (Figure 13). It is being marketed with the trade name Halaven. It is a synthetic analog of Halichondrin B which was discovered by Uemura and co-workers in 1986 from a sponge *Halichondria okadai* in very minute concentrations. The total synthesis of halichondrin B was reported in 1992 comprising of 90 steps starting from commercially available materials. The discovery of the synthetic route led to the development of synthetic analogs of halichondrin B with reduced molecular weight and
comparable antitumor effect. Eribulin mesylate, the methane sulfonate salt of a terminal alcohol in halichondrin B as shown in the structure above was found to be the most potent. Mechanistic studies revealed that the drug binds tubulin and thereby arrests the cell cycle during mitosis.

![Halichondrin B](image)

**Figure 13**: Halichondrin B and its synthetic analog, eribulin mesylate

Phase I studies of eribulin mesylate against solid tumors were reported to be favorable at tolerable doses. Phase II trial results as a monotherapy against refractory breast cancer were published in 2006 followed by a number of other reports demonstrating the efficacy of the drug in anthracyclines or taxane pretreated patients. Phase II trial reports against squamous cell carcinoma were not very encouraging. Phase III results against metastatic breast cancer, EMBRACE (Eisai Metastatic Breast Cancer Study Assessing Physician’s Choice Versus Eribulin) showed that heavily pre-treated patients who were administered Eribulin mesylate showed an improved median
overall survival (OS) of 13.12 months compared with Treatment of Physician’s Choice (TPC) of 10.65 months.\textsuperscript{56, 57}

**Marine natural products and analogs currently in clinical trials:**

Pharmacological value of the marine alkaloids is illustrated by the fact that several marine alkaloids are currently undergoing preclinical or investigations in various phases of human clinical trials for treatment of different cancers.\textsuperscript{36, 37, 40, 58-66} A few examples of these are depicted in Table 1

**Table 1: Marine alkaloids in clinical phase trials**

<table>
<thead>
<tr>
<th>Natural Product</th>
<th>Marine organism</th>
<th>Species</th>
<th>Clinical trial Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Didemnin B</td>
<td>Tunicate</td>
<td>Trididemnium.solidum</td>
<td>Withdrawn</td>
</tr>
<tr>
<td>Aplidine</td>
<td>Tunicate</td>
<td>Aplidium.albicans</td>
<td>Phase II</td>
</tr>
<tr>
<td>Kahalalide F</td>
<td>Sea slug</td>
<td>Elysia rufescens</td>
<td>Phase II</td>
</tr>
<tr>
<td>Bryostatin</td>
<td>Bryozoan</td>
<td>Bugula neritana</td>
<td>Phase II as co-drug</td>
</tr>
</tbody>
</table>

Didemnin B and aplidine were both isolated from tunicates by Rinehart \textit{et al} in early 80’s and 90’s respectively.\textsuperscript{37} On account of good \textit{in vitro} and \textit{in vivo} activity, Didemnin B was submitted for Phase I and Phase II clinical trials against a number of cancers. However, these trials resulted in neuromuscular toxicity, severe fatigue and anaphylaxis and hence were terminated.\textsuperscript{40} Aplidine, is a structural variant of Didemnin B with almost similar activities and better toxicity profile is currently undergoing Phase II clinical trials.\textsuperscript{40}
Kahalalide F was isolated from a sacoglossan (sea slug) named *Elysia Rufescens* by the Scheuer group from the University of Hawaii in 1993. Kahalalide F exhibited excellent *in vitro* activity against a number of tumors. In addition, it was thoroughly investigated on account of its ability of not affecting stem cells (low toxicity) thus indicating a high therapeutic index. Phase II trials of Kahalalide F against advanced malignant melanoma did not result in a good response in patients. However, clinical trials on androgen-refractory prostate cancer were successful and demonstrated efficacy of the compound. Further trials are in progress.
Bryostain 1 was isolated from bryozoan *Bugula neritana* in the Gulf of Mexico in 1968 by Pettit and co-workers.\(^{37}\) In addition to potent *in vitro* and *in vivo* activities against various cancer cell lines, synergistic effects with other drugs such as ara-C, paclitaxel, tamoxifen, dolastatin, vincristine doxorubicin and prednisone lead to its advancement in numerous phase I clinical trials either alone or in combination with other drugs.\(^{40}\) Phase II trials of Bryostatin 1 with paclitaxel against advanced pancreatic carcinoma were not successful,\(^{67}\) however, with vincristine in treatment of non-Hodgkin’s lymphoma was reported to be efficacious.\(^{68}\) Further reports on trials with bryostatin as co-drug are awaited.

![Chemical Structure of Bryostatin 1](image)

**Figure 16**: Bryostatin 1

**N-containing marine natural products**: This part of the introduction emphasizes on the importance of marine alkaloids as an important source of leads for drug discovery. Considerable numbers of these compounds are N-containing heterocycles. Following will be a concise description of some of the classes of N-containing marine alkaloids.
Pyrrole alkaloids of marine origin: These include lamellarins, ningalins, lukianols, polycitones, polycitrins, purpurone, rigidins and storniamides of which lamellarins are the most cytotoxic. The lamellarins A-D were first reported by Faulkner’s group in 1985 and were isolated from the Paluan mollusk Lamellaria sp. Lamellarins E-H were isolated in 1988 from an ascidian Didemnum chartaceum in the Indian ocean. Lamellarins I-M and triacetate of lamellarin N were obtained from ascidian Didemnum sp in Australia, lamellarins O,P,Q and R were isolated from a marine sponge, Dendrilla in Australia. Lamellarin S was isolated from an Australian ascidian Didemnum sp. Sulfated lamellarins T, U, V and Y were isolated from an unnamed ascidian from the Arabian Sea in the Indian subcontinent. Five lamellarin analogs which included the 20-sulfated derivatives of lamellarins B, C and L, the 8-sulfated derivative of lamellarin G and lamellarin Z were reported in 1999 from the Australian ascidian Didemnum chartaceum. In 2004, lamellarins γ, α and ε were reported to be isolated from the ascidian Didemnum obscurum followed by lamellarins ζ, η, φ and χ in 2005. A number of reviews on synthetic efforts towards these molecules have been published.

Figure 17: Examples of pyrrole alkaloids exhibiting cytotoxic activity
All lamellarins exhibited some level of cytotoxicity, however lamellarins D, X, ε, M and N showed the highest cytotoxic activity against all the cell lines tested. Lamellarins were also reported to be useful in treatment of MDR tumors on account of their own cytotoxic action in addition to synergistic increase in cytotoxicity of doxorubicin against MDR cells. Pharma Mar patented the use of the lamellarins for the treatment of MDR tumours. Studies on mechanisms of actions suggest topoisomerase I inhibition, cell death as a result of interaction with cancer cell mitochondria and inhibition of protein kinases to be the reasons for the cytotoxicity of these compounds.

β-carboline / isoquinoline alkaloids: Manzamine A was the first β-carboline to be isolated from a sponge *Haliclona* sp. in 1986. However, pharmacologically the most important of these compounds are ecteinascidins, first reported in 1990 from the colonial marine ascidian *Ecteinascidia turbinata*. A member of this class of compounds, ET-743 has very recently been approved for clinical use and has been described in detail earlier in the introduction.

![Manzamine A](image)

**Figure 18:** Representative example of a β-carboline alkaloid
The other important bioactive subclass is manzamines. Higa’s group reported manzamines A and B-F, from the Okinawan marine sponge *Haliclona sp* and *Xestospongia sp.*, respectively. These exhibited activities ranging from 0.5-6 µg/mL in breast, colon, lung and P388 cancer cell lines.\(^7^2\) Manzamine G (KB human epidermoid carcinoma cell line IC\(_{50}\), 0.03µg/mL) was obtained from the Indonesian marine sponge *Pachypellina sp*. Manzamines H and J reported by Higa’s group, from the Okinawan marine sponge *Ircinia sp* exhibited cytotoxicity against L1210 murine leukaemia and KB human epidermoid cells with IC\(_{50}\) values ranging from 1-10 µg/mL.\(^7^2\) Kobayashi’s group reported manzamine X and Y from *Xestospongia sp*, while 3,4-dihydromanzamine A, keramaphidins B and C, keramamine C, ircinols A and B, manzamine L and M from the Okinawan marine sponge *Amphimedon sp*. All of these showed cytotoxic activity against P388 and KB cell lines in the range of 0.28-11.8 µg/mL.\(^7^2\) Synthesis of these compounds has been recently reviewed.\(^8^3\) Latest research focuses on use of these compounds as antimalarial agents.\(^8^3\)

**Pyridopyrrolopyrimidine alkaloids:** Variolin A, variolin B, N(3’)-methyl tetrahydrovariolin B and variolin D belonging to the pyridopyrrolopyrimidine class were isolated from the Antarctic sponge *Kirkpatrickia varialosa* in 1994.\(^8^4,^8^5\) Cytotoxicity of the two major variolins against the P388 cell line was reported as IC\(_{50}\) value of 3.8 mg/mL for variolin A and 210 ng/mL for variolin B. N-(3’)-methyl tetrahydrovariolin B showed *in vitro* activity against the HCT 116 cell line (IC\(_{50}\), 0.48 mg/mL), but only modest *in vivo* activity against the P388 cell line.
**Indole alkaloids**: Indole alkaloids isolated from marine invertebrates could be an important source of lead molecules for cancer drug discovery. A subclass of indole alkaloids is halogenated indole alkaloids like psammopemmins, aplicyanins, meridianins, aplysinopsins and leptodiniclamine. Of these, meridianins and aplicyanins have been reported to exhibit cytotoxic effects. Meridianins are marine alkaloids isolated from ascidian *Aplidium meridianum*. Meridianins B,C,D and E display cytotoxicity toward murine mammalian adenocarcinoma cell line with IC$_{50}$ values ranging from 9-33µM. The mechanism of action is inhibition of various kinases.

**Figure 19**: Example of a pyridopyrrolopyrimidine alkaloid

**Figure 20**: Representative examples of indole alkaloids
Aplicyanins are a new family of indole alkaloids isolated from Antarctic tunicate *Aplidium cyaneum* by Reyes and co-workers. Aplicyanins B, D and F are cytotoxic to the human tumor cell lines MDA-MB-231, A549 and HT-29 in submicromolar range and also exhibit antimitotic activity.\(^\text{88}\) Very recently another group of indole alkaloids named as grandilodines A and C were reported to be active against vincristine resistant KB cells with an IC\(_{50}\) value of 4 \(\mu\text{g/mL}\).\(^\text{89}\)

**Pyridoacridines**: Amphimedine 1, isolated from the pacific sponge *Amphimedon* *sp* in 1983 was the first example of a pyridoacridine alkaloid with antitumorigenic effects.\(^\text{90}\) In 1999, neoamphimedine, was isolated from two specimen of *Xestospongia* *sp*. and is a topoisomerase II inhibitor as established by plasmid DNA catenation assay.\(^\text{91}\) Deoxyamphimedine was isolated by from *Xestospongia* *sp*. and exhibited cytotoxicity as a result of DNA damage via production of reactive oxygen species (ROS).\(^\text{92}\) Scheuer’s group reported shermilamine A and B while shermilamine D, E and cycloshermilamine were isolated from tunicate *Cystodytes violatinctus* by Kashman *et al.*\(^\text{93}\) Shermilamine C was isolated in 1994 by McDonald *et al* from an ascidian *Cystodytes sp.* in Fiji.\(^\text{93}\) Shermilamine D exhibiting cytotoxicity against P-388, A-549, HT-29 and MEL-28 cancer cell lines was the most potent of all shermilamines with IC\(_{50}\) values ranging from 0.3-2.6\(\mu\text{m}\). Kuanoniamines also belong to the rare benzothiazole class of alkaloids.\(^\text{94}\) Kuanoniamines A–D were reported by Caroll and Scheuer from a mollusk *Chelynotus semperi*.\(^\text{93}\) Kuanoniamines A and C were also isolated from marine sponge *Oceanapia sagittaria*.\(^\text{95}\) Kuanonamine A was the most active compound of the group reportedly inhibiting the proliferation of KB (human pharyngeal cancer) cell lines *in vitro* with an
IC$_{50}$ value of 1 µg/mL.$^{95}$ Kuanoniamine A was also found to cause extensive reduction of MCF-7 cells in G2/M phase.

Figure 21: Representative examples of pyridoacridine alkaloids.

Mechanism of action was reported to be inhibition of DNA synthesis and apoptosis.$^{93}$ Kuanoniamines D exhibited cytotoxic potencies against HCT cells in vitro with an IC$_{50}$ value of 8 µM.$^{93}$ Kuanoniamine C was less potent but showed high selectivity towards the estrogen receptor positive (ER+) breast cancer cell lines.$^{95}$ Thus, pyridoacridine alkaloid analogs can be interesting as potential anti-cancer agents with unique mechanistic pathways.

**Pyrroloquinolines:** Pyrroloquinolines containing a 1,3,4,5-tetrahydropyrrolo[4,3,2-de]quinoline ring system, were first reported in 1986, with the structural elucidation of discorhabdin C from the New Zealand sponge *Latrunculia cf. bocagei*. Discorhabdin C is an example of a fully substituted iminoquinone unit. Several other trisubstituted and fully substituted iminoquinones were isolated and evaluated for their antitumor properties. These included other discorhabdins, prianosins, batzellines, isobatzellines, damirones, makaluvamines, epinardins, tsitsikammamines, wakayin and
Discorhabdin C was isolated from the sponge *Latrunculia cf. bocagei.*

Discorhabdins A, B, O and discorhabdin Q were isolated from different subgenera of *Latrunculia* namely *brevis, purpurea, apicalis, bocagei* and *biformis* and also from *Zygya marsailis and fuliginosa.*

The origins have been reviewed in great detail by Urban *et al.* and recently by Hu *et al.*

Discorhabdin P was isolated from a deep water Caribbean *Batzella* sp. The discorhabdin series of alkaloids have been reported to exhibit impressive *in vitro* cytotoxicity against a variety of cancer cell lines such as P388, L1210, HCT-116 and KB. Among the most active of discorhabdins are A, B, C and I exhibiting an activity of 10, 18, 40 and 55 ng/mL respectively against P388 cell lines.

Discorhabdins S, T and U were isolated from a deep water marine sponge of the genus *Batzella.* Discorhabdins S, T and U exhibited IC$_{50}$ values of 3.08, >5 and 0.17 µM respectively against cultured murine P388 tumor cells, IC$_{50}$ values of >5, >5 and 0.17 µM respectively against A549 human lung adenocarcinoma cells and IC$_{50}$ value of 2.6, 0.7 and 0.069 µM respectively against PANC-1 human pancreatic cells.

Discorhabdin V was isolated from a new South African sponge species, *Tsitsikamma pendunculata* and exhibited cytotoxicity with an IC$_{50}$ value of 1.26 µM against HCT 116 cell lines.

Discorhabdin W, isolated from a New Zealand *Latrunculia sp.* sponge is a symmetrical dimer with potent *in-vitro* activity against the P388 murine leukemia cells (0.084 µM).

Discorhabdin X was isolated from South Australian marine sponge of the genera *Higginsia* and *spongisorites.* Discorhabdin Y isolated from a new deep water sponge species of the genus *Latrunculia* exhibited a wide range of antiviral and antimicrobial effects but no cytotoxic effect was observed.
Discorhabdin Z was obtained from dark green sponge *Sceptrella sp.* in Korean waters and exhibited cytotoxic activity of 2.2 µM against K562 leukemia cell line.\(^{102}\) Latest review summarizing origin and syntheses of discorhabdins and related alkaloids have been published recently.\(^{96}\) The batzellines A-C have been reported to exhibit anticancer activities in pancreatic cell lines.\(^{103}\) Isobatzellines A-D were isolated from a sponge of the genus *Batzella* in the Bahamas and are moderately active against leukaemia P388 cell line.\(^{72}\) The secobatzellines A and B, were also identified from a deep water Bahamian *Batzella* species. Secobatzelline A is particularly active against P388 and A549 cell lines.\(^{72}\)

**Figure 22:** Pyrroloquinoline alkaloids.

The alkaloids of our interest, makaluvamines and zyzzyanones will be discussed in detail in the various chapters of this dissertation and hence have not been included in
the general introduction. Several of these compounds described in this section of the dissertation are being extensively investigated in attempts to facilitate their transformation into successful drugs. Many of these compounds are likely to progress into clinical trials. This introduction thus further justifies the unique and role played by marine natural products in drug design and discovery.

**Role of synthesis in development of marine derived anticancer agents:**

Besides the fact that many of these classes of natural products have been obtained from their natural sources or biosynthesis, chemical and semi-synthesis plays a major role in their progress towards development into a clinically useful drug. The importance of chemical synthesis has been further highlighted by the immense collection of literature available till date. However, besides the complex structures in natural products being intellectually stimulating and challenging to organic / medicinal chemists there are practical advantages to chemical synthesis which include but are not limited to the following:

- Isolation in minute quantities is a drawback in terms of a thorough biological evaluation.
- Ecologically disadvantageous and hence impermissible to harvest large quantities of marine creatures.
- Emulation of the natural ecosystems of marine organisms to facilitate their growth may not be feasible.
- Synthesis or semisynthesis increases accessibility to larger quantities of bioactive compounds.
• Customize synthesis to incorporate groups on natural product that favors desirable biological properties and remove the groups that are unfavorable.

RESEARCH OVERVIEW

As a part of the ongoing research in our laboratory directed towards identifying drug leads from marine natural products, we were interested in the synthesis of marine natural products, their analogs and evaluating them for their potential antitumor activity. We are particularly interested in the class of marine alkaloids containing pyrroloquinoline ring system. This dissertation focuses on development of synthetic analogs of makaluvamines, development of synthetic method for bispyrroloquinone ring system using an oxidative free radical cyclization mediated by CAN or Mn(OAc)$_3$, total syntheses of zyzzyanones A-D and proposes future directions towards total syntheses of tsitsikammamines and wakayin.

**Synthesis and biological evaluation of makaluvamine analogs:** The first chapter of this dissertation focuses on the synthesis of makaluvamine analogs. This synthesis is a direct application of total synthesis of makaluvamine D accomplished using the 4,6,7-trimethoxyindole approach. Synthesis of all the analogs required an N-tosyl-6-methoxy tricyclic pyrroloiminoquinone intermediate as the precursor which is synthesized according to the reported procedure. This key intermediate is subjected to amination and deprotection steps to obtain the makaluvamine analogs. Previous work carried out in our laboratory has accomplished the synthesis of several analogs of makaluvamines and reported their preliminary *in vitro* cytotoxic activities. All of the synthesized analogs showed excellent *in vitro* activity against a number of cancer cell lines which warranted
further investigations towards synthetic as well as \textit{in vivo} studies. Hence additional analogs were synthesized using a similar strategy. Detosylation step in the synthesis of makaluvamine analogs was a low yielding step. So, efforts were made towards developing new synthetic methodology for detosylation reaction and the results of the same are reported. In addition, a concise account of \textit{in vitro} and \textit{in vivo} evaluation anticancer activities of selected makaluvamine analogs is presented.

\textit{Synthesis of bispyrroloquinone ring system via CAN mediated oxidative cyclization:} In the second chapter, we have developed a new oxidative free radical reaction between 6-benzylamionindolole-4,7-quinones and 1,3-dicarbonyl compounds mediated by CAN to form a tricyclic bispyrroloquinone ring system. Bispyrroloquinone and bispyrroloiminoquinone are two common polycyclic structural scaffolds present in a number of biologically active marine alkaloids such as zyzzyanones, tsitsikammamines and wakayin. So, this methodology will be useful in the synthesis of these alkaloids and/or their analogs. A plausible mechanism for this transformation is proposed based on previous literature reports of similar reactions. In order to demonstrate the generality of this synthetic methodology, the oxidative free radical cyclization reaction was extended to different combinations of aminoquinones and \(\beta\)-dicarbonyl compounds. The methodology was also extended to explore the use of \(\beta\)-ketsulfides, \(\beta\)-ketosulfoxides and \(\beta\)-ketosulfones in the place of \(\beta\)-dicarbonyl compounds. The reaction worked well in the case of \(\beta\)-ketosulfones. However, it failed to give expected products in the case of \(\beta\)-ketsulfides and \(\beta\)-ketosulfoxides. Synthetic procedures for the removal of protecting groups such as N-benzyl and N-tosyl from the bispyrroloiminoquinone ring system have also been standardized.
**Total Syntheses of Zyzzyanones A-D**: Chapter 3 describes the first total syntheses of four marine alkaloids, zyzzyanones A-D. Our first objective was to apply the CAN mediated oxidative cyclization for the synthesis of zyzzyanone core ring system. As zyzzyanones contained a phenolic ring, we had to modify our CAN mediated oxidative cyclization, we developed in chapter 2. This is mainly because CAN mediated oxidative cyclization reaction using 6-benzylamniñoindole-4,7-quinone with 1,3-diacrboyl compounds was not suitable for introducing aromatic rings on the bispyrroloiminoquinone ring. Our effort in overcoming this problem resulted in the identification of Mn(OAc)$_3$ as an alternative reagent and use of acetal as an alternative for 1,3-dicarbonyl compound used in the oxidative cyclization reaction. We prepared the required acetal and demonstrated that the oxidative cyclization of reaction of 6-benzylamniñoindole-4,7-quinone with 4-benzyloxyphenyl acetaldehyde acetal in the presence of Mn(OAc)$_3$ worked well in a model system. More specifically, an oxidative cyclization of 6-benzylamniñoindole-4,7-quinone derivative with 4-benzyloxyphenyl acetaldehyde acetal in the presence of Mn(OAc)$_3$ resulted in the formation of a bispyrroloquinone with appropriate substitutions in place. All four zyzzyanones were prepared from this key intermediate in a few steps. An in-depth discussion of the steps involved in this synthesis, including the setbacks and the strategies we used to overcome the setbacks is presented.
SYNTHESIS AND BIOLOGICAL EVALUATION OF MAKALUVAMINE ANALOGS

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In preparation for Medicinal Chemistry

Format adapted for dissertation
Abstract

Several analogs of makaluvamines have been synthesized. All the synthesized analogs were characterized using $^1$H NMR, $^{13}$C NMR and mass spectral analysis. In an effort to improve the yield of the makaluvamine analogs synthesized in this project, a novel azide mediated detosylation of N-tosylpyrroloiminoquinones was developed. Most of the synthesized analogs exhibited impressive cytotoxic activities against a number of cancer cell lines. Several investigative experiments were carried out to study mechanism of action of these analogs. *In vitro* testing on three different lung cancer cell lines revealed 7-(4-chlorobenzylamino)-3,4-dihydro-1-(toluene-4-sulfonyl)-pyrrolo[4,3,2-de]quinolin-8(1H)-one to be a very potent and selective agent against lung cancer. 7-(benzylamino)- 3,4-dihydropyrrolo[4,3,2-de]quinoline-8(1H)-one and 7-(4-fluorobenzylamino)- 3,4-dihydropyrrolo[4,3,2-de]quinoline-8(1H)-one were especially active against breast and prostate cancer cell lines respectively. *In vivo* studies of these two analogs were conducted in mice bearing MCF-7 and MDA-MB-468 xenograft tumors. 7-(4-fluorobenzylamino)- 3,4 -dihydropyrrolo[4,3,2-de]quinoline-8(1H)-one was also found to be active against OVCAR-3 cells and testing was done *in vitro* and *in vivo* to establish the same. Additional *in vitro* testing demonstrated the antiproliferative potency of these compounds against gliomas and pancreatic cancer cell lines. Overall, makaluvamine analogs were demonstrated to be impressive potential therapeutic agents against a variety of cancers.
Introduction

Makaluvamines is a group of 16 marine alkaloids bearing a pyrrolo[4,3,2-de]quinoline skeleton. Isolation and structural characterization of makaluvamines A to F were first reported by Radisky et al in 1993 from the methanolic extract of Fijian sponge *Zyzzya cf. marsailis*. Makaluvamine G was isolated along with known makaluvamines A and C from Indonesian sponge of genus *Histodermella* by Carney et al. Makaluvamines H to M were isolated from Pohnpeian sponge species of *Zyzzya fuliginosa* along with known makaluvamines C, D and G by Schmidt et al in 1995. In 1997, Venables et al reported the isolation and structure determination of makaluvamine N from a Philippine specimen of *Zyzzya fuliginosa*. The extraction procedure for isolation of makaluvamine N also resulted in isolation of known makaluvamines A, C, D, E and I. Makaluvamine O was isolated from Jamaican sponge *Smenospongia aurea* by Hu et al in 2002. The last of the known makaluvamines i.e. makaluvamine P was isolated from *Zyzzya fuliginosa* collected off the coast of the Vanatu islands along with known makaluvamines G, J, K and L by Casapullo et al in 2001. Figure 1 shows the structures of 16 naturally occurring makaluvamines discovered so far from different marine sponges.

Makaluvamines have exhibited an array of biological activities, primarily cytotoxicity against cancer cells. The cytotoxic action of makaluvamines was initially attributed to the interaction of these alkaloids with the enzymes topoisomerase I and II enzymes which are involved in DNA stabilization and relaxation mechanisms. Makaluvamines A to F along with other structurally related alkaloids such as makaluvone and Damirone B, were evaluated against human colon tumor cell line, HCT-
A Chinese hamster ovary (CHO) cell line, Xrs-6 that is sensitive to anticancer agents which exhibit their activity by creating DNA double stranded breaks. Makaluvamines A and F were the most active among the reported makaluvamines against HCT-116.

A decatenation assay demonstrated that all makaluvamines exhibited varying degrees of topoisomerase II inhibitory activity. The analysis of cytotoxic activity and topoisomerase II inhibitory activity of makaluvamines and related alkaloids such as

**Figure 1**: Structures of 16 known Makaluvamines A-P.
makaluvone and damirone B revealed an important structure activity relationship among these alkaloids. Makaluvamine B and makaluvone and damirone are structurally related to makaluvamines. However, they are either inactive or less active in cytotoxic as well as topoisomerase II decatenation assays, while all other makaluvamines demonstrated activity ranging from good to excellent. An examination of the structures of the inactive compounds revealed an important structure activity relationship. Makaluvamine B comprises of an aromatic ring and can be considered to be an oxidized form of makaluvamine A. Makaluvone and damirone B contain an o-quinonoid structure instead of p-iminoquinonoid structure present in makaluvamines (Figure 2). The fact that these structural features of makaluvamine B, makaluvone and damirone B are non-conducive to activity of these molecules indicates that the p-iminoquinonoid structural feature that is present in makaluvamines is responsible for the anticancer activity of this class of compounds.

![Chemical structures](image)

**Figure 2:** Makaluvamines A, makaluvone and damirone B.

Preliminary *in vivo* evaluation of makaluvamines A and C have also been conducted in xenograft models of P388 murine leukemia and ovcar 3 in athymic nude mice.¹ Both makaluvamine A and C suppressed tumor growth in the ovcar 3 solid tumor
model and caused slight life extension in P388 leukemia model. DNA intercalation studies were performed by monitoring the changes in the absorbance spectra of the compounds with increasing amounts of calf thymus DNA and comparing the results with known DNA intercalating agents as controls. Both makaluvamines A and C produced 53% and 66% absorption hypochromism and red shifts of 6 nm which are comparable to the controls.\(^1\) Dijoux et al reported the anticancer activity of several makaluvamines along with isobatzellines, batzellines and damirones in 2005.\(^9\) Among these Makaluvamine H was found to be the most potent followed by Makaluvamine C. Both these alkaloids were tested against ten cancer cell lines from the NCI panel. Topoisomerase II action of makaluvamines H and C were compared with etoposide as a control. Topo II, DNA and makaluvamine ternary complex stabilization was observed in these experiments, however was not as effective as in case of etoposide.\(^{9}\) This report also proved that reductive activation of makaluvamines can result in DNA damage in vitro and the ability of makaluvamines to intercalate with DNA was also reconfirmed.\(^9\) These studies suggested that makaluvamines exhibit their cytotoxic action via promotion of topoisomerase II cleavage, DNA intercalation and direct DNA damage under reductive activation conditions.

A number of efforts by different groups towards the total synthesis of makaluvamines have been reported in the literature. Two major approaches employed for the synthesis of the tricyclic pyrroloiminoquinone core of makaluvamines are 1) the indole approach\(^{10-21}\) and 2) the quinoline\(^{22-24}\) approach. All makaluvamines possess the pyrroloiminoquinone ring system with an amino group at the 7- position of the ring. This amine group gives these alkaloids the dark red or purple color and also confers hydrolytic
stability to the iminoquinone moiety. As discussed earlier, the \( p \)-iminoquinone moiety present in makaluvamines is the main factor for the activity of these compounds. Hence, most studies on making synthetic analogs were focused on derivatization keeping the tricyclic pyrroloiminoquinone system intact. In 1996, Zhao et al reported the synthesis and biological evaluation of compounds produced by combining the pyrroloimino-quinone moiety with DNA minor groove binding agent lexitropsin.\(^{25}\) The strategy was to improve DNA affinity, sequence selectivity and increase cellular uptake of makaluvamines.\(^{25,26}\) Beneteau et al reported the synthesis of pyrrolothiazio analogs of the pyrroloiminoquinone pharmacophore in 2001.\(^{27}\) This synthesis was a good example of incorporation of polyheterocyclic systems on the main pharmacophore. The final products however had poor solubility making their biological evaluation difficult. In 2006, Legentil et al reported the pyrazolic analogs of pyrroloiminoquinone alkaloids and their topoisomerase II inhibition and \textit{in vitro} evaluation of anticancer activity against three human tumor cell lines and two murine cell lines.\(^{28,29}\) In 2000, Besson et al reported the synthesis and antiproliferative evaluation of several 7-amino substituted pyrroloiminoquinone derivatives.\(^{30}\)

Our research on marine natural products of this class involves synthesis of novel makaluvamine analogs and evaluation of their potential anticancer activity. Our approach to this research was to synthesize non-natural analogs of these alkaloids with simple substitutions at the C-7 position and test the resulting compounds for their cytotoxicity. Earlier studies from our laboratory have resulted in the identification of a number of makaluvamine analogs with substitutions at the 7-position with hydrophilic, hydrophobic and sterically bulky groups.\(^{31,32}\) The current work is a continuation of this previous work.
which reinforces most of the results initially published with the synthesis of several newer analogs and their biological evaluation. We have also demonstrated the in vivo activity of several of our analogs in xenograft models of breast, prostate and ovarian cancers. During the synthesis of makaluvamine analogs we were faced with a low yielding detosylation step which affected the overall yield of the final compounds. In order to circumvent this problem, we discovered a new detosylation reagent NaN$_3$, which worked very well for compounds containing N-tosylpyrroloiminoquinones.

**Results and Discussion**

The research described here includes the synthesis and evaluation of newer makaluvamine analogs along with the resynthesis of some older analogs that has already been reported by our group in order to carry out additional in vitro and in vivo biological evaluations. List of compounds synthesized are given in Figure 3.

As many pyrroloiminoquinone alkaloids with proven cytotoxicities were found to have substitutions at the 7-position of the ring, we decided to explore substitutions with increased steric bulk, hydrophobicity and hydrophilicity at the 7-position of the pyrroloiminoquinone ring and study their effects on cytotoxicity. Of the 28 compounds listed in Figure 2, compounds 1b-f and 2o-v are new makaluvamine analogs synthesized and compounds 1a and 2a-n are the analogs resynthesized in small / larger quantities for additional in vitro / in vivo evaluations and mechanistic studies. Synthesis of these makaluvamine analogs followed the same procedure as reported from our laboratory previously.$^{31, 32}$ This involves the amination of N-tosyl-7-methoxypyrroloiminoquinone.
intermediate (3) with various amines followed by the removal of the tosyl group from the aminated product.

![Chemical structures]

<table>
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<td>2n</td>
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**Figure 3**: Makaluvamine analogs synthesized
N-tosyl-7-methoxypyrrroloiminoquinone intermediate (3): The starting compound required for the synthesis of makaluvamine analogs is a methoxy substituted tricyclic pyrroloiminoquinone derivative 3 which was synthesized using a previously reported 13 step synthetic procedure as shown in the Figure 4.

![Chemical Diagram]

**Figure 4: Synthesis of N-Tosyl-7-methoxytricyclic pyrroloiminoquinone (3).**
The synthesis started from commercially available 2,4,5-trimethoxybenzaldehyde (4) which was converted to 4,6,7-trimethoxyindole (8) in four steps. 2,4,5-trimethoxybenzaldehyde was condensed with methyl azidoacetate to afford the methyl azidocinnamate (5) thermolysis of which in refluxing xylenes gave 4,6,7-trimethoxyindole-2-carboxylate (6) in 98% yield. Hydrolysis of the carboxylate in 82% yield followed by decarboxylation of the resulting acid 7 using BaO afforded 4,6,7-trimethoxyindole (8) in 70% yield. Reaction of indole with oxalyl chloride gave the glyoxalyl intermediate which was then reacted with dibenzyl amine to afford the glyoxalyl amide 9 in 69% yield. Reduction of the amide using LiAlH₄ yielded the N,N-dibenzyl tryptamine derivative 10 in 99% yield. Protection of indole nitrogen of compound 10 was carried out by treatment with Ts₂O in the presence of KH/THF to afford the N-tosyl derivative 11 in 84% yield. Reductive debenzylation of the resulting intermediate using Pd black in the presence of HCOONH₄ afforded the free tryptamine 12 in 94% yield which was converted to BOC-protected compound 13 in 78% yield by treatment with (BOC)₂O. Oxidation of this compound using CAN in the presence of Bu₄NHSO₄ afforded the quinone 14. Removal of the BOC group from the quinone by treatment with TFA resulted in the formation of the pyrroloiminoquinone intermediate 3 in 98% yield.

Conversion of compound 3 to makaluvamine analogs:

Synthesis of proposed target compounds is outlined in Figure 5. All target compounds were synthesized in two steps starting from the known tricyclic pyrroloiminoquinone compound, 3.
Our initial attempts of amination of compound 3 always used an excess of the amine reagent, which caused the amination and detosylation in one step resulting in the formation of final products 2, directly. Excess amine was facilitating the detosylation in these reactions. So, for these compounds the intermediate N-tosyl derivatives were not isolated. However, later efforts using lesser equivalents of amine reagent (1-1.2eq) helped us isolate the tosyl intermediate compounds 1a-f in good purity. Remaining tosyl intermediates were subjected to detosylation without isolation of the intermediate product. Removal of tosyl protecting group from the tosylated compounds was accomplished by treatment with NaOMe in MeOH to obtain the final products 2a-v in 15 - 29% yield over two steps. All final products were completely characterized. Among the different derivatives synthesized were aliphatic substituents NHCH₃, aromatic benzyl and phenethyl amino substituents with different functional groups at 3, 4 and 5 positions and amino substituents containing aromatic heterocyclic systems such as furan and thiophene. Thus two groups of makaluvamine analogs were synthesized. First group contains a p-toluenesulfonyl (tosyl) group on the pyrrole ring nitrogen, while the second group contains a free pyrrole N-atom. Even though these reactions worked well to prepare the final products in sufficient quantities and purity required for biological evaluations, the
yield of the detosylation step suffered from the poor yield. Detosylation reaction of this class of compounds has been reported to be poor yielding in the literature. We have rectified this problem by discovering a new method for the removal of N-tosyl groups from N-tosylpyrroloiminoquinones using a neutral reagent NaN₃ in polar aprotic solvents such as DMF and DMSO.

**Alternative procedure for carrying out the detosylation using NaN₃:**

Even though we were able to make sufficient quantities of makaluvamine analogs by the method described earlier, our synthesis had a major drawback of getting only a low yield (15 to 29 %) over two steps. Hence we made an attempt to investigate alternative methods to introduce an amino group at the 7-position of pyrroloiminoquinone compound 3. One such alternative method is to introduce an azide functionality at the 7-position of 3. Reduction of azide to amine and further reductive amination of the primary amine formed would allow us to prepare a variety of aminated makaluvamine analogs. With this objective we treated compound 3 with NaN₃ in DMF as shown in Scheme 3. However, when this reaction was attempted we were not able to get the expected azide product 15. Instead, it led to formation of the detosylated product 16 in 83 % yield. This reaction was surprisingly clean without the formation of any byproducts. Presumably, the azide ion attacks the sulfur atom of the tosyl group to effect the detosylation reaction. As evidence to this mechanism, the byproduct, p-toluenesulfonyl azide, formed during this reaction was also isolated and characterized.
Even though the reaction did not work as expected, the result of the reaction prompted us to utilize this method as an alternative synthetic methodology for detosylation of tricyclic N-tosyl pyrroloiminoquinone compounds. During the synthesis of makaluvamine analogs, after the introduction of substituents at the 7-position, we needed to detosylate the intermediates to obtain the final products of our interest. Our detosylation reactions of these intermediates using traditional reagents (NaOH, NaOMe and NH₄OH) were complicated by the formation of side products and always resulted in low yields. Similar difficulties in detosylation of N-tosyl pyrroloiminoquinones have been reported in the past in the case of a pyrroloiminoquinone alkaloid analogue synthesis. In this particular literature report, the detosylation was carried out in 5-20% yields using the reagent TBAF. In order to circumvent this problem we decided to explore the possibility of using NaN₃ to facilitate detosylation of the aminated N-tosylpyrroloiminoquinones. We have examined the detosylation of N-tosyl-7-amino-substituted pyrroloiminoquinones containing 4-fluorobenzylamino-, 3,4-methylenedioxyphenethylamino-, and 3,4-dimethoxyphenethylamino- substituents under
different conditions to obtain the detosylated products 2n, 2f and 2s respectively (Figure 7).

![Chemical Structures](image)

**Figure 7:** NaN₃ mediated detosylation of makaluvamine analogs.

The deprotection reaction was carried out in polar aprotic solvents such as DMF and DMSO. The results are summarized in Table 1. The deprotection was found to be effective in 64–98% yield in polar aprotic solvents such as DMF and DMSO.

**Table 1:** NaN₃ mediated detosylation of N-tosylpyrroloiminoquinones

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Traditional detosylation agents known in literature are NaOMe³³, Sodium hydroxide³⁴, thioglycolate³⁵, amines¹³ and TBAF²⁷. This is the first ever report of using
NaNO₃ as a reagent for detosylation of N-tosyl groups. The following are the advantages of this detosylation method. 1) This detosylation reaction was very clean without formation of too many byproducts. 2) The reaction is carried out at room temperature under mild conditions. 3) NaNO₃ is a neutral reagent. The reaction does not require strongly basic reaction conditions as the most commonly used reagents for this reaction. 4) It is especially useful for the deprotection of N-tosyl pyrroloiminoquinones containing base-sensitive groups.

**Biological evaluation of makaluvamine analogs**

A preliminary *in vitro* biological evaluation of a number of these compounds was published previously from our group which involved cytotoxic evaluation against a number of cancer cell lines such as HCT-116 (Human Colon Tumor), MCF-7 (ER + breast cancer cell line) MDA-MB435 (ER- breast cancer cell line) and Topoisomerase II inhibition activity.³¹,³² However, later studies have shown that topoisomerase II inhibition is only one of the several possible mechanisms by which these compounds exhibit their cytotoxic activity. The following description comprises of *in vitro* and *in vivo* evaluation of some of the compounds synthesized in this series of makaluvamine analogs. *In vitro* results summarize the important observations regarding IC₅₀ values against different types of cancer cell lines, % cell survival, mechanism of action involving impact of compounds on cell cycle arrest or apoptosis and expression of proteins such as E2F1, Bcl-2, cyclin D1, cdk2, cdk4, cdk6, Bax and p53. Cyclins and cyclin dependent kinases (cdks) are important proteins that regulate the different stages of cell cycle and a decrease in the expression of these is an indicator of non-functional cells
or apoptosis. Bcl-2 is the cellular protein responsible in preventing apoptosis and hence decreased expression of Bcl-2 is a biomarker for increased apoptosis. Bax protein is also a promoter of cell death. In vivo evaluations summarize important results of experiments conducted using mouse xenograft models of breast cancer, ovarian cancer and mechanistic insight obtained by examining oncoprotein expression levels.

*In vitro* activities of Makaluvamine analogs against lung cancer

The data presented in this section is based on a published journal article titled “Synthesis and *In vitro* Anti-Lung Cancer Activity of 1,3,4,8-Tetrahydropyrrolo [4,3,2-de]quinolin-8(1H)-one Alkaloid Analogs” by Nadkarni, D.H.; Wang, F.; Wang, W.; Rayburn, E.R.; Ezell, S.J.; Murugesan, S.; Velu, S.E. and Zhang, R, 2009, *Medicinal Chemistry*, 5, 227-236. Seventeen analogs shown in Figure 3 were tested against lung cancer cell lines A549, H838 and H1299. The results are presented in Table 2. A549 cell lines express the p53 gene and H1299 are genetically modified and are known to be p53 null. The cells were exposed to various concentrations of the compounds for 72 hours followed by MTT assay. All assays were performed in triplicate. The values are expressed as IC$_{50}$ determined with triplicate mean values. Compound 3 was used as a control for the core structure of these compounds and did not show any major activity and the IC$_{50}$ values for all the tested cell lines were more than 10µM. Thus this result reinforced that amino N-atom at the 7-position of the pyrroloiminoquinone ring enhances the activity of the makaluvamine analogs. The most active analog identified from these studies was the N-tosyl analog 1b.
Table 2: *In vitro* activity (IC$_{50}$, µM) of makaluvamine analogs against lung cancer cell lines

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<td>3.01</td>
<td>1.80</td>
<td>1.22</td>
</tr>
<tr>
<td>1e</td>
<td>3.10</td>
<td>2.93</td>
<td>2.81</td>
<td>2i</td>
<td>0.56</td>
<td>0.59</td>
<td>0.42</td>
</tr>
<tr>
<td>1f</td>
<td>3.01</td>
<td>2.61</td>
<td>1.69</td>
<td>2j</td>
<td>8.82</td>
<td>9.71</td>
<td>&gt;10</td>
</tr>
<tr>
<td>2b</td>
<td>2.10</td>
<td>0.93</td>
<td>0.91</td>
<td>2k</td>
<td>2.21</td>
<td>1.05</td>
<td>0.67</td>
</tr>
<tr>
<td>2c</td>
<td>1.95</td>
<td>1.51</td>
<td>0.81</td>
<td>2l</td>
<td>3.33</td>
<td>1.29</td>
<td>1.84</td>
</tr>
</tbody>
</table>


Compound 1b exhibited an IC$_{50}$ value of 0.39 µM, 1.41 µM and 0.58 µM against A549, H838 and H1299 cell lines, respectively. In most cases, N-tosyl substitution did not significantly alter compound activity. But, in this case, the N-tosyl derivative 1b was found to be more active than the N-H derivatives. Analogs with -OH substituent on the side chain phenyl ring i.e. 2g and 2j were the least active against lung cancer. Compound 2g showed an IC$_{50}$ value of >10 µM, 4 µM and > 10 µM while 2j showed IC$_{50}$ value of 8.82 µM, 9.71 µM and >10 µM against A549, H838 and H1299 cell lines, respectively.

Examination of cell cycle effects were conducted using the compound 1b against A 549, H1299 and normal fibroblasts like IMR-90 and normal lung epithelial cells like BEAS-2B. It was observed that compound 1b caused major arrest in S-phase of the A549 cells but did not show considerable cell cycle impact in H1299 cells. Also A549 and BEAS-2B showed slight cell cycle arrest in S-phase and G2/M phase respectively. It should be noted that except H1299, which is p53 null, all other cell lines express p53. Thus, compound 1b could possibly be exhibiting the cell cycle arrest by interaction with p53.
Even though, compound 1b does not cause significant cell cycle arrest in H1299 cells it still exhibited substantial activity. This might be due to the increase in apoptotic index caused by the compound in both p53 wt and p53 null cells. The protein expression test revealed increase in cleaved PARP, caspase 8 and caspase 9 and decrease in MDM2—all strong indicators of apoptosis. Thus anti-cancer activity appears to occur via both p53-dependent and independent pathways as both A549 (p53 wt) and H1299 (p53 null) cells were affected by the compounds. Thus it is hypothesized that these compounds have the potential to be effective anti-lung cancer agents subject to further pharmacological and toxicological evaluations.

**Anti-breast cancer activity of compound 2a**

Compound 2a was selected as one of the lead compounds based on our initial screenings. So, extensive biological evaluations were carried out on this compound. The data presented in this section on the *in vitro* and *in vivo* activity of compound 2a is based on the published journal article titled “A novel synthetic iminoquinone, BA-TPQ, as an anti-breast cancer agent: *in vitro* and *in vivo* activity and mechanisms of action” by Wang, W; Rayburn, E. R; Velu, S.E; Chen, D; Nadkarni, D. H; Murugesan, S; Chen, D and Ruwen Zhang, 2010, *Breast Cancer Res.Treat.*123, 321-331. Compound 2a was tested on three human breast cancer cell lines (MCF-7/p53 wt; MCF-7/p53 KD and MDA-MB-468/p53 wt) and non-malignant breast epithelial cell line (MCF-10A) at concentrations 0 to 1.0 µM for 72 hours. The use of MCF-7 and MDA-MB-468 cell lines was justified by the non-similar expression of proteins and receptors in both these cell lines observed in advanced breast cancers and hence helped evaluate the activity of the
compound against different breast cancers. The inhibitory effects of compound 2a on these cell lines are summarized in the table 3.

**Table 3: In vitro anti-breast cancer activity of 2a**

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>Observed least concentration for growth inhibition (µM)</th>
<th>% inhibition at 1µM</th>
<th>Increase in apoptotic index at 0.75µM in comparison to control (vehicle treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>0.01</td>
<td>94.3% (P &lt; 0.01)</td>
<td>16 fold (P &lt; 0.01)</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>0.1</td>
<td>81.8 % (P &lt; 0.01)</td>
<td>4.5 fold (P &lt; 0.01)</td>
</tr>
<tr>
<td>MCF-7-p53KD</td>
<td>0.1</td>
<td>99.6 % (P &lt; 0.01)</td>
<td>6 fold (P &lt; 0.01)</td>
</tr>
</tbody>
</table>

At a concentration of 1 µM, compound 2a induced 94.3 %, 81.8 % and 99.6 % of cell growth inhibition in the MCF-7, MDA-MB-468 and MCF-7-p53KD cell lines. In MCF-10A which are considered to be “normal” breast cells, no significant cell growth inhibition was observed thus indicating a good therapeutic index. Increase in apoptotic index is also indicative of the inhibitory effects of the compound. In the p53 wt MCF-7 and p53 KD MCF-7 cells, a 0.75 µM concentration of compound 2a increased the apoptotic index 16-fold and 6-fold higher as compared to control (vehicle-treated) cells, respectively. In the MDA-MB-468 cells, compound 2a resulted in a 4.5 fold increase in apoptotic index. Effects of compound 2a on different phases of the cell growth were investigated. It was observed that at 0.75 µM, in MCF-7 wt (p53 positive) cells, compound 2a induced cell growth inhibition in the G1 phase which may be attributed to tumor suppressor gene p53 activation. In cell lines devoid of p53 i.e. MCF-7-p53KD and MDA-MB-468, the cell growth was arrested in the S-phase due to inhibition of other cell cycle regulators like cyclins. Decrease in expression levels of proteins E2F1, Bcl-2, cyclin-D1, cdk2, cdk4, and cdk6 and increased expression of Bax and p53 (in the MCF-7 cells) was observed thus justifying the increase in apoptosis observed in the experiments.
This was further supported by the increase in levels of cleaved PARP1 and cleaved caspases 3, 8 and 9 which are markers for apoptosis.\textsuperscript{39}

Compound \textit{2a} was evaluated against both MCF-7 and MDA-MB-468 mouse xenograft model of breast cancer.\textsuperscript{39} Body weight loss of the animal was used as a surrogate marker for toxicity. MCF-7 xenografts were administered doses of 5 and 10 mg/kg/day, 3 days/week for 3 days by i.p injection. Decrease in tumor growth was observed starting on the 6\textsuperscript{th} day after administration of the highest dose and about 60 \% reduction in tumor mass was observed by the 18\textsuperscript{th} day. In the MDA-MB-468 model, compound \textit{2a} was administered by i.p. injection at doses of 1 mg/kg/day and 10 mg/kg/day, 5 days/week for 6 weeks. 46 \% reduction in tumor growth was observed at the end of 6 weeks, however host toxicity was observed at the 10 mg/kg dose in both the models.\textsuperscript{39}

\textit{In vitro} evaluation of makaluvamine analogs \textit{2n}, \textit{2r}, \textit{2s}, \textit{2f} against several cancer cell lines and \textit{in vivo} investigations of \textit{2n}

Selected makaluvamine analogs, \textit{2n}, \textit{2r}, \textit{2s} and \textit{2f} were evaluated against breast, pancreatic, prostate, lung cancer and glioma cell lines. The data presented in this section is the results of \textit{in vitro} studies conducted on breast, pancreatic, prostate, lung cancer and glioma cell lines on compounds \textit{2n}, \textit{2r}, \textit{2s} and \textit{2f} published in the article titled “\textit{In vitro} and \textit{in vivo} anticancer activity of novel synthetic makaluvamine analogs” by Wang, W.; Rayburn, E.R.; Velu, S.E.; Nadkarni, D.H.; Murugesan, S.; and Zhang, R, 2009, Clin. Cancer. Res.15, 3511-3518. The four compounds were first evaluated for their \textit{in vitro}
cytotoxicity using MTT assay. The activities against various cell lines are reported as IC<sub>50</sub> values. All experiments were performed in triplicate.\textsuperscript{40}

**Table 4: In vitro activity of analogs 2n, 2r, 2s and 2f against various cell lines**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell line</th>
<th>(2n)</th>
<th>(2r)</th>
<th>(2s)</th>
<th>(2f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>MCF-7</td>
<td>0.097</td>
<td>0.435</td>
<td>0.709</td>
<td>1.220</td>
</tr>
<tr>
<td></td>
<td>MDA-MB-468</td>
<td>0.125</td>
<td>0.101</td>
<td>0.428</td>
<td>0.277</td>
</tr>
<tr>
<td>Prostate</td>
<td>LNCaP</td>
<td>1.290</td>
<td>1.742</td>
<td>3.959</td>
<td>24.451</td>
</tr>
<tr>
<td></td>
<td>PC3</td>
<td>0.978</td>
<td>2.037</td>
<td>2.236</td>
<td>2.619</td>
</tr>
<tr>
<td>Lung</td>
<td>A549</td>
<td>0.569</td>
<td>1.226</td>
<td>1.247</td>
<td>1.736</td>
</tr>
<tr>
<td></td>
<td>H358</td>
<td>0.170</td>
<td>0.398</td>
<td>1.204</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>H838</td>
<td>0.110</td>
<td>0.260</td>
<td>0.665</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>H1299</td>
<td>0.968</td>
<td>1.708</td>
<td>2.478</td>
<td>2.524</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>HPAC</td>
<td>0.535</td>
<td>1.131</td>
<td>2.920</td>
<td>2.852</td>
</tr>
<tr>
<td></td>
<td>Panc-I</td>
<td>0.104</td>
<td>0.258</td>
<td>0.587</td>
<td>0.720</td>
</tr>
<tr>
<td></td>
<td>MIA-PaCa-2</td>
<td>0.315</td>
<td>0.982</td>
<td>2.740</td>
<td>1.320</td>
</tr>
<tr>
<td>Glioma</td>
<td>U87MG</td>
<td>1.420</td>
<td>1.978</td>
<td>2.993</td>
<td>1.675</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>IMR90</td>
<td>1.488</td>
<td>3.774</td>
<td>5.698</td>
<td>2.880</td>
</tr>
</tbody>
</table>


The cytotoxic activities of these compounds showed a noticeable structure-activity relationship for the synthetic makaluvamine analogs. In general, for most cell lines, the order of activity of compounds was 2n > 2r > 2s > 2f. The compound 2n consistently appeared to show the best activity.\textsuperscript{40} This proves that a benzyl analog is favorable for the activities. Investigations on apoptotic index revealed that compounds 2n, 2r and 2f induced apoptosis in MDA-MB-68 cells in proportion to the concentrations exposed. However, compound 2s did not lead to significant apoptosis at 1 µM. Also the MCF-7 cells were found to be less sensitive to the apoptotic affect of these compounds.\textsuperscript{40} Cell cycle studies seemed to resonate with the observations related to apoptosis. Again
the effect of cell cycle arrest was prominently seen in MDA-MB-468 cells. Compounds 2n and 2r caused cell cycle arrest in the S-phase of these cells, with 2n showing a larger accumulation of cells at lower concentration of 0.1 µM. Compounds 2s and 2f showed the cell cycle arrest in S-phase at 1 µM. With MCF-7 cells, compound 2n showed considerable arrest in the S-phase, compound 2r in the G2/M phase and 2s in G1 phase at 1 µM concentration. Therefore, the effect of these compounds on MCF-7 and MDA-MB-468 cells based on presence or absence of p53 tumor suppressor gene respectively is clearly observed in the different impacts on various stages of cell cycle.

Further mechanistic studies were conducted in vitro. Compound 2n was active against both MCF-7 (p-53wt) and MDA-MB-468 (p-53 mutant) cell lines. In the MCF-7 cells, compound 2n activated p53; increased the cleavage of PARP, caspase-3, caspase-8, and caspase-9; activated ATM/p-ATM and ATR (at the lower concentrations); and led to increased phosphorylation of Chk1, Chk2, p53, and H2AX. The compound downregulated MDM2, E2F1, Cdk2, Cdk4, Cdk6, Cyclin D1, Cdc2, Cdc25c and Bcl-2 proteins. In the MCF-7, p53-/− cells and MDA-MB-468 (p53 mutant) cells, similar effects were observed for almost all of the proteins, indicating that compound 2n can exert its effects via the DNA damage response, inhibition of cell cycle progression or increase in apoptosis which is not dependent on p53 tumor suppressor gene.

Compound 2n was evaluated in a mouse MCF-7 xenograft model of breast cancer. Three doses were administered at 5 mg/kg/day, 3 days/week for 3 weeks; 10mg/kg/day, 3 days/week for 2 weeks; or 20 mg/kg/day, 3 days/week for 1 week. The lowest dosage of 5mg/kg indicated the beginning of tumor growth inhibition with minimal toxicity in terms of reduction in body weight. Best in vivo result in the form of
72% decrease in tumor size was obtained on day 18 with the highest dose. However, there was considerable loss in body weight indicating the compound was toxic when administered at doses higher than 5 mg/kg.  

Additional in vitro studies of 2n on prostate cancer cell lines

The in vitro effects of compound 2n on human (LNCaP and PC3) and murine (TRAMP C1) prostate cancer cells were described by us earlier in an article titled “FBA-TPQ, a novel marine derived compound as experimental therapy for prostate cancer”, Wang, F; Ezell, S. J; Zhang, Y; Wang, W; Rayburn, E. R; Nadkarni, D. H; Murugesan, S; Velu, S. E and Ruiwen Zhang, 2010, Invest. New Drugs, 28, 234-241. LNCaP and PC3 belong to different class of prostate cancer cell lines representing the early and late stage of cancer. LNCaP are known to retain their prostate specific properties like androgen-dependent cell growth. PC3 is not only insensitive to androgen but also does not respond to common growth factors like EGF, FGF thus proving to be the more aggressive counterpart. TRAMP C1 is hormone independent prostate cancer cell line. At a concentration of 1 μM, compound 2n greatly reduced the viability of LNCaP cells, PC3 cells, and TRAMP C1 cells (IC50 0.26 μM) thus proving its effect on different phenotypes of the same cancer cell line. Normal fibroblasts were less sensitive (IC50 5.82 μM) to the inhibitory effects of compound 2n compared to prostate cancer cells indicating a good therapeutic index. Experiments conducted to determine the mechanism of action proved that compound 2n inhibits prostate cancer cell proliferation by inducing cell cycle arrest resulting into cell death. Overall, compound 2n exhibited greater inhibitory effect on PC3 cells as compared to LNCaP cells. PC3 cell cycle growth
is inhibited in G2/M phase while LNCaP in S-phase. Mechanism of action in both cell lines was through induction of apoptosis as indicated by considerable increase in apoptosis at 1 μM concentration. TRAMP C1 cells were found to be most sensitive to the effect of compound 2n showing almost complete inhibition of cell proliferation at 1 μM concentration, arresting the cell cycle in both S-phase and G2/M phase and also depicting 5 fold increase in apoptotic index. The cell cycle is regulated by numerous proteins, including p53, MDM2, E2F1 and p21 interacting in specific programmed cascades. Observed cell cycle arrest may be attributed to decreased MDM2 expression leading to apoptosis. It was also observed that compound 2n inhibited the expression of the AR, PSA and PSA promoter activity which in turn justifies the mechanism of MDM2 inhibition due to possible interaction between PSA promoter and MDM2.

**In vitro and in vivo studies using compound 2n on ovarian cancer cell lines**

The description of *in vitro* and *in vivo* activity of compound 2n is based on the journal article published in collaboration with our research laboratory as “Experimental Therapy of Ovarian Cancer with Synthetic Makaluvamine Analog: *In Vitro* and *In Vivo* Anticancer Activity and Molecular Mechanisms of Action.” by Chen, T; Xu, Y; Guo, H; Liu, Y; Hu, P; Yang, X; Li, X; Ge, S; Velu, S. E; Nadkarni, D. H; Wang, W; Zhang, R; and Hui Wang , 2011, *PLoS ONE*, 6,e20729. Compound 2n was evaluated on two ovarian cancer cell lines (A2780 and OVCAR-3) and human ovarian IOSE 144 (Immortalized non-tumorigenic Ovarian Surface Epithelial cells). The IC₅₀ values and cell viability percentages are summarized in Table 5.
Table 5: Results of the cell viability assay for determination of *in vitro* activity of 2n against ovarian cancer cell lines for 48 hours using an MTS assay (Promega).

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>IC₅₀ (µM)</th>
<th>% inhibition at 0.5 µM (P &lt; 0.05)</th>
<th>% inhibition at 1 µM (P &lt; 0.05)</th>
<th>% inhibition at 2.5 µM (P &lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2780</td>
<td>1.78</td>
<td>25.2 %</td>
<td>45.7 %</td>
<td>65.8 %</td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>0.98</td>
<td>33.9 %</td>
<td>56.7 %</td>
<td>73.7 %</td>
</tr>
<tr>
<td>IOSE144</td>
<td>&gt;10</td>
<td>2.7 %</td>
<td>7.7 %</td>
<td>25.6 %</td>
</tr>
</tbody>
</table>

OVCAR-3 cell lines were more sensitive to compound 2n with an IC₅₀ value of 0.98 µM closely followed by A2780 with 1.78 µM. IOSE144, which are non-tumorigenic, exhibited an IC₅₀ of more than 10 µM thus clearly establishing the selectivity of compound 2n for the cancer cells with a good therapeutic index value. The cell survival assays reiterated the observations, with % cell growth inhibition increasing from 25.2 % to 45.7 % to 65.8 % as concentration increased from 0.5 µM to 1µM to 2.5 µM in case of A2780 cells. Compound 2n was further investigated for its apoptotic effect on OVCAR-3 cell lines and was found to induce a fourfold increase in the apoptotic index at 1.5 µM. The experiments conducted to study the impact of this compound on cell cycle arrest showed the increase in number of cells in the G2/M phase at 1.5 µM concentration and an S-phase arrest at 2.5 µM concentration. An additional interesting study on the cellular ROS (Reactive Oxygen species) stress induction by compound 2n was also performed by monitoring production of ROS over several concentrations up to 3µM. This test showed increase in ROS levels with increasing concentrations thus leading to the conclusion that ROS-induced stress may be causing DNA damage ultimately leading to cell death. Another observation in support of apoptosis as the mechanism of action is the change in the potential caused by the disturbances to the mitochondrial membrane. In this case a decrease in the membrane
potential was observed which was measured by the changes in intensity caused by the “JC-1” dye. Protein expression experiments showed increase in amount of PARP, caspase-3 and Bax and decrease in Bcl-2 all associated with cell death. As seen in similar studies conducted against other cancer cell lines described earlier a down-regulation of key proteins like Cdc25, CyclinB1, CDK1, E2F1 and MDM2 and up-regulation of p53, p21 and p27 associated with tumor suppression further reinforce the anti-proliferative action of compound 2n by apoptosis, cell cycle inhibitory effects.

In vivo evaluations were conducted on an OVCAR-3 mouse xenograft model. Mice were separated into three groups, one receiving vehicle treatment (control group), other group a dose of 1mg/kg a day for 5 days and still another group receiving 10 mg/kg a day for 5 days by i.p. Weight loss was the surrogate marker for toxicity. The reduction in tumor growth was evident from the ninth day. In comparison to the control group a 20.5 % reduction in tumor growth was observed in the group that was administered the lower dose and 69.4 % tumor growth inhibition in the higher dose group. No considerable body weight loss was observed pointing to a satisfactory safety profile.

Conclusions

Several analogs of makaluvamine natural alkaloids were synthesized and characterized. A novel method of detosylation was developed to improve the yield of the final steps in the synthesis. In vitro evaluation of several of these analogs was performed on a number of varied cancer cell lines like breast cancer, lung cancer, prostate cancer, pancreatic cancer and gliomas. Most of these compounds exert their effects primarily by
inhibiting cell cycle progression and inducing apoptosis, making use of both p53-dependent and p53-independent pathways. It is also possible that these compounds activate the DNA damage response and inhibit topoisomerase II, although the effects on apoptosis and the cell cycle appear to be the most important mechanisms of action for the compound. **2a** and **2n** were subjected to *in vivo* studies in mouse xenograft models of MCF-7 and MDA-MB-468 breast cancer cell lines. This multi-faceted activity may prove to be a major benefit while using these analogs in clinic. Further studies of these analogs include synthesis of analogs with improved drug delivery capacity so as to further reduce the minimal concentration of activity hence reducing toxicity. Also studies of their activity against drug-resistant tumors will further shed light on their mechanism of action and contribute to better design and development of these molecules into better leads or drugs.

**Experimental**

**General Considerations:** Melting points were determined using an Electrothermal MEL-TEMP apparatus and are uncorrected. All $^1$H and $^{13}$C NMR spectra were recorded on a Bruker ARX 400 or DPX 300 spectrometer using TMS as internal standard. The value of chemical shifts (δ) are given in parts per million and coupling constants ($J$) in Hz. Mass spectra were recorded on Micromass Platform LCC instrument. Reactions were monitored by TLC (Whatmann silica gel, UV 254, 25 μM plates), and flash column chromatography was performed using Baker silica gel (40 μM) in the solvent systems indicated. Anhydrous solvents used for reactions were purchased in Sure-Seal bottles from Aldrich Chemical Co. Other reagents were purchased from Aldrich, Fisher
Scientific, or Acros Chemical Companies and used as received. Compounds 3 and 5-14 were reported previously and the $^1$H-NMR matched well with the reported data.$^{13}$ All pyrroloiminoquinone compounds 3, 1a-f, 2a-v were isolated as TFA salts.

**Methyl 2,4,5-trimethoxy-$\alpha$-azidocinnamate (5)**

A solution of NaOMe (25.0 g, 463 mmol) in anhydrous MeOH (120 mL) was cooled to -18 °C using freezing mixture under N$_2$. A solution of 2,4,5-trimethoxybenzaldehyde (18 g, 92 mmol) and methyl azidoacetate (46.0 g, 513 mmol) in a mixture of MeOH (110 mL) and anhydrous THF (90 mL) was added drop wise with stirring to the methoxide solution, maintaining the temperature at -10 to -15°C for 1 hour. The mixture was stirred for an additional 3.5 hours while the temperature was maintained below 0°C. The resulting heterogeneous mixture was poured over ice (1 kg) and stirred. The precipitate formed was filtered, washed with water, and dried over CaCl$_2$ in vacuum desiccators to obtain compound 5 (19.0 g, 70%); $^1$H-NMR (CDCl$_3$) δ 3.86 (s, 3H), 3.90 (s, 6H), 3.93 (s, 3H), 6.48 (s, 1H), 7.38 (s, 1H), 7.92 (s, 1H).

**Methyl 4,6,7-trimethoxyindole-2-carboxylate(6)**

Methyl 2,4,5-trimethoxy-$\alpha$-azidocinnamate 5 (18 g, 61 mmol) was added slowly to refluxing xylenes (500 mL) with stirring over a period of 1 hour using a solid addition funnel. (Adequate shield protection was used). The addition was carried out in small portions ensuring no excess evolution of nitrogen occurred. The reaction was refluxed for 6 hours and then allowed to attain room temperature. Xylenes were evaporated off from
the reaction mixture to obtain the pure compound 6 (16 g, 98%); \(^1\)H-NMR (CDCl\(_3\)): \(\delta\) 3.92 (s, 9H), 3.95 (s, 3H), 6.27 (s, 1H), 7.26 (d, 1H, \(J = 2.3\) Hz), 8.86 (bs, 1H).

\textbf{4,6,7-Trimethoxyindole-2-carboxylic acid (7)}

To a solution of methyl 4,6,7-Trimethoxyindole-2-carboxylate 6 (18 g, 61 mmol) in THF (250 mL) and MeOH (50 mL), NaOH (2N, 150 mL) was added and the suspension was stirred until it became a clear solution and the reaction mixture was continued to stir at room temperature for 24 hours. TLC examination using EtOAc/hexane (1:1) indicated the completion of reaction. The solvents were completely removed from the reaction mixture and the residue was diluted with water (300 mL). The aqueous solution was washed with CH\(_2\)Cl\(_2\) (2 x 150 mL) and then acidified with 1 N HCl. The precipitate formed was filtered, washed with water (500 mL), and dried over CaCl\(_2\) in vacuum desicator to obtain the acid 7 (14 g, 82%); \(^1\)H-NMR (CDCl\(_3\)): \(\delta\) 3.93 (s, 3H), 3.94 (s, 3H), 3.96 (s, 3H), 6.28 (s, 1H), 7.39 (d, 1H, \(J = 2.2\) Hz), 9.02 (s, 1H) and 12.60 (bs, 1H).

\textbf{4, 6,7-Trimethoxyindole (8)}

4,6,7-Trimethoxyindole-2-carboxylic acid 7 (15 g, 60 mmol) was intimately mixed with BaO (2 g, 13 mmol), and the reaction mixture was heated in a Kugelrohr at 240°C for 1 hour. The reaction mixture was cooled down and the residue was purified on a column of Si gel using EtOAc/hexanes (1:3) to afford white crystals of 4,6,7-trimethoxyindole 8 (8.6 g, 70%); \(^1\)H-NMR (CDCl\(_3\)): \(\delta\) 3.93 (s, 9H), 6.29 (s, 1H), 6.56 (t, 1H, \(J = 2.4\) Hz), 7.02 (t, 1H, \(J = 2.4\) Hz), 8.21 (bs, 1H).
N,N-Dibenzyl-2-(4,6,7-trimethoxy-1H-indol-3-yl)-2-oxoacetamide (9)

A solution of 4,6,7-trimethoxyindole 8 (8.00 g, 38.6 mmol) in anhydrous ether (100 mL) was cooled to 0 °C under N₂ atmosphere. A solution of oxalyl chloride (6.30 g, 50.2 mmol) in the same solvent (50 mL) was added to this over a period of 10 minutes. After being stirred for 3 hours at room temperature, dibenzylamine (26.7 g, 135.2 mmol) was added to this over a period of 15 minutes, and the resulting mixture was stirred for an additional 20 hours at room temperature. The solid obtained in the reaction mass was filtered off, washed with ether (100 mL). The solid obtained was dissolved in CHCl₃ (800 mL) washed with 3N HCl (3 × 250 mL), water (1 × 250 mL) and brine (1 × 250 mL). The organic extract was dried over anhydrous Na₂SO₄. The drying agent was filtered off and the solvent was evaporated off to obtain the crude product, which was purified by column chromatography over Si gel using EtOAc / CHCl₃ (1:5) to obtain pure compound 9 (11.0 g, 69%); ¹H-NMR (CDCl₃) δ 3.74 (s, 3H), 3.94 (s, 3H), 3.95 (s, 3H), 4.47 (s, 2H), 4.59 (s, 2H), 6.41 (s, 1H), 7.20-7.40 (m, 10H), 7.89 (d, 1H, J = 2.3Hz), 8.97 (bs, 1H).

N,N-Dibenzyl-2-(4,6,7-trimethoxy-1H-indol-3-yl)ethanamine (10)

A solution of oxoacetamide 9 (8.00 g, 17.5 mmol) in anhydrous THF (100 mL), a suspension of LiAlH₄ (5.30 g, 140 mmol) in anhydrous ether (200 mL) was added and the reaction mixture was refluxed for 18 hours under N₂ atmosphere. TLC examination using EtOAc / hexanes (1:1) revealed that the reaction was complete. It was cooled to 0°C and a saturated Na₂SO₄ solution was slowly added to destroy excess LiAlH₄. Inorganic salts were allowed to settle down and removed by filtration through celite 545. The filtrate was concentrated under reduced pressure, and the residue obtained was
dissolved in CH$_2$Cl$_2$ (200 mL), washed with water (3 × 75 mL), brine (1 × 75 mL) and dried over anhydrous Na$_2$SO$_4$. Removal of solvent from the dried extract furnished a greenish thick oil which was purified by passing through a pad of silica gel to obtain pure tryptamine derivative 10 (7.40 g, 99%); $^1$H-NMR (CDCl$_3$) δ 2.77 (t, 2H, $J$ = 8 Hz), 3.03 (t, 2H, $J$ = 8 Hz), 3.66 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 6.14 (s, 1H), 6.68 (d, 1H, $J$= 2 Hz), 7.18-7.36 (m, 10H), 8.00 (bs, 1H).

**N,N-Dibenzyl 2-[4,6,7-trimethoxy-1-tosyl-1H-indol-3-yl]ethanamine (11)**

A solution of compound 10 (7.40 g, 17.2 mmol) in anhydrous THF (120 mL) was added to a suspension of KH (9.20 g, 68.8 mmol) in the same solvent (50 mL) maintained under N$_2$ atmosphere slowly over a period of 30 minutes. The reaction mixture was then stirred at room temperature for 1 hour and then cooled to 0 °C. A solution of p-toluenesulfonic anhydride (8.41 g, 25.8 mmol) in the same solvent (100 mL) was slowly added to this, and the reaction mixture was further stirred for 30 minutes at 0 °C and an additional 20 hours at room temperature. TLC examination EtOAc/hexanes (1:1) revealed the completion of the reaction. The reaction mixture was cooled to 0 °C and the excess KH was destroyed by very slow addition of distilled water. The solvent was then completely removed and water (200 mL) was added to the residue. It was extracted with CH$_2$Cl$_2$ (3 × 200 mL) and the extract was washed with water (1 × 200 mL) and dried over anhydrous Na$_2$SO$_4$. Drying agent was removed and the crude product obtained was purified by chromatography over Si gel using EtOAc / CHCl$_3$ (1:10) to obtain compound 11 (7.23 g, 74%); $^1$H-NMR(CDCl$_3$): δ 2.33 (s, 3H), 2.77 (t, 2H, $J$= 7.7 Hz),

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2.97(t, 2H, J = 7.7 Hz), 3.59 (s, 3H), 3.64 (s, 4H), 3.74 (s, 3H), 3.83(s, 3H), 6.22 (s, 1H), 7.19 (d, 2H, J = 8.3 Hz), 7.23-7.37 (m, 11H), 7.73(d, 2H, J = 8.3 Hz).

2-[4,6,7-Trimethoxy-1-tosyl-1H-indol-3-yl]ethanamine (12)

To a solution of compound 11 (7.23 g, 12.4 mmol) in anhydrous EtOH (300 mL) HCOONH₄ (11.0 g, 174 mmol) and Pd black (2.0 g, 19 mmol) were added and the reaction mixture was refluxed under N₂ for 15 hours. The reaction mixture was then allowed to attain room temperature and filtered through Celite 545 to remove the catalyst and insoluble salts. The filtrate was concentrated and dissolved in CHCl₃ (300 mL). The CHCl₃ extract was washed with water (3 × 100 mL) and of NaHCO₃ (1 × 50 mL) and brine (1 × 50 mL). The extract was dried over anhydrous Na₂SO₄. Removal of solvent from the dried (Na₂SO₄) extract gave debenzylated product 12 as a gum which was used as such for the next reaction. (4.60 g, 94% ); ¹H-NMR (CDCl₃): δ 2.35 (s, 3H), 2.90-2.93 (m, 2H), 2.98-3.10 (m, 2H), 3.76 (s, 3H),3.85 (s, 3H), 3.86 (s, 3H), 6.34 (s, 1H), 7.22 (d, 2H, J = 8.4 Hz), 7.40 (s, 1H), 7.74 (d, 2H, J = 8.4 Hz).

tert-Butyl-2-[4,6,7-trimethoxy-1-tosyl-1H-indol-3-yl]ethyl carbamate (13)

To a solution of the crude compound 12 (4.80 g, 11.9 mmol) in CH₂Cl₂ (100 mL) 4-(N,N-dimethylamino) pyridine (0.140 g, 1.18 mmol) and Et₃N (3.0 g, 30 mmol) were added and cooled to 0 °C. A solution of (BOC)₂O (6.8 g, 30 mmol) in CH₂Cl₂ (50 mL) was then added drop wise. The reaction mixture was stirred at 0 °C for 1 hour and then allowed to attain room temperature and stirring continued for another 24 hours. TLC examination EtOAc / hexanes (1:1) revealed the completion of the reaction. The reaction
mixture was diluted with CH₂Cl₂ (150 mL) and washed with water (2 × 150 mL) and brine (1 × 100 mL) and dried over anhydrous Na₂SO₄. After the removal of drying agent, the solution was concentrated under reduced pressure to afford the crude product which was purified by column chromatography over Si gel using EtOAc / CHCl₃ (1:15) to furnish pure compound 13 (4.60 g, 78%); ¹H-NMR (CDCl₃): δ 1.44 (s, 9H), 2.35 (s, 3H), 2.94 (t, 2H, J = 6.5 Hz), 3.42 (q, 2H, J = 6.3 Hz), 3.75 (s, 3H), 3.85 (s, 6H), 4.68 (bs, 1H), 6.34 (s, 1H), 7.21 (d, 2H, J = 8.4 Hz), 7.40 (s, 1H), 7.74 (d, 2H, J = 8.4 Hz).

tert-Butyl 2-(4,7-dihydro-6-methoxy-4,7-dioxo-1-tosyl-1H-indol-3-yl)ethylcarbamate (14)

To a solution of carbamate 13 (1.00 g, 1.98 mmol) in CH₂Cl₂ (70 mL), Bu₄NHSO₄ (1.35 g, 3.96 mmol) was added and the resulting mixture was stirred for 5 minutes. Then solid CAN (2.18 g, 3.96 mmol) was added and the suspension was stirred for 5 minutes. One drop of water was added and the reaction mixture was stirred for 5 minutes. After every 5 minutes a drop of water was added and the progress of the reaction was monitored by TLC. It required about ten drops of water and 1 hour of stirring for the reaction to go to completion (note: controlled addition of water is crucial since excess of water can lead to side products). TLC examination EtOAc / hexanes (1:1) revealed completion of the reaction. The mixture was then diluted with water (100 mL) and extracted with CH₂Cl₂ (2 × 100 mL). The CH₂Cl₂ extract was washed with water (3 × 50 mL) brine (1 × 50 mL) and dried over anhydrous Na₂SO₄. The solvent was evaporated off and the residue obtained was purified by column chromatography over Si gel using EtOAc / hexanes (1:3) to obtain pure compound 14 (0.500 g, 54%); ¹H-NMR (CDCl₃)
δ1.41 (s, 9H), 2.42 (s, 3H), 2.96 (t, 2H, \( J = 6.8 \)), 3.39 (q, 2H, \( J = 6 \) Hz), 3.77 (s, 3H), 4.72 (bs, 1H), 5.70 (s, 1H), 7.33 (d, 2H, \( J = 8 \) Hz), 7.67 (s, 1H), 8.04 (d, 2H, \( J = 8 \) Hz).

3,4-Dihydro-7-methoxy-1-tosyl-pyrrolo[4,3,2-de]quinolin-8(1H)-one (3)

To a stirred solution of the quinone 14 (1.50 g, 3.70 mmol) in \( \text{CH}_2\text{Cl}_2 \) (20 mL), a solution of TFA and \( \text{CH}_2\text{Cl}_2 \) (1:1) (40 mL) was added drop wise, at room temperature. The reaction mixture was stirred at room temperature for 2 hours. The solvent was evaporated under reduced pressure. Traces of TFA were removed under reduced pressure by co-evaporation with \( \text{CH}_2\text{Cl}_2 \) (3 × 50 mL) to give the compound 3 as a greenish yellow trifluoroacetate salt (1.48 g, 98% yield); \(^1\text{H}-\text{NMR} (\text{CD}_3\text{COCD}_3): \delta 2.43 (s, 3H), 3.27 (t, 2H, \( J = 7.3 \) Hz), 3.81 (s, 3H), 4.10 (t, 2H, \( J = 7.3 \) Hz), 5.8 (s, 1H), 7.47 (d, 2H, \( J = 8 \) Hz), 7.96 (s, 1H), 8.06 (d, 2H, \( J = 8 \) Hz).

General procedure for amination

To a solution of compound 3 (1 equiv.) in anhydrous \( \text{MeOH} \) (25 mL), a solution of amine (1.2 equiv.) in anhydrous \( \text{MeOH} \) (5 mL) was added drop-wise over a period of 10-15 minutes. The resulting solution was stirred at room temperature for 20 hours. TLC analysis \( \text{MeOH} / \text{CHCl}_3 \) (1:20) revealed the completion of reaction. The reaction mixture was then cooled to 0°C and quenched by adding TFA (1.2 equiv.). It was allowed to attain room temperature and stirred for 10-15 minutes. Then the solvent was removed under reduced pressure, co-evaporated with \( \text{CHCl}_3 \) to remove excess TFA and the residue was purified by flash column chromatography on silica gel with \( \text{MeOH} / \text{CHCl}_3 \) (1:40) as eluent to furnish the tosylated makaluvamines 1a-f.
7-(Benzylamino)-3,4-dihydro-1-tosyl-pyrrolo[4,3,2-de]quinolin-8(1H)-one (1a)

Compound 1a was prepared by following the general procedure starting from compound 3 (0.090 g, 0.19 mmol) and benzyl amine (0.026 g, 0.24 mmol). (0.035 g, 34%); \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) 2.47 (s, 3H), 3.00 (t, 2H, \(J = 7.2\) Hz), 4.01 (t, 2H, \(J = 7.2\) Hz), 4.46 (d, 2H, \(J = 5.6\) Hz), 6.33 (s, 1H), 7.00 (bs, 1H), 7.30–7.35 (m, 2H), 7.38–7.45 (m, 5H), 7.69 (s, 1H) and 8.04 (d, 2H, \(J = 8\) Hz); \(^{13}\)C-NMR (CDCl\(_3\)) \(\delta\) 18.0, 21.9, 42.5, 48.3, 87.9, 117.9, 123.2, 127.7, 128.1(2C), 128.4, 128.8, 129.0(2C), 129.3(2C), 130.2(2C), 132.9, 134.2, 147.3, 150.1, 156.9 and 166.7; MS (ES+) m/z (M+) 432.

7-(4-Chlorobenzylamino)-3,4-dihydro-1-tosyl-pyrrolo[4,3,2-de]quinolin-8(1H)-one (1b)

Compound 1b was prepared by following the general procedure starting from compound 3 (0.10 g, 0.21 mmol) and 4-chlorobenzylamine (0.036 g, 0.25 mmol). (0.040 g, 32%); \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) 2.44 (s, 3H), 2.93-3.04 (m, 2H), 3.80-4.15 (m, 2H), 4.42 (bs, 2H), 6.20 (bs, 1H), 7.02(bs, 1H), 7.20-7.27 (m, 2H), 7.35-7.40 (m, 4H), 7.66 (s, 1H), 8.01 (d, 2H, \(J = 8.1\) Hz); \(^{13}\)C-NMR (CDCl\(_3\)) \(\delta\) 17.9, 21.8, 42.7, 47.4, 88.0, 118.1, 123.1, 127.7, 128.2, 129.0(2C), 129.4(2C), 129.8(2C), 130.2(2C), 132.8, 132.9, 134.7, 147.4, 150.2, 157.0 and 166.5; MS (ES+) m/z (M+) 466.

3,4-Dihydro-7-(3,4-dimethoxybenzylamino)-1-(tosyl)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (1c)

Compound 1c was prepared by following the general procedure, starting from compound 3 (0.080 g, 0.17 mmol) in MeOH (18 mL) and 3,4-dimethoxybenzylamine
(0.034 g, 0.20 mmol). (0.036 g, 35%); $^1$H-NMR (CDCl$_3$) δ 2.45 (s, 3H), 2.97 (t, 2H, $J = 7.4$ Hz), 3.88 (s, 6H), 3.98 (t, 2H, $J = 7.4$ Hz), 4.38 (d, 2H, $J = 5.6$ Hz), 6.28(s, 1H), 6.81(s, 1H), 6.86 (s, 2H), 7.02 (bs, 1H), 7.38 (d, 2H, $J = 8.2$ Hz), 7.67 (s, 1H) and 8.02 (d, 2H, $J = 8.2$ Hz); $^{13}$C-NMR (CDCl$_3$) δ 18.0, 21.9, 42.5, 48.2, 55.9, 56.0, 87.7, 111.4, 111.5, 118.0, 121.0, 123.1, 126.5, 127.8, 128.2, 129.0(2C), 130.2(2C), 132.9, 147.3, 149.5, 149.4, 150.1, 156.9 and 166.6; MS (ES+) m/z (M+) 492.

3,4-Dihydro-7-(4-fluorophenethylamino)-1-(tosyl)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (1d)

Compound 1d was prepared by following the general procedure, starting from compound 3 (0.080 g, 0.17 mmol) in MeOH (15 mL) and 4-fluorophenethylamine (0.028 g, 0.21 mmol). (0.045 g, 46%); $^1$H-NMR (CDCl$_3$) δ 2.46 (s, 3H), 2.90-3.00 (m, 4H), 3.50-3.70 (m, 2H), 3.98 (bt, 2H, $J = 7.2$ Hz), 6.32 (s, 1H), 6.84 (bs, 1H), 7.04 (t, 2H, $J = 8.5$ Hz), 7.15-7.25 (m, 2H), 7.39 (d, 2H, $J = 8.4$ Hz), 7.63 (s, 1H) and 8.02 (d, 2H, $J = 8.4$ Hz); $^{13}$C-NMR (CDCl$_3$) δ 18.0, 21.9, 33.3, 42.5, 45.0, 87.6, 115.8(2C) and 116.0 (C-F coupling), 118.0, 123.1, 128.1, 129.0 (2C), 129.8(2C) and 130.3(C-F coupling), 130.2 (2C), 132.4, 132.5, 133.0, 147.3, 150.4, 156.8, 160.4, 166.4; MS (ES+) m/z (M+) 464.

3,4-Dihydro-7-(4-methoxyphenethylamino)-1-(tosyl)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (1e)

Compound 1e was prepared by following the general procedure, starting from compound 3 (0.084 g, 0.18 mmol) in MeOH (15 mL) and 4-methoxyphenethylamine (0.032 g, 0.22 mmol). (0.050 g, 48%); $^1$H-NMR (CDCl$_3$) δ 2.46 (s, 3H), 2.85-3.05 (m,
4H), 3.52-3.58 (m, 2H), 3.81 (s, 3H), 3.97 (bt, 2H, J = 6.9 Hz), 6.15 (bs, 1H), 6.87 (d, 2H, J = 8.7 Hz), 7.13 (d, 2H, J = 8.7 Hz), 7.38 (d, 2H, J = 8.4 Hz), 7.65 (s, 1H) and 8.02 (d, 2H, J = 8.4 Hz); \(^{13}\)C-NMR (CDCl\(_3\)) \(\delta\) 18.0, 21.9, 33.2, 42.5, 45.2, 55.3, 87.4, 114.5(2C), 118.0, 123.2, 127.9, 128.1, 128.7, 129.0(2C), 129.7(2C), 130.2(2C), 133.1, 147.3, 150.6, 156.7, 158.8 ,166.5; MS (ES+) m/z (M+) 476.

7-(2-(Benzo[d][1,3]dioxol-5-yl)ethylamino)-3,4-dihydro-1-(tosyl)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (1f)

Compound 1f was prepared by following the general procedure, starting from compound 3 (0.080 g, 0.17 mmol) in MeOH (15 mL) and 3,4 methylenedioxyphenethylamine (0.041 g, 0.20 mmol). (0.040 g, 39%); \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) 2.45 (s, 3H), 2.88 (t, 2H, J = 6.6 Hz), 2.97 (t, 2H, J = 6.6 Hz), 3.50-3.70 (m, 2H), 3.90-4.10 (m, 2H), 5.96 (s, 2H), 6.06 (bs, 1H), 6.60-6.70 (m, 2H), 6.70-6.80 (m, 2H), 7.38 (d, 2H, J = 8.2 Hz), 7.65 (s, 1H) and 8.01 (d, 2H, J = 8.2 Hz); \(^{13}\)C-NMR (CDCl\(_3\)) \(\delta\) 18.0, 21.9, 33.8, 42.6, 45.1, 87.6, 101.1, 108.7, 108.8, 118.0, 121.8, 123.2, 127.8, 128.1, 129.0(2C), 129.9, 130.2(2C), 130.4, 133.1, 146.8, 147.3, 148.3, 150.5, and 166.3; MS (ES+) m/z (M+) 490.

General procedure for detosylation using NaOMe:

To a solution of N-tosyl makaluvamines 1 (1 equiv.) in anhydrous MeOH, NaOMe (20 equiv.) was added and stirred for 45 minutes. TLC analysis MeOH/CHCl\(_3\) (1:20) revealed that the reaction was complete. The resulting solution was cooled to 0 °C and quenched at with TFA (30 equiv.) and stirred further at room temperature for 30
minutes. The solvent was evaporated off under reduced pressure and the residue was co-evaporated 3 times with CHCl₃ to remove excess TFA. The crude compound was then purified by flash column chromatography over Si gel using MeOH / CHCl₃ (1:20) as eluent to obtain the pure detosylated makaluvamines 2a-v (15-29% yields over two steps from compound 3). Compound 2m was directly obtained without isolating the intermediate N-tosyl pyrroloiminoquinone product by heating the reaction mixture at 65 °C for 10 min. For synthesis of compounds 2f, 2g, 2j, 2k and 2p where the commercially available amine precursors were hydrochloride or hydrobromide salts, Et₃N (1.2 eq) was used to release the free amine in the reaction mixture.

**General procedure for detosylation using NaN₃:**

3,4-Dihydro-7-methoxy-pyrrolo[4,3,2-de]quinolin-8(1H)-one (16)

A solution of N-tosyl pyrroloiminoquinone 3 (0.050 g, 0.10 mmol) in DMF (2 mL) was stirred with NaN₃ (0.0080 g, 0.13 mmol) at room temperature for 4 hours. TLC examination using MeOH / CHCl₃ (1:10) revealed the completion of the reaction with the formation of one major spot. Solvent was completely removed under high vacuum and the crude product purified by column chromatography over Si gel using MeOH / CHCl₃ (1 : 20) as eluent to obtain 16. (0.028 g, 83% ); ¹H NMR (CD₃OD) δ 3.05 (t, 2H, J = 6.9 Hz), 3.22 (t, 2H, J = 6.9), 3.83 (s, 3H), 5.78 (s, 1H), 7.10 (s, 1H); ¹³C NMR (CD₃OD) δ 24.9, 40.7, 57.2, 108.4, 121.4, 124.7, 127.9, 131.7, 161.5, 172.6, 186.3; MS (ES⁺) m/z 204 (M+H); HRMS (EI at 70 ev) m/z Found: 202.0741; Calcd for C₁₁H₁₀N₂O₂: 202.0742 (M⁺).
7-(Benzylamino)-3,4-dihydro-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2a)

Compound 2a was prepared by following the general procedure, starting from compound 3 (0.20 g, 0.42 mmol) in MeOH (30 mL) and benzylamine (0.054 g, 0.51 mmol) to give 0.15 g (0.27 mmol) of tosyl intermediate which was detosylated using NaOMe (0.30 g, 5.5 mmol) in MeOH (15 mL) to afford the product (0.035 g, 21%); $^1$H NMR (CD$_3$OD) $\delta$ 2.94 (t, 2H, $J$ = 7.8 Hz), 3.82 (t, 2H, $J$ = 7.8 Hz), 4.60 (s, 2H), 5.40 (s, 1H), 7.15 (s, 1H) and 7.25–7.40 (m, 5H); $^{13}$C NMR (CD$_3$OD) $\delta$ 19.5, 44.2, 48.0, 86.4, 120.2, 123.7, 125.7, 127.1, 128.3 (2C), 129.0, 130.0 (2C), 137.3, 155.2, 159.9, 168.8; MS (ES$^+$) m/z 278 (M$^+$); HRMS (EI at 70 ev) m/z Found: 277.1218; Calcd for C$_{17}$H$_{15}$N$_3$O: 277.1215 (M$^+$-H).

3,4-Dihydro-7-(3,4,5-trimethoxybenzylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2b)

Compound 2b was prepared by following the general procedure, starting from compound 3 (0.16 g, 0.34 mmol) in MeOH (25 mL) and 3,4,5-trimethoxybenzylamine (0.081 g, 0.41 mmol) to obtain 0.084 g (0.13 mmol) of tosyl intermediate which was detosylated using NaOMe (0.142 g, 2.64 mmol) in MeOH (15 mL) to afford the product (0.033 g, 20%); $^1$H NMR (CD$_3$OD) $\delta$ 2.94 (t, 2H, $J$ = 7.5 Hz), 3.73 (s, 3H), 3.76 (s, 6H), 3.86 (t, 2H, $J$ = 7.5 Hz), 4.52 (s, 2H), 5.44 (s, 1H), 6.65 (s, 2H), 7.15 (s, 1H); $^{13}$C NMR (CD$_3$OD) $\delta$ 19.4, 44.2, 48.6, 56.6 (2C), 61.0, 86.4, 105.6 (2C), 120.2, 123.7, 125.7, 127.1, 133.3, 138.6, 155.0 (2C), 155.1, 159.9 and 168.8; MS (ES$^+$) m/z 368 (M$^+$); HRMS (EI at 70 ev) m/z Found: 367.1534; Calcd for C$_{20}$H$_{21}$N$_3$O$_4$: 367.1532 (M$^+$-H).
3,4-Dihydro-7-(4-fluorophenethylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2d)

Compound 2d was prepared by following the general procedure, starting from compound 3 (0.16 g, 0.34 mmol) in MeOH (25 mL) and 4-fluorophenethylamine (0.056 g, 0.40 mmol) to obtain 0.085 g (0.15 mmol) of tosyl intermediate which was detosylated using NaOMe (0.16 g, 2.9 mmol) in MeOH (15 mL) to afford the product (0.032 g, 23%); $^1$H NMR (CD$_3$OD) δ 2.90–3.10 (m, 4H), 3.57 (t, 2H, $J = 7.2$ Hz), 3.84 (t, 2H, $J = 7.2$ Hz), 5.42 (s, 1H), 6.95–7.10 (m, 2H), 7.15 (s, 1H) and 7.20–7.35 (m, 2H); $^{13}$C NMR (CD$_3$OD) δ 19.5, 34.2, 44.2, 46.1, 85.3, 116.2 and 116.5 (C-F coupling) (2C), 120.2, 123.9, 125.5, 127.2, 131.6 and 131.7 (C-F coupling) (2C), 135.4, 155.1 and 159.8 (C-F coupling), 161.7, 164.9, 168.5; MS (ES$^+$) m/z 310 (M$^+$); HRMS (EI at 70 ev) m/z Found: 309.1270; Calcd for C$_{18}$H$_{16}$FN$_3$O: 309.1277 (M$^+$-H).

3,4-Dihydro-7-(4-methoxyphenethylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2e)

Compound 2e was prepared by following the general procedure, starting from compound 3 (0.16 g, 0.35 mmol) in MeOH (25 mL) and 4-methoxyphenethylamine (0.060 g, 0.40 mmol) to obtain 0.061 g (0.10 mmol) of tosyl intermediate which was detosylated using NaOMe (0.11 g, 2.0 mmol) in MeOH (15 mL) to afford the product (0.040 g, 26%); $^1$H NMR (CD$_3$OD) δ 2.80–3.00 (m, 4H), 3.55 (t, 2H, $J = 7.5$ Hz), 3.75 (s, 3H), 3.82 (t, 2H, $J = 7.2$ Hz), 5.39 (s, 1H), 6.85 (d, 2H, $J = 8.0$ Hz), 7.16 (s, 1H), 7.17 (d, 2H, $J = 8.0$ Hz). $^{13}$C NMR (CD$_3$OD) δ 19.5, 34.3, 44.2, 46.4, 55.7, 85.2, 115.2 (2C), 120.2, 123.9, 125.5, 127.2, 130.8 (2C), 131.2, 155.1, 159.7, 160.1 and 168.5. MS (ES$^+$) m/z 322 (M$^+$). HRMS (EI at 70 ev) m/z Found: 321.1465; Calcd for C$_{19}$H$_{19}$N$_3$O$_2$: 321.1477 (M$^+$-H).
7-(2-(Benzo[d][1,3]dioxol-5-yl)ethylamino)-3,4-dihydro-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2f)

Compound 2f was prepared by following the general procedure, starting from compound, 3 (0.20 g, 0.43 mmol) in MeOH (30 mL) and 3,4-methylenedioxyphenethylamine hydrochloride (0.10 g, 0.41 mmol), Et₃N (0.080 mL, 0.41 mmol) to obtain 0.099 g (0.16 mmol) of tosyl intermediate which was detosylated using NaOMe (0.18 g, 3.3 mmol) in MeOH (15 mL) to afford the product(0.045 g, 24%); Alternatively tosyl intermediate (0.018 g, 0.030 mmol), was detosylated using NaN₃ and DMSO following procedure used in preparation of compound 16 to obtain 2f (0.0090 g, 74%); ¹H NMR (CD₃OD) δ 2.89 (t, 2H, J = 7.5 Hz), 2.94 (t, 2H, J = 7.5 Hz), 3.55 (t, 2H, J = 7.2 Hz), 3.84 (t, 2H, J = 7.8 Hz), 5.41 (s, 1H), 5.89 (s, 2H), 6.60–6.80 (m, 3H), 7.14 (s, 1H); ¹³C NMR (CD₃OD) δ 19.5, 34.8, 44.1, 46.3, 85.3, 102.3, 109.3, 110.1, 120.2, 123.0, 123.9, 125.5, 127.2, 133.0, 147.9, 149.4, 154.9, 159.7 and 168.5; MS (ES⁺) m/z 336 (M⁺); HRMS (EI at 70 ev) m/z Found: 335.1276; Calcd for C₁₉H₁₇N₃O₃: 335.1270 (M⁺-H).

3,4-Dihydro-7-methylamino-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2m)

A solution of methylamine hydrochloride (0.036 g, 0.55 mmol) and Et₃N (0.054 g, 0.55 mmol) in MeOH (2 mL) was added to a solution of pyrroloiminoquinone (0.050 g, 0.11 mmol) in anhydrous MeOH (5 mL) at room temperature. The resulting solution was heated in an Orbital Shaker at 65 °C for 10 minutes. TLC analysis MeOH/CHCl₃ (1:20) revealed that the reaction was complete. The solution was concentrated under reduced pressure and the violet residue was purified by flash column chromatography on
silica gel with MeOH:CHCl$_3$ (1/20) as eluent to obtain 2m. (0.009 g, 27%); $^1$H NMR (CD$_3$OD) $\delta$ 2.97 (t, 2H, $J$ = 7.5 Hz), 3.02 (s, 3H), 3.86 (t, 2H, $J$ = 7.5 Hz), 5.41 (s, 1H) and 7.16 (s, 1H); $^{13}$C NMR (CD$_3$OD) $\delta$ 19.6, 30.5, 44.2, 84.8, 120.2, 124.1, 125.6, 127.1, 156.3, 159.7 and 168.6; MS (ES$^+$) m/z 202 (M$^+$); HRMS (EI at 70 ev) m/z Found: 201.0896; Calcd for C$_{11}$H$_{11}$N$_3$O: 201.0902 (M$^+$-H).

3,4-Dihydro-7-(4-fluorobenzylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2n)

Compound 2n was prepared by following the general procedure, starting from compound 3 (0.20 g, 0.43 mmol) in MeOH (25 mL) and 4-fluorobenzylamine (0.063 g, 0.51 mmol) to obtain 0.075 g (0.13 mmol) of tosyl intermediate which was detosylated using NaOMe (0.144 g, 2.66 mmol) in MeOH (15 mL) to afford the product (0.032 g, 18%); Alternatively, the detosylation was carried out using the NaN$_3$ in DMF using tosylated compound (0.025 g, 0.044 mmol) following the procedure used in the preparation of compound 16 to afford the compound 2n (0.011g, 84%). $^1$H NMR (CD$_3$OD) $\delta$ 2.94 (t, 2H, $J$ = 7.6 Hz), 3.83 (t, 2H, $J$ = 7.6 Hz), 4.57 (s, 2H), 5.40 (s, 1H), 7.01–7.20 (m, 3H) and 7.30 –7.40 (m, 2H); $^{13}$C NMR (CD$_3$OD) $\delta$ 19.8, 44.6, 47.7, 86.8, 117.0 and 117.2 (C-F coupling) (2C), 120.6, 124.0, 126.1, 127.5, 130.7 and 130.8 (C-F coupling) (2C), 133.6, 133.7, 155.5 and 160.4 (C-F coupling), 165.4, 169.2 MS (ES$^+$) m/z 296 (M$^+$); HRMS (EI at 70 ev) m/z Found: 295.1114; Calcd for C$_{17}$H$_{14}$FN$_3$O: 295.1121 (M$^+$-H).
3,4-Dihydro-7-(4-hydroxybenzylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2o)

Compound 2o was prepared by following the general procedure, starting from compound 3 (0.10 g, 0.21 mmol) in MeOH (25 mL), 4-hydroxybenzylamine hydrobromide (0.052 g, 0.25 mmol) and Et$_3$N (0.020 mL, 0.25 mmol) to obtain 0.084 g (0.15 mmol) of tosyl intermediate which was detosylated using NaOMe (0.16 g, 2.6 mmol) in MeOH (15 mL) to afford the product (0.025 g, 29%); $^{1}$H NMR (CD$_3$OD) $\delta$ 2.95 (t, 2H, $J = 7.6$ Hz), 3.83 (t, 2H, $J = 7.7$ Hz), 4.49 (s, 2H), 5.45 (s, 1H), 6.78 (d, 2H, $J = 8.4$ Hz), 7.15 (s, 1H), 7.17 (d, 2H, $J = 8.4$ Hz); $^{13}$C NMR (CD$_3$OD) $\delta$ 19.5, 44.2, 47.9, 86.2, 116.7(2C), 120.2, 123.8, 125.6, 127.1, 127.7, 129.9(2C), 155.0, 158.5, 159.8 and 168.8; MS (ES$^+$) $m/z$ 294 (M$^+$).

3,4-Dihydro-7-(4-isopropylbenzylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2p)

Compound 2p was prepared by following the general procedure, starting from compound 3 (0.10 g, 0.22 mmol) in MeOH (15 mL) and 4-isopropylbenzylamine (0.038 g, 0.26 mmol) to obtain 0.050 g (0.090 mmol) of tosyl intermediate which was detosylated using NaOMe (0.091 g, 1.7 mmol) in MeOH (15 mL) to afford the product(0.021 g, 23%); $^{1}$H NMR (CD$_3$OD) $\delta$ 1.23 (d, 6H, $J = 8.7$ Hz), 2.89 (septet, 1H, $J = 8.7$ Hz), 2.95 (t, 2H, $J = 7.6$ Hz ), 3.83 (t, 2H, $J = 7.6$ Hz), 4.56 (s, 2H), 5.41 (s, 1H), 7.15 (s, 1H,), 7.25 (s, 4H); $^{13}$C NMR (CD$_3$OD) $\delta$ 19.5, 24.4 (2C), 35.1, 44.2, 47.9, 86.4, 120.2, 123.8, 125.7, 127.1, 128.0(2C), 128.4(2C), 134.5, 150.0, 155.2, 159.9 and 168.8; MS (ES$^+$) $m/z$ 320 (M$^+$).
3,4-Dihydro-7-((4-dimethylamino)benzylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2q)

Compound 2q was prepared by following the general procedure, starting from compound 3 (0.10 g, 0.21 mmol) in MeOH (25 mL) and 4-dimethylaminobenzylamine (0.038 g, 0.26 mmol) 0.050 g (0.090 mmol) of tosyl intermediate which was detosylated using NaOMe (0.091 g, 1.7 mmol) in MeOH (15 mL) to afford the product (0.020 g, 22 %); ¹H NMR (CD₃OD) δ 2.93 (s, 6H), 2.96 (t, 2H, J = 7.6 Hz), 3.83 (t, 2H, J = 7.6 Hz), 4.48 (s, 2H), 5.46 (s, 1H), 6.78 (d, 2H, J = 8.4 Hz), 7.15 (s, 1H), 7.19 (d, 2H, J = 8.4 Hz); ¹³C NMR (CD₃OD) δ 19.5, 41.0, 44.2, 47.9, 86.2, 114.3(2C), 120.2, 123.9, 124.9, 125.6, 127.1, 129.5(2C), 151.9, 155.0, 159.7 and 168.9; MS (ES⁺) m/z 321 (M⁺).

3,4-Dihydro-7-phenethylamino-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2r)

Compound 2r was prepared by following the general procedure, starting from compound 3 (0.20 g, 0.43 mmol) in MeOH (25 mL) and phenethylamine (0.062 g, 0.51 mmol) 0.070 g (0.13 mmol) of tosyl intermediate which was detosylated using NaOMe (0.135 g, 2.50 mmol) in MeOH (15 mL) to afford the product (0.038 g, 22%); ¹H NMR (CD₃OD) δ 2.90–3.05 (m, 4H), 3.59 (t, 2H, J = 7.5 Hz), 3.84 (t, 2H, J = 7.5 Hz), 5.42 (s, 1H), 7.13 (s, 1H) and 7.20–7.40 (m, 5H); ¹³C NMR (CD₃OD) δ 19.5, 35.1, 44.1, 46.2, 85.3, 120.2, 123.8, 125.4, 127.2, 127.8, 129.8 (2C), 129.9 (2C), 139.3, 154.9, 159.7 and 168.4. MS (ES⁺) m/z 292 (M⁺). HRMS (EI at 70 ev) m/z Found: 291.1371; Calcd for C₁₈H₁₇N₃O: 291.1371 (M⁺-H).
3,4-Dihydro-7-(3,4-dimethoxyphenethylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2s)

Compound 2s was prepared by following the general procedure, starting from compound 3 (0.100 g, 0.21 mmol) in MeOH (25 mL) and 3,4-dimethoxyphenethyl-amine (0.047 g, 0.26 mmol) to obtain 0.052 g (0.080 mmol) of tosyl intermediate which was detosylated using NaOMe (0.090 g, 1.7 mmol) in MeOH (15 mL) to afford the product (0.020 g, 20%); Alternatively, the detosylation was carried out using the NaN₃ in DMF using tosylated compound (0.02 g, 0.03 mmol) following the procedure used in the preparation of compound 16 to afford the compound 2s (0.013 g, 88% yield). ¹H NMR (CD₃OD) δ 2.85–3.00 (m, 4H), 3.58 (t, 2H, J = 7.2 Hz), 3.79 (s, 3H), 3.80 (s, 3H), 3.83 (t, 2H, J = 7.5 Hz), 5.39 (s, 1H), 6.72–6.91 (m, 3H), 7.14 (s, 1H); ¹³C NMR (CD₃OD) δ 19.5, 34.8, 44.1, 46.3, 56.3, 56.4, 85.3, 113.3, 113.8, 120.2, 122.3, 123.9, 125.4, 127.3, 132.2, 149.5, 150.6, 155.0, 159.6 and 168.5; MS (ES⁺) m/z 352 (M⁺); HRMS (EI at 70 ev) m/z Found: 351.1571; Calcd for C₂₀H₂₁N₃O₃: 351.1583 (M⁺-H).

3,4-Dihydro-7-((furan-2-yl)methylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2t)

Compound 2t was prepared by following the general procedure, starting from compound 3 (0.100 g, 0.21 mmol) in MeOH (25 mL) and furfuryl amine (0.031 g, 0.26 mmol) to obtain 0.04 g (0.08 mmol) of tosyl intermediate which was detosylated using NaOMe (0.081 g, 1.5 mmol) in MeOH (20 mL) to afford the product (0.012 g, 15%); ¹H NMR (CD₃OD) δ 2.97 (t, 2H, J = 7.5 Hz), 3.88 (t, 2H, J = 7.5 Hz), 4.58 (s, 2H), 5.63 (s, 1H), 6.41 (s, 2H), 7.16 (s, 1H), 7.50 (t, 1H, J = 1.2 Hz); ¹³C NMR (CD₃OD) δ 19.5, 41.1,
3,4-Dihydro-7-((5-methylfuran-2-yl)methylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2u)

Compound 2u was prepared by following the general procedure, starting from compound, 3 (0.10 g, 0.21 mmol) in MeOH (25 mL) and 5-methylfurfurylamine (0.028 g, 0.26 mmol) to obtain 0.043 g (0.080 mmol) of tosyl intermediate which was detosylated using NaOMe (0.086 g, 1.6 mmol) in MeOH (15 mL) to afford the product (0.015 g, 18%); 1H NMR (CD3OD) δ 2.25 (s, 3H), 2.97 (t, 2H, J = 7.5 Hz), 3.63 (s, 3H), 3.69 (t, 2H, J = 3.9 Hz), 3.87 (t, 2H, J = 7.5 Hz), 4.51 (s, 2H), 5.64 (s, 1H), 5.98 (d, 1H, J = 3.0 Hz), 6.27 (d, 2H, J = 3.0 Hz), 7.17 (s, 1H); 13C NMR (CD3OD) δ 13.4, 19.5, 41.2, 44.3, 86.2, 107.5, 111.0, 120.2, 123.7, 125.6, 127.1, 148.3, 154.0, 154.9, 160.1 and 168.8; MS (ES+) m/z 282 (M+).

3,4-Dihydro-7-((thiophen-2-yl)methylamino)-pyrrolo[4,3,2-de]quinolin-8(1H)-one (2v)

Compound 2v was prepared by following the general procedure, starting from compound 3 (0.10 g, 0.21 mmol) in MeOH (25 mL) and thiophene-2-methylamine (0.036 g, 0.26 mmol) to obtain 0.058 g (0.11 mmol) of tosyl intermediate which was detosylated using NaOMe (0.11 g, 2.1 mmol) in MeOH (20 mL) to afford the product (0.020 g, 24%); 1H NMR (CD3OD) δ 2.95 (t, 2H, J = 7.6 Hz), 3.85 (t, 2H, J = 7.6 Hz), 4.76 (s, 2H), 5.56 (s, 1H), 6.99 (dd, 1H, J1 = 5.1 Hz, J2 = 1.2 Hz), 7.10 (d, 1H, J = 2.7

44.4, 86.3, 109.9, 111.6, 120.2, 123.6, 125.7, 127.1, 144.2, 150.3, 155.0, 160.2 and 168.8; MS (ES+) m/z 268 (M+).
Hz), 7.14 (s, 1H), 7.37 (dd, 1H, \( J_1 = 5.1 \text{ Hz}, J_2 = 1.2 \text{ Hz} \)); \(^{13}\)C NMR (CD\(_3\)OD) \( \delta \) 19.4, 43.0, 44.4, 86.5, 120.2, 123.6, 125.7, 126.9, 127.1, 127.9, 128.0, 139.6, 154.6, 160.1 and 168.8; MS (ES\(^+\)) \text{m/z} 284 (M\(^+\)).

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References:


SYNTHESIS OF BISPYRROLOQUINONE RING SYSTEM VIA CAN MEDIATED OXIDATIVE CYCLIZATION

DWAYAJA H. NADKARNI, MURUGESAN SRINIVASAN AND SADANANDAN E. VELU.

In preparation for Tetrahedron

Format adapted for dissertation
Abstract:

Bispyrroloquinone and bispyrroloiminoquinone are two polycyclic structural scaffolds present in a number of biologically active marine alkaloids such as zyzzyanones, tsitsikammamines and wakayin. A facile synthetic methodology to construct the tricyclic bispyrroloquinone ring structure is developed. The key step in the synthesis of this ring system involved the construction of a pyrrole ring on 6-benzylaminooindole-4,7-quinone by reaction with a \( \beta \)-dicarbonyl compound in the presence of ceric ammonium nitrate (CAN) in MeOH / CH\(_2\)Cl\(_2\). A plausible mechanism for this transformation is proposed based on previous literature reports of similar reactions. In order to demonstrate the generality of this synthetic methodology, the reaction was carried out on different combinations of aminoquinones and \( \beta \)-dicarbonyl compounds. The methodology was also extended to explore the use of \( \beta \)-ketosulfides, \( \beta \)-ketosulfoxides and \( \beta \)-ketosulfones in the place of \( \beta \)-dicarbonyl compounds. The reaction worked well in the case of \( \beta \)-ketosulfones. However, it failed to give expected products in the case of \( \beta \)-ketosulfides and \( \beta \)-ketosulfoxides. All of the newly synthesized compounds were characterized by \(^1\)H NMR, \(^{13}\)C NMR and Mass Spectral analysis. Development of this synthetic methodology is significant because of its possible application in the total synthesis of marine natural products containing bispyrroloquinone ring structure.
Introduction

Compounds containing a \( p \)-quinone moiety are widespread in nature representing important classes of biologically active molecules.\(^1\)\(^-\)\(^5\) Indole-4,7-quinones form an important structural unit present in many biologically active natural products such as calothrixins,\(^6\) murrayaquinones,\(^7\) zyzzyanones,\(^8\),\(^9\) tsitsikammamines\(^10\) and wakayin.\(^11\) As a part of our research interest directed towards the synthesis and biological evaluation of marine natural products, we have been particularly interested in marine natural products containing a bispyrroloquinone or a bispyrroloiminoquinone ring systems as core structures. A few examples of these marine natural products are zyzzyanones, tsitsikammamines and wakayin (Figure 1).

**Figure 1**: Marine alkaloids Tsitsikammamines, Wakayin and Zyzzyanones.
These natural products or their synthetic analogs may prove to be effective leads in development of potential anticancer agents. Hence the development of methodologies for the synthesis of these alkaloids is important. Since these alkaloids contain either a bispyrroloquinone or a bispyrroloiminoquinone ring system as core structures, we decided to look at chemistry that would lead to the synthesis of these core ring systems first. A possible step in the synthesis of these ring systems would be the formation of a pyrrole ring on to a bicyclic indolo-4,7-quinone or a tricyclic pyrroloiminoquinone ring system. Known synthetic methods to construct pyrrole rings on to quinones include annulation reactions which can be accomplished using reagents like DDQ as used in the synthesis of (+)-bismurrayquinone-A\textsuperscript{12} and the reactions catalyzed by AlCl\textsubscript{3} as used in the synthesis of indolocarbazole quinones.\textsuperscript{13}

Carbon-carbon bond formation reactions resulting in cyclization have been receiving increasing attention in the recent past. Of these reactions, oxidative free radical mediated carbon-carbon bond formation reactions have been developed into a very useful synthetic tool.\textsuperscript{14,15} Redox processes based on electron transfer have constituted a major part of the mechanism of these reactions. Chemical methods for electron transfer reactions involve the use of salts of high valent metals like Mn (III), Ce (IV), Cu(II), Ag (I), Co (III), Fe (III) etc.\textsuperscript{15,16} All of these metal salts have found applications as reagents in different reactions. Of these, Ce (IV) reagents are the more commonly used due to factors such as better solubility in organic solvents, relatively lower toxicity, easiness of handling, and experimental versatility.\textsuperscript{17} Known cerium (IV) reagents used in oxidative free radical cyclization include cerium (IV) trifluoracetate, cerium (IV)
methanesulfonate, cerium (IV) trifluoromethanesulfonate and cerium (IV) ammonium nitrate (CAN). The most extensively used cerium (IV) reagent in organic chemistry is CAN.\textsuperscript{16} The greater acceptance of this reagent as a single electron oxidant is attributed to its large electron reduction potential. The enormous growth in the use of this reagent in synthetic organic chemistry is evident from the large number of publications and several reviews on CAN mediated reactions.\textsuperscript{14,16,18-26} The usefulness of Ce (IV) reagents in C-C bond formation was established by Heiba and Dessau in 1971.\textsuperscript{16} The substrates for the formation of radicals can be 1,3-dicarbonyl compounds, silyl enol ethers, styrenes enamines, enamides etc.\textsuperscript{16} The free radical reactions involving 2-hydroxy-1,4-napthoquinones,\textsuperscript{27} 2-amino-1,4-naphthoquinones\textsuperscript{28-30} have also been reported. CAN was reportedly used in development of synthetic methods for homoallylic alcohols\textsuperscript{31} and β-ketoenol ethers.\textsuperscript{32} CAN has facilitated free radical chemistry in carbohydrate synthesis with glycols as substrates.\textsuperscript{33,34} CAN facilitated C-C bond formation has found successful applications in the total syntheses of a number of natural products such as norbisabolide,\textsuperscript{35} Kalafungins,\textsuperscript{36} (+)-subincandine F\textsuperscript{37} and (+)-γ- Rubromycin.\textsuperscript{38} CAN has been used in oxidative free radical cyclization reactions resulting in formation of pyrrole rings.\textsuperscript{28-30} Recently, we have reported a preliminary communication on the synthesis of bispyrroloquinone and bispyrroloiminoquinone ring systems present in wakayin, tsitsikammanines and zyzzyanones by a CAN mediated oxidative cyclization.\textsuperscript{39} As an extension of this work, we now report the application of this facile synthetic methodology to incorporate various functional groups such as esters, ketones, amides and sulfones. This is achieved by extending the synthetic methodology to other 1,3-dicarbonyl substrates such as 1,3-diketones, β-ketoamides and β-ketosulfones.
Results and Discussion

Our first objective was to develop a synthetic method for the bispyrroloquinone ring system present in marine natural products. This can be achieved by constructing a pyrrole ring on to indole-4,7-quinones. Construction of pyrrole rings on quinones has been previously reported. One of the procedures for this is by making use of an oxidative free radical reaction of quinones with \(\beta\)-dicarbonyl compounds catalyzed by CAN as reported previously by Tseng et al in 2002. Based on this methodology, we proposed the retrosynthesis of a substituted bispyrroloquinone ring system starting from a 6-aminoindole-4,7-quinone and 1,3-dicarbonyl compound as outlined in Figure 2.

![Figure 2: Retrosynthetic analysis of bispyrroloquinone ring system.](image)

The bispyrroloquinone 17 may be synthesized by reacting a 1,3-dicarbonyl compound 19 with a 6-aminoindolo-4,7-quinone derivative 20. Compound 20 can be synthesized from 4,6,7-trimethoxyindole 8. Synthesis of compound 8 is reported in
Chapter 1. 1,3-dicarbonyl compound 19 to be used in this synthesis are simple molecules that are commercially available. As starting reagents for this synthesis, we have first synthesized four derivatives of 6-benzylamino indolo-4,7-quinone unit (Figure 3, 21a-d). Benzylamino derivatives were chosen as starting material because of the ease of removal of the benzyl protecting group at a later stage of the synthesis, as might be needed in the synthesis of a natural product.

![Figure 3: 6-Benzylnamino-N-tosylindole-4,7-quinones](image)

All four of these compounds 21a-d were synthesized starting from 4,6,7-trimethoxy indole (8) following a procedure similar to that reported in the literature as outlined in Figure 4.

![Figure 4: Synthetic scheme for 6-benzylnamino-N-tosylindole-4,7-quinones, 21a-d](image)
The indole N-atom of 4,6,7-trimethoxyindole (8) was protected with a tosyl group by treatment with $\text{Ts}_2\text{O}$ in the presence of KH in anhydrous THF to obtain the N-tosyl derivative 22 in 85% yield. Compound 22 was then oxidized using an aqueous solution of CAN in CH$_3$CN as solvent at room temperature to afford N-tosyl-6-methoxyindole-4,7-quinone 23 in 92% yield. Amination of quinone 23 with substituted benzyl amines in anhydrous MeOH/THF at room temperature gave the aminated compounds 21a-d in excellent yields. This amination reaction takes place faster in MeOH. However, quinone 23 was not completely soluble in MeOH. So, we had to use a combination of MeOH and THF for these reactions. The reaction conditions and the yields for 6-amino derivatives of N-tosylindole-4,7-quinones 21a-d are given in Table 1.

![Diagram](image_url)

**Table 1**: 6-amino derivatives of N-tosylindole-4,7-quinones prepared (21a-d).

<table>
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<th>Entry</th>
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<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
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<td>H</td>
<td>MeOH/THF</td>
<td>25</td>
<td>20</td>
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<tr>
<td>21b</td>
<td>OMe</td>
<td>MeOH/THF</td>
<td>25</td>
<td>18</td>
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<tr>
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<td>3,4-OCH$_2$O-</td>
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<td>25</td>
<td>22</td>
<td>94</td>
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</tbody>
</table>

We chose ethyl acetoacetate as the 1,3-dicarbonyl compound for the first attempt on the synthesis of bispyrroloquinone ring system. The reaction is outlined in Figure 5. 6-benzylaminindole-4,7-quinone (21a) was treated with ethyl acetoacetate in the presence of CAN in CH$_2$Cl$_2$: MeOH (1:5) at 25°C to afford the bispyrroloquinone derivative 25a in
68% yield. Four equivalents of CAN were required for the completion of this reaction. CAN was added slowly in four equal portions during the reaction time. Attempts to carry out this reaction with lesser equivalents of CAN resulted in incomplete or no reaction.

![Reaction Scheme](image)

**Figure 5**: Synthesis of bispyrroloquinone derivative 25a

We believe this cyclization reaction follows a general mechanism similar to one that is reported in cases of aminoquinones such as 2-amino-1,4-naphthoquinones.\(^\text{28, 29}\) However, there is also a possibility of the reaction going through an anionic intermediate instead of the radical intermediate, as well. A proposed mechanism for the formation of compound 25a based on previous literature reports\(^\text{28, 29}\) of similar oxidative free radical cyclization is given in Figure 6.

Radical initiation occurs by loss of a single electron from the active methylene group of ethyl acetoacetate to form the radical 26. The initiation may also be favored by the fact that Ce(IV) facilitates formation of enolate,\(^\text{42}\) which further loses an electron to CAN to form the radical 26. This radical undergoes an intermolecular addition to the quinone 21a to obtain radical 27. Radical 27 loses another electron to CAN to obtain cation 28. Reformation of the quinone ring and the nucleophilic attack of the amine nitrogen to the carbonyl carbon of one of the ester groups led to the formation of 29. Intermediate 29 loses a proton to form 30 and 30 undergoes loss of water and restores the
aromaticity of the pyrrole ring to form product 25a. The factors such as stability associated with cyclization and oxophilicity of cerium salts\textsuperscript{28} favor this mechanistic pathway.

\textbf{Figure 6:} Proposed mechanism for bispyrroloquinone derivative 25a.

In order to confirm the proposed free radical mechanistic pathway we repeated the conversion of 21a to 25a in the absence of CAN. This reaction failed to give the product indicating that the presence of CAN is necessary for this reaction. This also rules out the possibility for an anionic mechanism. This mechanism is additionally supported by the fact that cerium has the ability to display adjacent oxidation states +3 and +4.\textsuperscript{16}
Generality of the synthetic methodology

This synthetic strategy was then applied to the remaining aminoquinones 21b-d. In order to examine the generality of this methodology, we extended our studies using substrates other than β-ketoesters. This includes substrates such as β-ketoamides, 1,3-diketones (symmetrical and unsymmetrical), monoketones, β-ketosulphones, β-ketosulfoxides and β-ketosulfides. Four different benzylamino indole-4,7-quinones (21a-d) were also used in these studies. The reaction conditions for the cyclization were the same as we used in the previous reaction.

Reaction with β-ketoester

Reactions with β-ketoesters were continued with three other substituted 6-benzylaminoindole-4,7-quinone derivatives 21b-d under the same reaction conditions as described previously (Figure 7). These reactions worked well to afford the products 25b-d in good yields. The yields and conditions for these three reactions are summarized in Table 2.

![Diagram](image)

Figure 7: Synthesis of bispyrroloquinone derivatives, 25b-d
Table 2: Synthesis of ester derivative of bispyrroloquinones

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25b</td>
<td>OMe</td>
<td>CH₂Cl₂/MeOH</td>
<td>25°C</td>
<td>1</td>
<td>71%</td>
</tr>
<tr>
<td>25c</td>
<td>NO₂</td>
<td>CH₂Cl₂/MeOH</td>
<td>25°C</td>
<td>1.5</td>
<td>60%</td>
</tr>
<tr>
<td>25d</td>
<td>3,4-OCH₂O-</td>
<td>CH₂Cl₂/MeOH</td>
<td>25°C</td>
<td>1</td>
<td>70%</td>
</tr>
</tbody>
</table>

**Reaction with β-diketones**

This synthetic methodology was further applied to 1,3-diketones. As a part of this study, we have used both symmetrical and unsymmetrical 1,3-diketones for our experiments. Reactions of various 6-benzylaminoindole-4,7-quinone derivatives 21a-c with different 1,3-diketones are given in Figure 8.

![Reaction schematic](image)

Three 6-benzylaminoindole-4,7-quinone derivatives 21a-c were used in this study. Two 1,3-diketones used are acetyl acetone and 1-phenylbutane-1,3-dione. Acetyl
acetone is a symmetrical dicarbonyl compound and 1-phenylbutane-1,3-dione is an
unsymmetrical dicarbonyl compound. Treatment of 6-benzylaminoindole-4,7-quinone
derivatives 21a-c with acetyl acetone in the presence of CAN in CH$_2$Cl$_2$ / MeOH (1:5) at
room temperature afforded compounds 25e-g. Similarly, treatment of 6-
benzylaminoindole-4,7-quinone derivatives 21a-c with 1-phenylbutane-1,3-dione in the
presence of CAN in CH$_2$Cl$_2$ / MeOH (1:5) at room temperature afforded compounds 25h-
j. The conditions and yields for these reactions are summarized in Table 3.

Table 3: Reaction of 6-benzylaminoindole-4,7-quinone derivatives 21a-c with different
1,3-diketones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R$_1$</th>
<th>R$_2$</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25e</td>
<td>H</td>
<td>CH$_3$</td>
<td>CH$_2$Cl$_2$/MeOH</td>
<td>25°C</td>
<td>1 hr</td>
<td>67</td>
</tr>
<tr>
<td>25f</td>
<td>OMe</td>
<td>CH$_3$</td>
<td>CH$_2$Cl$_2$/MeOH</td>
<td>25°C</td>
<td>1 hr</td>
<td>68</td>
</tr>
<tr>
<td>25g</td>
<td>NO$_2$</td>
<td>CH$_3$</td>
<td>CH$_2$Cl$_2$/MeOH</td>
<td>25°C</td>
<td>1.5 hr</td>
<td>58</td>
</tr>
<tr>
<td>25h</td>
<td>H</td>
<td>C$_6$H$_5$</td>
<td>CH$_2$Cl$_2$/MeOH</td>
<td>25°C</td>
<td>1 hr</td>
<td>67</td>
</tr>
<tr>
<td>25i</td>
<td>OMe</td>
<td>C$_6$H$_5$</td>
<td>CH$_2$Cl$_2$/MeOH</td>
<td>25°C</td>
<td>1 hr</td>
<td>65</td>
</tr>
<tr>
<td>25j</td>
<td>NO$_2$</td>
<td>C$_6$H$_5$</td>
<td>CH$_2$Cl$_2$/MeOH</td>
<td>25°C</td>
<td>1.5 hr</td>
<td>52</td>
</tr>
</tbody>
</table>

Mechanistically, there is only one possible product in the case of symmetrical 1,3-
diketone. Unsymmetrical 1,3-diketones can give us two different regioisomeric products.

For example, in the reaction with unsymmetrical 1-phenylbutane-1,3-dione, there is a
theoretical possibility of two regioisomeric products, 25j (A) and 25j (B) (Figure 9).

The regioisomer 25j (B) was found to be the only product formed in this reaction.
The structure of the regioisomeric product was confirmed by as determined by $^1$H-NMR,
$^{13}$C-NMR and NOESY experiments. The NOESY spectrum of compound 25j is shown in
Figure 10.
Figure 9: Two possible regioisomers of the compound 25j.

Figure 10: NOESY NMR of the compound 25j.
The three-proton singlet at 2.18 ppm is assigned to the methyl group at C-6 position. The two-proton singlet at 5.60 ppm is assigned to CH$_2$ group at N-7 position, which has a NOESY correlation with the singlet at 2.18 ppm. This assignment clearly shows that the more stable regioisomer B is formed as opposed to less stable regioisomer A. This might be due the fact that there is less steric hindrance in isomer B where the two phenyl groups are farther apart as compared to isomer A (Figure 10). Another NOESY correlation characteristic of this structure is between the –CH$_3$ (2.3 ppm) and the up-field proton on the p-nitrobenzyl ring at 7.2 ppm confirming the structure 25j (B).

**Reaction with ketones:**

There are reports in the literature for the reaction of quinonoid compounds with monocarbonyl compounds such as acetone.\textsuperscript{16} Hence, we have tried to extend this oxidative cyclization reaction of 6-benzylaminoindole-4,7-quinone derivatives to just ketones, instead of 1,3-diketones. In addition, in order to work out synthetic strategies for the natural products, an oxidative free radical cyclization of the aminoindolo-4,7-quinone with monocarbonyl substrates would be highly favorable.

![Figure 1](image-url)

**Figure 11:** Reaction of 6-benzylaminoindole-4,7-quinone derivatives 21a-c with 2-butanone.
With this in mind, we attempted reactions 6-benzylaminoindole-4,7-quinone derivatives \textit{21a-c} with 2-butanone in the presence of CAN in CH$_2$Cl$_2$: MeOH (1:5) at room temperature. However, this reaction did not proceed at room temperature. Heating of the reaction mixture resulted only in the formation of complex mixtures as indicated by TLC and NMR.

**Reaction with $\beta$-ketoamide**

Three 6-benzylaminoindole-4,7-quinone derivatives \textit{21a-c} were used in this study. The $\beta$-ketoamide chosen for this study is N, N-dimethylacetoacetamide. 6-benzylaminoindole-4,7-quinone derivatives \textit{21a-c} were treated with N, N-dimethyl acetoacetamide in the presence of CAN in CH$_2$Cl$_2$: MeOH (1:5) at room temperature. Reaction with amides proceeded smoothly just as in case of $\beta$-ketoesters and 1,3-diketones with similar reaction times and yields to afford the cyclized products, \textit{25k-m} as shown in Figure 12. The reaction conditions and the yields obtained for the products, \textit{25k-m} are summarized in Table 4.

![Figure 12: Reaction of 6-benzylaminoindole-4,7-quinone derivatives \textit{21a-c} with N, N-dimethylacetoacetamide](image)

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Table 4: Amide derivatives of bispyrroloquinones

<table>
<thead>
<tr>
<th>Entry</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25k</td>
<td>H</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;/MeOH</td>
<td>25°C</td>
<td>1</td>
<td>67</td>
</tr>
<tr>
<td>25l</td>
<td>OMe</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;/MeOH</td>
<td>25°C</td>
<td>1</td>
<td>68</td>
</tr>
<tr>
<td>25m</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;/MeOH</td>
<td>25°C</td>
<td>1.5</td>
<td>58</td>
</tr>
</tbody>
</table>

Reaction with β-ketosulfide, β-ketosulfones and β-ketosulfoxides:

As a continuation to study the impact of different substituents on β-keto compounds we further investigated effect of presence of sulfide, sulfoxide and sulfone on the oxidative cyclization reaction.

![Chemical structures](image)

**Figure 13**: Sulfur derivatives of bispyrroloquinones.
The resulting products from these reactions could prove to be valuable intermediates for the synthesis of zyzzyanones, tsitsikammamines and wakain or their analogs. With this objective in mind, we attempted reactions of compounds 21a-c with 1-methylthio-2-propanone (β-ketosulfide), 1-methylsulfinyl-2-propanone (β-ketosulfoxide) and 1-methanesulfonyl-2-propanone (β-ketosulfone) under the same reaction conditions described previously for 1,3-dicarbonyl compounds. The three reactions are given in Figure 13.

For sulfide and sulfoxide substrates, the reactions did not progress well in spite of adding several equivalents of CAN and heating. Heating actually has resulted in the decomposition of starting compounds as indicated by TLC and proton NMR. However, when 6-benzylaminoindole-4,7-quinone derivatives 21a-c were treated with β-ketosulfone (1-methanesulfonyl-2-propanone) in the presence of CAN in CH₂Cl₂: MeOH (1:5) at room temperature the reaction did progress giving expected products 25n-p with lower yields and longer reaction times. The yields and the reaction conditions are summarized in Table 5.

Table 5: Sulfone derivatives of bispyrroloquinones

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25n</td>
<td>H</td>
<td>CH₂Cl₂/MeOH</td>
<td>25°C</td>
<td>6 hr</td>
<td>23</td>
</tr>
<tr>
<td>25o</td>
<td>OMe</td>
<td>CH₂Cl₂/MeOH</td>
<td>25°C</td>
<td>6 hr</td>
<td>35</td>
</tr>
<tr>
<td>25p</td>
<td>NO₂</td>
<td>CH₂Cl₂/MeOH</td>
<td>25°C</td>
<td>7 hr</td>
<td>31</td>
</tr>
</tbody>
</table>

Thus, these experiments led us to the observation, that the radical formation and hence the reaction is favored by presence of electron withdrawing carbonyl or sulfone group at the β-position to the first carbonyl group. This observation was supported by the
fact that there was no reaction with mono ketones, β-ketosulfides and β-ketosulfoxides. The difference in the ability of carbonyl group and β-ketosulfone in stabilizing the radical is highlighted by the poor yield obtained in case of β-ketosulfones along with longer reaction times. Steric interactions exert a major influence on the formation of the product and the condensation occurs at the least hindered carbonyl groups.

Our next goal was the removal of tosyl and benzyl protecting groups present in bispyrroloquinone ring system obtained from the cyclization reactions, as such deprotection steps would be necessary when we apply this methodology in the synthesis of alkaloids.

**Figure 14:** Removal of tosyl and benzyl groups from compounds 25a and 25h

These deprotection experiments were carried out on a couple of our cyclization products (25a and 25h) as outlined in Figure 14. The detosylation was performed by
treatment of compound 25a and 25h with excess NaOEt and anhydrous EtOH at room temperature for 2 hours or alternatively by treatment with NaN₃ in DMF at room temperature for 4 hours. Both these methods worked well giving similar yields. Detosylation of 25a resulted in 67 % yield of 31a while 25h resulted in 79 % yield of 31h. Debenzylation reactions were first attempted using transfer hydrogenolysis using 5% and 10% Pd/C with HCOONH₄ in EtOH. These reactions failed to yield the expected debenzylated product. Hence a stronger Pd black catalyst was used in these reactions. Treatment of compounds 31a and 31h with HCOONH₄ and Pd black in refluxing EtOH yielded the debenzylated products 32a and 32h in 68 % and 64% yields respectively.

Conclusions

In conclusion, we have accomplished the synthesis of bispyrroloquinone ring system using a novel and efficient protocol. This protocol is the successful application of CAN mediated free radical oxidative cyclization to an indoloquinone system. While this methodology cannot be directly applied to the synthesis of the marine alkaloids, zyzzyanones, tsitsikammamines and wakayin, it will be useful in introducing different functional groups on to the core structure. These varied substitution patterns will in turn help in the development of synthetic analogs which eventually will facilitate structure activity relationship studies and optimization of lead molecules. Further efforts in this direction are currently in progress.
Experimental

**General Considerations:** Solvent evaporations were carried out *in vacuo* with rotary evaporator. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator (Whatmann, silica gel, UV254, 25 μm plates). Spots were visualized by UV light (254 and 365 nm). Flash column chromatography was performed using Dynamic Adsorbents silica gel (18-32 μM) in the solvent systems indicated. All $^1$H and $^{13}$C NMR spectra were recorded on a Bruker ARX 300 or ARX 400. NMR solvents used were deuterated chloroform CDCl$_3$ with TMS as internal standard. The value of chemical shifts (δ) are given in parts per million and coupling constants (J) in Hz. Mass spectra were recorded on Micromass Platform LCC instrument. Anhydrous solvents used for reactions were purchased in Sure-Seal bottles from Sigma-Aldrich Chemical Co. Deuterated solvents were purchased from Sigma-Aldrich Chemical Co. Other reagents were purchased from Fisher scientific, Aldrich or Acros Chemical Companies and used as received. Syntheses of compounds 22, 23 have been reported earlier.$^{41,43}$ The spectral data of these compounds matched well with the reported values.

**4,6,7-Trimethoxy-1-tosyl-1H-indole (22)**

A solution of 4,6,7-trimethoxyindole 8 (2.0 g, 9.7 mmol) in anhydrous THF (30 mL) was slowly added to a suspension of KH (30% by weight dispersion in mineral oil) (5.20 g, 38.6 mmol) in the same solvent (30 mL) maintained under N$_2$ and stirred at room temperature for 1 hour. A solution of (Ts)$_2$O (3.94 g, 12.1 mmol) in THF (20 mL) was slowly added to this at 0°C, and the reaction mixture was stirred at room temperature for 15 hours. TLC analysis (EtOAc/CHCl$_3$, 1:3) revealed the completion of the reaction.
Reaction mixture was quenched with slow addition of water until all of the excess KH was destroyed. The solvent was evaporated off under reduced pressure and resulting residue dissolved in CH$_2$Cl$_2$ (300 mL). The CH$_2$Cl$_2$ extract was washed with water (2 × 75 mL), brine (1 × 75 mL) and dried over anhydrous Na$_2$SO$_4$. Removal of solvent after filtering off the drying agent, afforded the crude product which was purified by flash chromatography over Si gel using EtOAc/CHCl$_3$ (1:4) as eluent to afford pure 4,6,7-trimethoxy-1-tosyl-1H-indole 22 (3.2 g, 92%); $^1$H-NMR (CDCl$_3$) $\delta$ 2.34 (s, 3H), 3.74 (s, 3H), 3.83 (s, 3H), 6.35 (s, 1H), 6.63 (d, 1H, $J$ = 3.5 Hz), 7.19 (d, 2H, $J$ = 8.4 Hz), 7.59 (d, 1H, $J$ = 3.8 Hz), 7.74 (d, 1H, $J$ = 8.4 Hz).

6-Methoxy-1-tosyl-1H-indole-4,7-dione (23)

To a solution of N-(p-toluenesulfonyl)-4,6,7-trimethoxyindole 22 (4.7 g, 13 mmol) in CH$_3$CN (150 mL), a solution of CAN (24.98 g, 45.57 mmol) in water (90 mL) was added and the reaction mixture was stirred at room temperature for 4 hours. TLC analysis (EtOAc / CHCl$_3$, 1:1) revealed that the reaction was complete. The solvent was evaporated under reduced pressure and the resulting residue was dissolved in CH$_2$Cl$_2$ (300 mL). Water (100 mL) was added and the organic layer was separated. The CH$_2$Cl$_2$ extract was washed with water (3 × 100 mL) and dried over anhydrous Na$_2$SO$_4$. The drying agent was filtered off and solvent was removed to obtain the crude product which was subjected to column chromatography over Si gel using EtOAc/CHCl$_3$ (1:5) as eluent to afford the pure quinone 23 as a yellow solid (3.0 g, 69%); $^1$H-NMR (CDCl$_3$) $\delta$ 2.42 (s, 3H), 3.78 (s, 3H), 5.75(s, 1H), 6.71(d, 1H, $J$ = 3.2Hz), 7.34(d, 1H, $J$ = 8.3 Hz), 7.82 (d, 1H, $J$ = 3.2 Hz), 8.05 (d, 1H, $J$ = 8.3 Hz).
6-Benzylamino-1-tosyl-1H-indole-4,7-dione (21a)

To a solution of 6-methoxy-1-tosyl-1H-indole-4,7-dione 23 (1.2 g, 3.3 mmol) in MeOH and THF (1:1, 50 mL) at room temperature, a solution of benzyl amine 21a (0.5 g, 5 mmol) in MeOH (4 mL) was added and stirred at room temperature for 20 hours. TLC analysis (EtOAc / CHCl₃, 1:1) revealed that the reaction was complete. The solvent was removed under reduced pressure to obtain the crude product as a reddish brown residue. It was purified by flash column chromatography over Si gel using EtOAc / CHCl₃ (1:10) as eluent to furnish the pure compound 21a. (1.3 g, 88%); ¹H NMR (CDCl₃) δ 2.43 (s, 3H), 4.24 (d, 2H, J = 5.7 Hz), 5.35 (s, 1H), 6.06 (bt, 1H, J = 5.7 Hz), 6.71 (d, 1H, J = 3.0 Hz), 7.15-7.20 (m, 2H), 7.25-7.45 (m, 5H), 7.8 (d, 1H, J = 3.0 Hz), 7.99 (d, 2H, J = 8.4 Hz); ¹³C NMR (CDCl₃) δ 21.8, 47.3, 97.5, 108.6, 127.0, 127.8 (2C), 128.2, 128.9(2C), 129.0(2C), 129.8(2C), 131.3, 134.0, 134.4, 135.7, 146.2, 147.9, 170.2, 181.4; and MS (ES⁺) m/z 407 (M+H).

6-(4-Methoxybenzylamino)-1-tosyl-1H-indole-4,7-dione (21b)

To a solution of 6-methoxy-1-tosyl-1H-indole-4,7-dione 23 (1.13 g, 3.41 mmol) in MeOH and THF (1:1, 100 mL) at room temperature a solution of 4-methoxybenzylamine (0.70 g, 5.1 mmol) in MeOH (4 mL) was added and the reaction mixture was stirred at room temperature for 20 hours. TLC analysis (EtOAc / CHCl₃, 1:1) revealed that the reaction was complete. The solvent was removed under reduced pressure and the reddish yellow residue obtained was purified by flash column chromatography over Si gel using EtOAc / CHCl₃ (1:10) as eluent to furnish pure compound 21b (1.40 g, 94%); ¹H NMR (CDCl₃) δ 2.43 (s, 3H), 3.80 (s, 3H), 4.16 (d, 2H, J = 6.0 Hz), 5.35 (s, 1H),
5.99 (bt, 1H, J = 6.0 Hz), 6.72 (d, 1H, J = 3.0 Hz), 6.87 (d, 2H, J = 8.7 Hz), 7.18 (d, 2H, J = 8.7 Hz), 7.34 (d, 2H, J = 8.4 Hz), 7.80 (d, 1H, J = 3.0 Hz), 7.98 (d, 2H, J = 8.4 Hz); $^{13}$C NMR (CDCl$_3$) δ 21.8, 46.8, 55.3, 97.3, 108.6, 114.4(2C), 126.9, 127.7, 128.9(2C), 129.2(2C), 129.8(2C), 131.3, 134.0, 134.4, 134.5, 146.2, 147.8, 159.5, 170.2, 181.4; and MS (ES$^+$) m/z 434 (M+H).

6-(4-Nitrobenzylamino)-1-tosyl-1H-indole-4,7-dione (21c)

To a solution of 6-methoxy-1-tosyl-1H-indole-4,7-dione 23 (0.700 g, 2.16 mmol) in MeOH and THF (1:1, 100 mL) at room temperature, a solution of 4-nitrobenzylamine hydrochloride (0.540 g, 3.24 mmol) in MeOH (4 mL) and Et$_3$N (0.330 g, 3.24 mmol) were added and stirred for 20 hours. TLC analysis (EtOAc / CHCl$_3$, 1:1) revealed that the reaction was complete. The solvent was removed under reduced pressure and the reddish yellow residue was purified by flash column chromatography over Si gel using EtOAc / CHCl$_3$ (1:10) as eluent to furnish pure compound 21c (0.700 g, 70% yield); $^1$H NMR (CDCl$_3$) δ 2.45 (s, 3H), 4.42 (d, 2H, J = 6.0 Hz), 5.23 (s, 1H), 6.19 (bt, 1H, J = 6.0 Hz), 6.72 (d, 1H, J = 3.0 Hz), 7.37 (d, 2H, J = 8.4 Hz), 7.42 (d, 2H, J = 8.7 Hz), 7.82 (d, 1H, J = 3.0 Hz), 8.01 (d, 2H, J = 8.4 Hz), 8.21 (d, 2H, J = 8.7 Hz); $^{13}$C NMR (CDCl$_3$) δ 21.8, 46.4, 98.5, 108.7, 124.2(2C), 126.9, 128.0, 129.0(2C), 129.8(2C), 131.5(2C), 133.9, 134.1, 143.2, 146.4, 147.6, 147.8, 170.1, 181.4; and MS (ES$^+$) m/z 451 (M+H).

6-((Benzo[d][1,3]dioxol-6-yl)methylamino)-1-tosyl-1H-indole-4,7-dione (21d)

To a solution of 6-methoxy-1-tosyl-1H-indole-4,7-dione 23 (0.0780 g, 0.240 mmol) in MeOH and THF (1:1, 10 mL) at room temperature, a solution of
piperonylamine (0.052 g, 0.34 mmol) in MeOH (2 mL) was added and stirred at room temperature for 22 hours. TLC analysis (EtOAc / CHCl₃, 1:1) revealed that the reaction was complete. The solvent was removed under reduced pressure and the reddish orange residue obtained was purified by flash column chromatography over Si gel with EtOAc / CHCl₃ (1:10) as eluent to furnish pure compound 21d (0.102 g, 94%); ¹H NMR (CDCl₃) δ 2.44 (s, 3H), 4.14 (d, 2H, J = 5.6 Hz), 5.32 (s, 1H), 5.96 (s,2H), 6.01 (bs, 1H), 6.65-6.75 (m, 3H), 6.75 (s, 1H), 7.35 (d, 2H, J = 8.4 Hz ), 7.8 (d, 1H, J = 4.0 Hz), 7.98 (d, 2H, J = 8.4 Hz); ¹³C NMR (CDCl₃) δ 22.0, 47.2, 97.6, 101.5, 108.3, 108.7, 108.8, 121.4, 127.0, 129.1(2C), 129.5, 130.0(2C), 131.5, 134.0, 134.5, 146.4, 147.6, 147.9, 148.3, 170.3, 181.6; and MS (ES⁺) m/z 451 (M⁺).

**CAN mediated oxidative cyclization: General procedure**

To a solution of bicyclic quinone 21a-d (1 equiv) and β-dicarbonyl compound (4 equiv) in MeOH and CH₂Cl₂ (5:1), CAN (4 equiv) was added in four equal portions at 10 min intervals. The reaction mixture was stirred for another 10 min at room temperature. TLC analysis (EtOAc / hexanes, 1:1) revealed completion of the reaction. Solvent was completely removed under reduced pressure, and the residue was dissolved in CH₂Cl₂ (75 mL), washed with water (3 × 50 mL), brine (1 × 50 mL) and dried over anhydrous Na₂SO₄. The drying agent was filtered and the solvent was evaporated under reduced pressure. The crude product obtained was purified by column chromatography over Si gel using EtOAc / hexanes (1:10) as eluent to obtain the pure bispyrroloquinones 25a-p in 50-72% yield.
1-Benzyl-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic acid ethyl ester (25a)

Following the general procedure, compound 21a (0.094 g, 0.23 mmol) was treated with ethyl acetoacetate (0.12 g, 0.92 mmol) and CAN (0.51 g, 0.92 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25a (0.081 g, 68%); ¹H NMR (CDCl₃) δ 1.39 (t, 3H, J = 7.2 Hz), 2.32 (s, 3H), 2.39 (s, 3H), 4.37 (q, 2H, J = 7.2 Hz), 5.66 (s, 2H), 6.73 (d, 1H, J = 3.2 Hz), 6.95-7.05 (m, 2H), 7.20 (d, 2H, J = 8.4 Hz), 7.25-7.30 (m, 3H), 7.71 (d, 1H, J = 3.2 Hz), 7.90 (d, 2H, J = 8.4 Hz); ¹³C NMR (CDCl₃) δ 10.9, 14.1, 21.7, 48.5, 61.1, 108.3, 113.9, 124.4, 126.5(2C), 127.6, 128.7(2C), 128.9(2C), 129.4(3C), 129.6, 130.1, 132.6, 134.0, 135.6, 141.2, 145.7, 164.5, 167.1, 177.0; and MS (ES⁺) m/z 515 (M⁺).

1-(4-Methoxybenzyl)-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indole-3-carboxylic acid ethyl ester (25b)

Following the general procedure, compound 21b (0.054 g, 0.12 mmol) was treated with ethyl acetoacetate (0.064 g, 0.50 mmol) and CAN (0.27 g, 0.50 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25b (0.047g, 71%); ¹H NMR (CDCl₃) δ 1.38 (t, 3H, J = 7.2 Hz), 2.33 (s, 3H), 2.41 (s, 3H), 3.78 (s, 3H), 4.35 (q, 2H, J = 7.2 Hz), 5.58 (s, 2H), 6.74 (d, 1H, J = 3.2 Hz), 6.77 (d, 2H, J = 8.7 Hz), 6.91 (d, 2H, J = 8.7 Hz), 7.24 (d, 2H, J = 8.1 Hz), 7.71 (d, 1H, J = 3.2 Hz), 7.93 (d, 2H, J = 8.1 Hz); ¹³C NMR (CDCl₃) δ 11.0, 14.2, 21.7, 48.1, 55.3, 61.0, 108.3, 114.0, 114.1(2C), 124.5, 127.8, 128.1(2C), 129.0(2C), 129.4, 129.5(2C), 129.6, 130.2, 132.6, 134.2, 141.1, 145.8, 159.1, 164.5, 167.1, 176.9; and MS (ES⁺) m/z 546 (M⁺).
1-(4-Nitrobenzyl)-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic acid ethyl ester (25c)

Following the general procedure, compound 21c (0.050 g, 0.11 mmol) was treated with ethyl acetoacetate (0.058 g, 0.44 mmol) and CAN (0.24 g, 0.44 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25c (0.037 g, 60%); ¹H NMR (CDCl₃) δ 1.40 (t, 3H, J = 7.2 Hz), 2.35 (s, 3H), 2.37 (s, 3H), 4.39 (q, 2H, J = 7.2 Hz), 5.72 (s, 2H), 6.76 (d, 1H, J = 3.3 Hz), 7.12 (d, 2H, J = 8.7 Hz), 7.17 (d, 2H, J = 8.3 Hz), 7.72 (d, 1H, J = 3.3 Hz), 7.86 (d, 2H, J = 8.3 Hz), 8.13 (d, 2H, J = 8.7 Hz); ¹³C NMR (CDCl₃) δ 10.8, 14.2, 21.6, 48.1, 61.2, 108.5, 114.4, 124.0 (2C), 124.7, 127.2 (2C), 128.9 (2C), 129.4 (3C), 129.7, 129.9, 132.9, 134.0, 140.8, 143.1, 146.1, 147.4, 164.2, 166.9, 176.7; and MS (ES⁺) m/z 562 (M⁺).

1-(Benzo [d][1,3]dioxol-6-yl)-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic acid ethyl ester (25d)

Following the general procedure, compound 21d (0.094 g, 0.21 mmol) was treated with ethyl acetoacetate (0.109 g, 0.84 mmol) and CAN (0.4 g, 0.92 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25d (0.066 g, 57%); ¹H NMR (CDCl₃) δ 1.39 (t, 3H, J = 7.2 Hz), 2.34 (s, 3H), 2.44 (s, 3H), 4.37(q, 2H, J = 7.2 Hz), 5.55 (s, 2H), 5.95 (s, 2H, 6.45-6.55 (m, 2H), 6.70 (d, 1H, J = 7.8 Hz), 6.75 (d, 1H, J = 3.0 Hz), 7.28 (d, 2H, J = 8.4 Hz), 7.73 (d, 2H, J = 8.4 Hz), 7.96 (d, 2H, J = 8.4 Hz); ¹³C NMR (CDCl₃) δ111.0, 14.1, 21.7, 48.3, 49.1, 61.1, 101.2, 107.4, 108.3(2C), 114.0, 120.3, 124.5, 129.0 (2C), 129.3, 129.5(3C), 129.6, 130.1, 132.6, 134.1, 141.1, 145.9, 147.1, 148.1, 164.5, 167.1, 177.0; and MS (ES⁺) m/z 560 (M⁺).
3-Acetyl-1-benzyl-2-methyl-7-tosyl-1H,7H-pyrrolo[3,2-f] indole-4,8-dione (25e)

Following the general procedure, compound 21a (0.050 g, 0.12 mmol) was treated with acetyl acetone (0.049 g, 0.48 mmol) and CAN (0.27 g, 0.48 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25e (0.040 g, 67%); ¹H NMR (CDCl₃) δ 2.26 (s, 3H), 2.40 (s, 3H), 2.65 (s, 3H), 5.66 (s, 2H), 6.74 (d, 1H, J = 2.4 Hz), 6.90-7.00 (m, 2H), 7.21 (d, 2H, J = 8.0 Hz), 7.20-7.40 (m, 3H), 7.73 (d, 1H, J = 2.4 Hz), 7.93 (d, 2H, J = 8.0 Hz); ¹³C NMR (CDCl₃) δ 10.9, 21.8, 31.6, 48.5, 108.2, 122.6, 123.7, 126.5 (2C), 127.6, 128.7(2C), 129.0(2C), 129.5(3C), 129.6, 130.1, 132.3, 133.9, 135.6, 140.8, 145.9, 167.0, 178.3, 199.1; and MS (ES⁺) m/z 487 (M⁺).

3-Acetyl-1-(4-methoxybenzyl)-2-methyl-7-tosyl-1H,7H-pyrrolo[3,2-f] indole-4,8-dione (25f)

Following the general procedure, compound 21b (0.070 g, 0.16 mmol) was treated with acetyl acetone (0.064 g, 0.64 mmol) and CAN (0.35 g, 0.64 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25f (0.056 g, 68%); ¹H NMR (CDCl₃) δ 2.28 (s, 3H), 2.41(s, 3H), 2.63 (s, 3H), 3.78 (s, 3H), 5.58 (s, 2H), 6.73 (d, 1H, J = 3.0 Hz ), 6.78 (d, 2H, J = 8.0 Hz), 6.93 (d, 2H, J = 8.5 Hz), 7.25 (d, 2H, J = 8.0 Hz), 7.73 (d, 1H, J = 3.0 Hz), 7.95 (d, 2H, J = 8.5 Hz); ¹³C NMR (CDCl₃) δ 10.9, 21.7, 31.6, 48.0, 55.3, 108.2, 114.1(2C), 122.7, 123.8, 127.7, 128.1(2C), 129.0,129.1(2C), 129.5(2C), 129.6, 130.3, 132.3, 134.1, 140.7, 145.9, 159.1, 167.1, 178.3, 199.0; and MS (ES⁺) m/z 517 [M⁺].
3-Acetyl-2-methyl-1-(4-nitrobenzyl)-7-tosyl-1H,7H-pyrrolo[3,2-f] indole-4,8-dione (25g)

Following the general procedure, compound 21c (0.045 g, 0.10 mmol) was treated with acetyl acetone (0.040 g, 0.40 mmol) and CAN (0.22 g, 0.40 mmol) in anhydrous MeOH / CH₂Cl₂ (15 mL + 3 mL) to furnish compound 25g (0.027 g, 51%); ¹H NMR (CDCl₃) δ 2.30 (s, 3H), 2.37 (s, 3H), 2.67 (s, 3H), 5.72 (d, 2H), 6.75 (d, 1H, J = 3.3 Hz), 7.14 (d, 2H, J = 8.7 Hz), 7.19 (d, 2H, J = 8.1 Hz), 7.74 (d, 1H, J = 3.3 Hz), 7.88 (d, 2H, J = 8.1 Hz), 8.14 (d, 2H, J = 8.7 Hz); ¹³C NMR (CDCl₃) δ 10.8, 21.6, 31.7, 48.0, 108.5, 122.9, 124.0, 124.1, 127.2(2C), 128.9, 129.0(2C), 129.4(2C), 129.7(2C), 129.9, 132.5, 133.7, 140.4, 143.0, 146.2, 147.4, 166.9, 178.1, 198.8; and MS (ES⁺) m/z 532 [M+H].

3-Benzoyl-1-benzyl-2-methyl-7-tosyl-1H,7H-pyrrolo[3,2-f] indole-4,8-dione (25h)

Following the general procedure, compound 21a (0.10 g, 0.25 mmol), 1-phenyl-2-propanone (0.16 g, 0.99 mmol) and CAN (0.47 g, 0.99 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25h (0.090 g, 67%); ¹H NMR (CDCl₃) δ 2.19 (s, 3H), 2.41 (s, 3H), 5.69 (s, 2H), 6.59 (d, 1H, J = 2.4 Hz), 6.90-7.10 (m, 2H), 7.20-7.35 (m, 5H), 7.40(t, 2H, J = 7.2 Hz), 7.54 (t, 1H, J = 7.2 Hz), 7.68 (d, 1H, J = 2.4 Hz), 7.82 (d, 2H, J = 7.5 Hz), 7.95 (d, 2H, J = 8.1 Hz); ¹³C NMR (CDCl₃) δ 10.7, 21.8, 48.7, 108.2, 120.9, 125.1, 126.6(2C), 127.7, 128.4(2C), 128.7, 128.8(2C), 128.9, 129.0(2C), 129.2(2C), 129.5(2C), 130.6, 132.0, 133.1, 134.1, 135.7, 138.4, 139.7, 145.8, 167.0, 177.3, 192.9; and MS (ES⁺) m/z 549 (M⁺).
3-Benzoyl-1-(4-methoxybenzyl)-2-methyl-7-tosyl-1H,7H-pyrrolo[3,2-f] indole-4,8-dione (25i)

Following the general procedure, compound 21b (0.080 g, 0.18 mmol) was treated with 1-phenyl-2-propanone (0.090 g, 0.72 mmol) and CAN (0.36 g, 0.22 mmol) anhydrous MeOH / CH₂Cl₂ (15 mL + 3 mL) to furnish compound 25i (0.074g, 70%); ¹H NMR (CDCl₃) δ 2.21 (s, 3H), 2.43 (s, 3H), 3.8 (s, 3H), 5.61 (s, 2H), 6.59 (d, 1H, J = 3.3 Hz), 6.81 (d, 2H, J = 8.4 Hz), 7.01 (d, 2H, J = 8.4 Hz), 7.25-7.30 (m, 2H), 7.40 (t, 2H, J = 8.0 Hz), 7.54 (t, 1H, J = 8.0 Hz), 7.68 (d, 1H, J = 3.3 Hz), 7.81 (d, 2H, J = 8.0 Hz), 7.97 (d, 2H, J = 8.0 Hz); ¹³C NMR (CDCl₃) δ10.8, 21.8, 48.1, 55.3, 108.2, 114.1(2C), 120.8, 125.1, 127.8, 128.2(2C), 128.3(2C), 128.6, 129.1(2C), 129.2(2C),129.4, 129.5(2C), 130.6, 131.9, 133.1, 134.1, 138.3, 139.7, 146.0, 159.1, 167.1, 177.3, 192.9; and MS (ES⁺) m/z 579 [M⁺].

3-Benzoyl-2-methyl-1-(4-nitrobenzyl)-7-tosyl-1H,7H-pyrrolo[3,2-f] indole-4,8-dione (25j)

Following the general procedure, compound 21c (0.050 g, 0.11 mmol) was treated with 1-phenyl-2-propanone (0.072 g, 0.44 mmol) and CAN (0.24 g, 0.44 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25j (0.034 g, 52%); ¹H NMR (CDCl₃) δ 2.23 (s, 3H), 2.39 (s, 3H), 5.76 (s, 2H), 6.61 (d, 1H, J = 2.4 Hz), 7.15-7.25 (m, 4H), 7.43 (t, 2H, J = 8.0 Hz), 7.57 (t, 1H, J = 7.6 Hz), 7.69 (d, 1H, J = 2.4 Hz), 7.82 (d, 2H, J = 7.6 Hz), 7.90 (d, 2H, J = 8.0 Hz), 8.18 (d, 2H, J = 8.4 Hz); ¹³C NMR (CDCl₃) δ 10.6, 21.6, 48.1, 108.4, 121.1, 124.1(2C), 125.3, 127.3 (2C), 128.4, 128.6(2C),
129.0(2C), 129.2(2C), 129.4(2C), 129.8, 130.2, 132.2, 133.3, 133.9, 138.1, 139.2, 143.1, 146.1, 147.5, 166.9, 177.0, 192.5; and MS (ES^+ m/z 594 [M+H]

1-Benzyl-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydropyrrolo[3,2-f] indole-3-carboxylic acid dimethylamide (25k)

Following the general procedure, compound 21a (0.050 g, 0.12 mmol) was treated with N,N-dimethylacetoacetamide (0.064 g, 0.49 mmol) and CAN (0.27 g, 0.49 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25k (0.043 g, 68%); ^1H NMR (CDCl₃) δ 2.15 (s, 3H), 2.41 (s, 3H), 2.88 (s, 3H), 3.13 (s, 3H), 5.55-5.75 (m, 2H), 6.70 (d, 1H, J = 3.0 Hz), 6.90-7.10 (m, 2H), 7.20-7.30 (m, 5H), 7.71 (d, 1H, J = 3.0 Hz), 7.93 (d, 1H, J = 8.4 Hz); ^13C NMR (CDCl₃) δ 10.6, 21.8, 34.8, 38.0, 48.6, 108.0, 117.5, 123.3, 126.7(2C), 127.6, 128.5, 128.7(2C), 129.0 (2C), 129.4(3C), 131.0, 131.9, 134.0, 135.8, 137.1, 145.8, 165.5, 167.0, 178.0; and MS (ES^+) m/z 516 (M^+).

1-(4-Methoxybenzyl)-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indole-3-carboxylic acid dimethylamide (25l)

Following the typical procedure, compound 21b (0.070 g, 0.16 mmol) was treated with N,N-dimethylacetoacetamide (0.083 g, 0.64 mmol) and CAN (0.35 g, 0.64 mmol) in anhydrous MeOH / CH₂Cl₂ (15 mL + 3 mL) to furnish compound 25l (0.063g, 72%); ^1H NMR (CDCl₃) δ 2.17 (s, 3H), 2.42 (s, 3H), 2.86 (s, 3H), 3.13 (s, 3H), 3.79 (s, 3H), 5.40-5.60 (m, 2H), 6.69 (d, 1H, J = 3.3 Hz), 6.78 (d, 2H, J = 7.6 Hz), 6.96 (d, 2H, J = 8.5 Hz), 7.2-7.3 (m, 2H), 7.71(d, 1H, J = 3.3 Hz), 7.95 (d, 2H, J = 8.5 Hz); ^13C NMR (CDCl₃) δ 10.7, 21.7, 34.8, 38.0, 48.1, 55.3, 107.9, 114.1(2C), 117.6, 123.3, 127.9(2C), 128.3(2C),
128.4, 129.0, 129.4(3C), 131.0, 131.9, 134.2, 137.0, 145.8, 159.1, 165.6, 167.0, 178.0; and MS (ES⁺) m/z 546 [M⁺].

2-Methyl-1-(4-nitrobenzyl)-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic acid dimethylamide (25m)

Following the typical procedure, compound 21c (0.045 g, 0.10 mmol) was treated with N,N-dimethylacetamide (0.051 g, 0.40 mmol) and CAN (0.22 g, 0.40 mmol) in anhydrous MeOH / CH₂Cl₂ (10 mL + 2 mL) to furnish compound 25m (0.031g, 56%); ¹H NMR (CDCl₃) δ 2.20 (s, 3H), 2.38 (s, 3H), 2.90 (s, 3H), 3.15 (s, 3H), 5.60-5.80 (m, 2H), 6.71 (d, 1H, J = 3.3 Hz), 7.15-7.25 (m, 4H), 7.72 (d, 1H, J = 3.3 Hz), 7.88 (d, 2H, J = 8.4 Hz), 8.14 (d, 2H, J = 8.7 Hz); ¹³C NMR (CDCl₃) δ 10.5, 21.6, 34.9, 38.1, 48.1, 108.1, 117.9, 123.6, 124.0 (2C), 127.4, 128.4(2C), 129.0, 129.4(2C), 129.7(2C), 130.4, 132.1, 133.9, 136.8, 143.1, 146.1, 147.4, 165.1, 166.7, 177.7; and MS (ES⁺) m/z 561 [M+H].

1-Benzyl-3-methanesulfonyl-2-methyl-7-tosyl-1H,7H-pyrrolo[3,2-f] indole-4,8-dione (25n)

Following the general procedure, compound 21a (0.090 g, 0.22 mmol) was treated with 1-methanesulfonyl-2-propanone (0.12 g, 0.88 mmol) and CAN (0.49 g, 0.88 mmol) in anhydrous MeOH / CH₂Cl₂ (20 mL + 4 mL) to furnish compound 25n (0.027 g, 31%); ¹H NMR (CDCl₃) δ 2.44 (s, 3H), 2.90 (s, 3H), 3.25 (s, 3H), 4.70 (q, 1H, J = 14.4 Hz), 6.43 (d, 1H, J = 3.0 Hz), 7.19–7.30 (m, 2H), 7.33-7.50 (m, 5H), 7.92(d, 1H, J = 3.0 Hz), 8.11(d, 2H, J = 8.4 Hz); ¹³C NMR (CDCl₃) δ 21.8, 46.5, 51.3, 53.5, 87.8, 108.1,
126.8, 127.7, 127.8, 128.2(2C), 128.4, 129.2, 129.6(2C), 129.8 (2C), 129.9(2C), 132.5, 133.4, 135.7, 136.3, 146.6, 159.6, 163.8, 166.5 and MS (ES$^+$) $m/z$ 523 [M+H]

3-Methanesulfonfnyl-1-(4-methoxybenzyl)-2-methyl-7-tosyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (25o)

Following the general procedure, compound 21b (0.057 g, 0.13 mmol) was treated with 1-methanesulfonyl-2-propanone (0.067 g, 0.52 mmol) and CAN (0.27 g, 0.52 mmol) in anhydrous MeOH / CH$_2$Cl$_2$ (10 mL + 2 mL) to furnish compound 25o (0.022 g, 25%); $^1$H NMR (CDCl$_3$) $\delta$ 2.44 (s, 3H), 2.90 (s, 3H), 3.30(s, 3H), 3.76 (s, 3H), 4.64 (m, 2H), 6.42 (d, 1H, $J = 3.2$ Hz), 6.77 (d, 2H, $J = 8.4$ Hz), 7.30-7.55 (m, 4H), 7.91 (d, 1H, $J = 3.2$ Hz), 8.10 (d, 2H, $J = 8.4$ Hz); $^{13}$C NMR (CDCl$_3$) $\delta$ 21.8, 46.0, 51.4, 53.6, 55.2, 87.7, 108.1, 113.5(2C), 126.7, 127.9, 129.6(2C), 129.7 129.9(2C), 131.3, 131.5, 132.5(2C), 133.3, 136.3, 146.6, 159.1, 159.5, 163.9, 166.6 and MS (ES$^+$) $m/z$ 553 [M+H].

3-Methanesulfonfnyl-2-methyl-1-(4-nitrobenzyl)-7-tosyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (25p)

Following the general procedure, compound 21c (0.054 g, 0.12 mmol) was treated with 1-methanesulfonyl-2-propanone (0.067 g, 0.48 mmol) and CAN (0.26 g, 0.48 mmol) in anhydrous MeOH / CH$_2$Cl$_2$ (10 mL + 2 mL) to furnish compound 25p (0.021 g, 31%); $^1$H NMR (CDCl$_3$) $\delta$ 2.45 (s, 3H), 2.88 (s, 3H), 3.42 (s, 3H), 4.60-4.90 (m, 2H), 6.45 (d, 1H, $J = 3.2$ Hz), 7.37 (d, 2H, $J = 8.4$ Hz), 7.57 (d, 2H, $J = 8.4$ Hz), 7.95 (d, 1H, $J = 3.2$ Hz), 8.10-8.20 (m, 4H); $^{13}$C NMR (CDCl$_3$) $\delta$ 21.9, 46.2, 51.6, 53.9, 88.3, 108.3,
123.5(2C), 126.7, 129.6(2C), 130.0(2C), 130.3(2C), 130.5, 132.9, 133.2, 135.1, 135.7, 143.0, 146.8, 147.4, 159.7, 163.2, 166.4 and MS (ES\(^+\)) \(m/z\) 568 [M+H].

1-Benzyl-2-methyl-4,8-dioxo-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic acid ethyl ester (31a)

Method A: To a solution of compound 25a (0.038 g, 0.07 mmol) in anhydrous EtOH (10 mL), NaOEt (0.050 g, 0.074 mmol) was added and the reaction mixture was stirred at room temperature for 45 minutes. TLC analysis (EtOAc / CHCl\(_3\), 1:3) indicated completion of the reaction. The reaction mixture was quenched with water and solvent was evaporated off. The resulting aqueous layer was extracted with EtOAc (2 × 10 mL). The organic extract was washed with water (3 × 5 mL), brine (1 × 5 mL) and dried over anhydrous Na\(_2\)SO\(_4\). The drying agent was filtered and the solvent was removed to obtain the crude product, which was purified by column chromatography over Si gel using EtOAc / CHCl\(_3\) (1:10) as eluent to yield compound 31a (0.016 g, 65%);

Method B: Alternatively, to a solution of compound 25a (0.015 g, 0.03 mmol) in DMF, NaN\(_3\) (0.0023 g, 0.035 mmol) was added and the reaction stirred at room temperature for 4 hours. TLC analysis (EtOAc / CHCl\(_3\), 1:3) indicated completion of the reaction. The reaction mixture was diluted with water (5 mL). EtOAc (10 mL) was added and the aqueous layer was separated out. The EtOAc layer was washed with water (3 × 5 mL) brine (1 × 5 mL) and dried over anhydrous Na\(_2\)SO\(_4\). The drying agent was filtered and the solvent was removed to obtain the crude product, which was purified by column chromatography over Si gel using EtOAc / CHCl\(_3\) (1:10) as eluent to yield compound 31a (0.0050 g, 58%); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 1.41 (t, 3H, \(J = 7.2\) Hz), 2.29 (s, 3H), 4.40 (q, 2H,
$J = 7.2$ Hz), 5.64 (s, 2H), 6.57-6.59 (m, 2H), 7.02 (d, 2H, $J = 6.6$ Hz), 7.23-7.33 (m, 3H) and 10.66 (bs, 1H); $^{13}$C NMR (CDCl$_3$) δ 10.6, 14.2, 48.6, 61.1, 108.9, 115.0, 125.2, 126.0(2C), 126.5, 127.5, 127.7, 129.0, 129.3(2C), 131.6, 135.8, 140.5, 164.9, 169.5 and 177.8; MS (ES-) m/z 361 (M-H); and HRMS (EI at 70 ev) m/z Found: 362.1267; Calculated for C$_{21}$H$_{18}$N$_2$O$_4$: 362.1273.

3-Benzoyl-1-benzyl-2-methyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (31h)

Method A: To solution of compound 25h (0.026 g, 0.035 mmol) in anhydrous EtOH (10 mL), NaOEt (0.024 g, 0.35 mmol) was added and the reaction mass was stirred at room temperature for 45 minutes. TLC analysis (EtOAc / CHCl$_3$, 1:3) indicated the completion of the reaction. The reaction mixture was then quenched with water, solvent was evaporated off. The resulting aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic extract was washed with water (3 × 5 mL), brine (1 × 5 mL) and dried over anhydrous Na$_2$SO$_4$. The solvent was evaporated after filtration of the drying agent to obtain the crude product, which was purified by column chromatography over Si gel using EtOAc / CHCl$_3$ (1:10) as eluent to yield the compound 31h (0.014 g, 78%).

Method B: Alternatively, to a solution of 25h (0.015 g, 0.027 mmol) in DMF, NaN$_3$ (0.0021 g, 0.033 mmol) was added and the reaction stirred at room temperature for 4 hours. TLC analysis (EtOAc/ CHCl$_3$, 1:3) indicated completion of the reaction. The reaction mixture was diluted with water (5 mL). EtOAc (10 mL) was added and the aqueous layer was separated out. The EtOAc layer was washed with water (3 × 5 mL) brine (1 × 5 mL) and dried over anhydrous Na$_2$SO$_4$. The drying agent was filtered and the solvent was removed to obtain the crude product, which was purified by column
chromatography over Si gel using EtOAc / CHCl₃ (1:10) as eluent to yield compound 31h (0.0080 g, 68%); ¹H NMR (CDCl₃) δ 2.21 (s, 3H), 5.93 (s, 2H), 6.45 (d, 1H, J = 2.7 Hz), 6.86 (d, 1H, J = 2.7 Hz), 7.15 (d, 2H, J = 6.9 Hz), 7.30-7.55 (m, 6H), 7.88 (d, 2H, J = 6.9 Hz) and 9.87 (bs, 1H); ¹³C NMR (CDCl₃) δ 10.5, 48.7, 108.9, 121.9, 124.4, 126.3(2C), 126.5, 127.4, 127.9, 128.6(2C), 128.8, 129.1(2C), 129.3(2C), 132.3, 133.1, 135.8, 138.5, 139.1, 169.4, 178.0 and 193.4; MS (ES+) m/z 395 (M+H); and HRMS (EI at 70 ev) m/z Found: 394.1302; Calculated for C₂₅H₁₈N₂O₃: 394.1317.

2-methyl-4,8-dioxo-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic acid ethyl ester (32a)

To a solution of compound 31a (0.015 g, 0.040 mmol) in anhydrous EtOH (15 mL), HCOONH₄ (0.05 g, 0.8 mmol) and Pd black (0.043 g, 0.40 mmol) were added and the reaction mixture was refluxed for 15 hours. TLC analysis (MeOH / CHCl₃, 1:20) indicated the completion of the reaction. The reaction mixture was filtered through a pad of celite 585 and washed with EtOAc / CHCl₃ (1:1, 20 mL). The combined filtrate was concentrated under reduced pressure. The organic residue obtained was redissolved in EtOAc (10 mL) and washed with water (2 × 5 mL), brine (1 × 5 mL) and dried over anhydrous Na₂SO₄. The drying agent was filtered and the solvent was removed to afford the crude product which was purified by column chromatography over Si gel using MeOH / CHCl₃ (1:25) as eluent to furnish compound 32a (0.0076 g, 68%); ¹H NMR (CD₃OD) δ 1.39 (t, 3H, J = 7.1 Hz), 2.42 (s, 3H), 4.33 (q, 2H, J = 7.1 Hz), 6.55 (d, 1H, J = 2.7 Hz) and 7.00 (d, 1H, J = 2.7 Hz); ¹³C NMR (CD₃OD) δ 11.3, 13.1, 60.3, 108.0, 112.7, 124.9, 125.0, 128.2, 130.8, 132.1, 139.8, 164.9, 168.2 and 179.1; MS (ES-) m/z
271 (M-H); and HRMS (EI at 70 ev) m/z Found: 272.0797; Calculated for C_{18}H_{12}N_{2}O_{4}: 272.0792.

3-benzoyl-2-methyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (32h)

To a solution of compound 31h (0.02 g, 0.05 mmol) in anhydrous EtOH (15 mL), HCOONH_{4} (0.05 g, 0.75 mmol) and Pd black (0.05 g, 0.5 mmol) were added and the reaction mixture was refluxed for 15 hours. TLC analysis (MeOH / CHCl_{3}, 1:20) indicated the completion of the reaction. The reaction mixture was filtered through a pad of celite 585 and washed with EtOAc / CHCl_{3} (1:1, 20 mL). The combined filtrate was concentrated under reduced pressure. The residue obtained was redissolved in EtOAc (10 mL) and washed with water (2 × 5 mL), brine (1 × 5 mL) and dried over anhydrous Na_{2}SO_{4}. The drying agent was filtered and the solvent was evaporated to obtain the crude product which was purified by column chromatography over Si gel using MeOH / CHCl_{3} (1:25) as eluent to furnish compound 32h (0.009 g, 64%); {^{1}H-NMR (CD_{3}OD) \delta 2.27 (s, 3H), 6.45 (d, 1H, J = 2.7 Hz), 6.98 (d, 1H, J = 2.7 Hz), 7.42-7.49 (m, 2H), 7.55-7.62 (m, 1H) and 7.82-7.86 (m, 2H); {^{13}C-NMR (CD_{3}OD) \delta 10.3, 107.8, 120.2, 124.8, 125.8, 127.3, 128.0(2C), 129.0(2C), 131.1, 131.6, 132.7, 137.5, 138.7, 168.3, 179.6 and 194.1; MS (ES-) m/z 303 (M-H); and HRMS (EI at 70 ev) m/z Found: 304.0848; Calculated for C_{18}H_{12}N_{2}O_{3}: 304.0842.
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References:


TOTAL SYNTHESES OF ZYZZYANONES A-D

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Format adapted for dissertation
Abstract:

Marine natural products provide a vast array of organic compounds with unique chemical structures and interesting biological properties. These natural products are generally isolated from marine invertebrate animals such as sponges, ascidians, tunicates, mollusks and marine bacteria. Nitrogen containing natural products are called alkaloids. The largest numbers of bioactive marine alkaloids with novel structures have been isolated from marine sponges. Pharmacological value of these marine bioactive alkaloids compounds has ensured their progress in various phases of clinical trials. While a number of these alkaloids have been isolated in quantities sufficient to ascertain their biological profile, many with unique structures are available only in minute quantities, preventing their thorough biological evaluations. Laboratory synthesis of these alkaloids and their analogs provides access to these materials in larger quantities. Zyzzyanones A-D are the first representatives of a new class of marine alkaloids containing a unique pyrrolo[3,2-f]indol-4,8(1H,7H)-dione skeleton. These alkaloids were isolated from Australian sponge *zyzza fuliginosa* by Utkina et al in 2004-2005. Zyzzyanones A-D exhibited moderate cytotoxic activity against mouse Ehrlich carcinoma cells. As a part of an ongoing project in our laboratory directed towards the synthesis and biological evaluation of marine alkaloids, we have developed synthetic methods for the first total syntheses of zyzzyanones A-D. The key step in this synthesis is the formation of a bispyrroloquinone ring system via an oxidative free radical cyclization reaction of an acetal with 6-benzylaminoinoindole-4,7-quinone mediated by Mn(OAc)$_3$. 
**Introduction**

Zyzzyanones A-D are the first representatives of a new class of marine alkaloids containing a pyrrolo[3,2-$f$]indol-4,8(1$H$,7$H$)-dione skeleton. These alkaloids were discovered in 2004-2005 by Utkina et al, in the course of their search for biologically active metabolites from marine sponges. They first investigated the aqueous EtOH extract of the Australian marine sponge *Zyzzya fuliginosa* in 2004. This investigation resulted in the isolation and characterization of *zyzzyanone A*.\(^1\) Subsequently, *zyzzyanone B-D* were also isolated and characterized by the same group in 2005.\(^2\) Chemical structures of *Zyzzyanones A – D* are shown in Figure 1.

![Chemical structures of Zyzzyanones A-D](image)

- R = Me, *Zyzzyanone A*
- R = H, *Zyzzyanone B*
- R = Me, *Zyzzyanone C*
- R = H, *Zyzzyanone D*

**Figure 1:** Structures of *zyzzyanones A-D*.

Biosynthetically, *zyzzyanones* may have an interrelationship with other sponge derived marine alkaloids such as makaluvamines, tsitsikammamines and wakayin. It is possible that makaluvamine, wakayin and tsitsikammamines are either biosynthetically derived from or transformed into a *zyzzyanone* skeleton (Figure 2).\(^1\)
Initial biological evaluation of zyzzyanones revealed that they exhibit moderate cytotoxic activity against mouse Ehrlich carcinoma cells (IC\textsubscript{50}, 25 µg/mL) and inhibited the cell division of fertilized sea urchin eggs at concentration of 25 µg/mL.\textsuperscript{1,2} These alkaloids possess a unique bispyrroloquinone ring system as a common core structure. In spite of their discovery in 2004-05, no synthesis for these alkaloids has been reported in the literature till date. Considering the fact that zyzzyanones have demonstrated moderate cytotoxic properties and most other alkaloid metabolites isolated from similar sponge species have exhibited a wide range of bioactivities; it is of interest to synthesize this class of compounds and their analogs and further explore their biological activity.

As a part of our ongoing investigations on the synthesis of novel marine alkaloids and their analogs, we have previously published the total synthesis of secobatzelline B\textsuperscript{3} and synthesis and biological evaluations of several analogs of makaluvamines.\textsuperscript{4,5} We have also previously reported a facile synthesis of bispyrroloquinone ring system present in zyzzyanones.\textsuperscript{6} This manuscript describes the first total syntheses of marine alkaloids, zyzzyanones A-D.
The second chapter of this dissertation has laid the foundation for the synthesis of zyzzyanones by standardizing conditions for an oxidative free radical cyclization reaction of 6-aminoindoloe-4,7-quinones with 1,3-dicarbonyl compounds mediated by CAN leading to the formation of a bispyrroloquinone ring system. However, the bispyrroloquinone product (Figure 3) obtained from this reaction did not contain the phenolic ring and the aminoethyl side chain present in zyzzyanones. In addition, bispyrroloquinone product of the CAN mediated cyclization had additional substituents on the ring such as a methyl and ethoxycarbonyl groups that are not present in zyzzyanones (Figure 3). Thus, it was clear that a synthetic modification of this reaction is needed to accomplish the total synthesis of zyzzyanones.

**Figure 3:** Comparison of the product obtained from CAN mediated reaction with zyzzyanone A

Our initial efforts were focused on extending the CAN mediated oxidative free radical cyclization to incorporate a phenolic group to the bispyrroloquinone ring. We searched the literature for other similar reactions that could possibly be used to introduce an aromatic ring to the bispyrroloquinone. Fortunately, we were able to find an oxidative
free radical cyclization reaction of an aminoquinone with acetaldehyde acetal mediated by Mn(OAc)$_3$ which might be an alternative for the CAN mediated oxidative cyclization. There are several literature reports of the use of Mn(OAc)$_3$ as an oxidizing agent in organic reactions.$^7$-$^9$ Use of Mn(OAc)$_3$ as a one electron oxidant is similar to other one electron oxidants such as Co (III), Ce (IV) and some two electron oxidants, Tl (III) and Pb (IV).$^8$ The reaction that is most helpful in forming C-C bond is the one in which Mn(OAc)$_3$ facilitates the formation of an intermediate adduct free radical by reacting with an enolizable compound (electron acceptor) followed by addition of this radical to the substrate (electron donor which is an electron rich carbon-carbon double bond).$^{10}$ Some of the common electron acceptors are $\beta$-dicarbonyl compounds,$^7$-$^9$-$^{12}$ dimedone,$^{13}$ acylacetonitriles,$^{14}$-$^{15}$ methylthioacetanilides,$^{16}$-$^{18}$ alkylsulfonyl and alkylthio substituted aromatic amides,$^{19}$ alkynitroacetates$^{20}$-$^{21}$, nitroketones and nitroamides.$^{22}$ Some of the common electron donors are quinones such as quinolinones and napthoquinones.$^{23}$ Different types of free radical cyclizations with active methylene compounds have been reported by Chuang et al using napthoquinones and benzoquinones as substrates.$^{20}$-$^{24}$-$^{32}$ Interestingly, the use of monocarbonyl compounds as electron acceptors have also been reported. The example of an oxidative free radical reaction which was helpful in our synthetic strategy towards zyzzyanones is the one in which aldehyde acetals are used as electron acceptors and quinone units as electron donors.$^{33}$

**Results and Discussion**

The retrosynthetic analysis of zyzzyanone A is shown in Figure 4. Zyzzyanone A can be prepared from 4-hydroxyphenyl acetaldehyde (33) and the 6-aminoindole-4,7-
quinone derivative 34. 6-aminoindole-4,7-quinone derivative 34 can be prepared from corresponding methoxy derivative 35. Compound 35 can be prepared from the tryptamine derivative 36, which in turn can be prepared from 4,6,7-trimethoxyindole (8). Synthesis of 4,6,7-trimethoxyindole (8) starting from 2,4,5-trimethoxybenzaldehyde (4) is already reported in the literature\textsuperscript{34} and is also described in detail in Chapter 1 of this dissertation.

Figure 4: Retrosynthetic analysis of zyzyzanone A.

Since we have already standardized conditions for an oxidative cyclization of 6-aminoindole-4,7-quinones with carbonyl compounds (chapter 2) using CAN, we first attempted the CAN mediated oxidative cyclization of N-tosyl-6-benzylaminooindole-4,7-quinone (21a) with phenyl acetaldehyde as a model reaction (Figure 5). This reaction did not proceed to form the expected product 37 in spite of increasing the reaction temperature and adding excess reagent. We also tried this reaction in other solvents such as MeOH / CH\textsubscript{2}Cl\textsubscript{2}, EtOH, THF and CH\textsubscript{3}COOH instead of CH\textsubscript{3}CN, without success. All
of these attempted reactions resulted only in the formation of complex mixtures which might be due to the formation of oligomeric compounds formed from the aldehyde under these reaction conditions as observed previously by others.\textsuperscript{35}

**Figure 5:** Attempted oxidative cyclization of 21a with phenyl acetaldehyde.

Failure of this reaction and the formation of the possible oligomeric byproducts indicated that we needed a less reactive or a protected form of the aldehyde as the electron acceptor. Literature has rare instances of using aldehydes as electron acceptors in this type of oxidative cyclization reactions. This may be attributed to the ability of aldehydes to polymerize easily.\textsuperscript{36} However, examples of successful use of acetals, i.e. the protected form of aldehydes in oxidative free radical reactions are reported in the literature.\textsuperscript{24} One such example similar to our system was reported by Shanab \textit{et al}, where 6-aminoazanaphto-quinone (38) was reacted with acetaldehyde acetal in the presence of Mn(OAc)\textsubscript{3} and acetic acid (Figure 6).\textsuperscript{33}

**Figure 6:** Oxidative cyclization reaction of 6-aminoazanaphtoquinone with acetaldehyde diethyl acetal.
Based on this literature report, we modified our model reaction by using N-tosyl-6-benzylaminoindole-4,7-quinone (21a) and 4-hydroxyphenyl acetaldehyde diethyl acetal and Mn(OAc)$_3$. 4-Hydroxyphenyl acetaldehyde diethyl acetal was not commercially available. In order to synthesize this compound we had to synthesize 4-hydroxyphenyl acetaldehyde. This was first attempted by oxidizing tyrosol (40) using PCC/PDC following a reported procedure.$^{37}$ The literature precedence does describe of the aldehyde being unstable and the use of the crude aldehyde without any purification for further steps. However, we did not get good yield of the aldehyde from this reaction. This problem is attributed to the presence of the reactive phenolic hydroxyl present in the molecule.

![Chemical Diagram](image)

**Figure 7**: Synthesis of 4-benzylxyphnyl acetaldehyde (43)

In order to address this problem, the hydroxyl group of tyrosol (40) was protected with a benzyl group to afford 4-benzyloxy tyrosol (41) in 98 % yield (Figure 7). Simultaneous literature search for other oxidation methods for primary alcohols led to the use of the reagent, IBX in DMSO as a favorable alternative to PCC/PDC.$^{38}$ Using this literature procedure, we oxidized 4-benzyloxy tyrosol (41) in the presence of IBX in DMSO to afford the expected aldehyde 42 in 95 % yield. The aldehyde 42 was converted
to acetal 43 by treatment with anhydrous EtOH in the presence of catalytic amount of conc. H₂SO₄ in 90% yield following a similar literature procedure.³⁹

The aminoquinone 21a was then reacted with the acetal 43 in the presence of excess Mn(OAc)₃ in CH₃CN as shown in Figure 8. This reaction worked well to afford the expected cyclized product, 44a in 64% yield. A variation of this reaction using a different aminoquinone 21b in the presence of excess Mn(OAc)₃ in CH₃CN also afforded expected product 44b in 70% yield. An excess of Mn(OAc)₃ was required for the this reaction. Use of 7 equivalents of Mn(OAc)₃ was found to be the optimum for the success of this reaction. Our attempts of this reaction with lesser equivalents of Mn(OAc)₃ (3 and 5 eq) resulted in lower yields of the product.

Figure 8: Oxidative cyclization of 6-benzylaminooindole-4,7-quinones with acetal in the presence of Mn(OAc)₃.

We found that CH₃CN was a best choice of solvent for this reaction compared to more acidic solvents such as CH₃COOH, CF₃CH₂OH and HCOOH. This is presumably
due to slow rate of conversion of acetal to aldehyde in CH$_3$CN which favors the cyclization with quinone as opposed to the formation of polymeric side products.

Based on previous literature reports on the mechanism of similar reactions, we have proposed a possible reaction mechanism for this transformation as outlined in Figure 9.

\[ \text{Figure 9: Proposed reaction mechanism for Mn(OAc)$_3$ mediated oxidative free radical cyclization of aminoquinone 21a with acetal 43.} \]

This reaction could proceed via a free radical oxidative cyclization mechanism.$^{24, 25, 30, 32}$

Literature precedence suggest that oxidative cyclization reactions using Mn(OAc)$_3$ involve the formation of enolate as an intermediate.$^{8, 10}$ Based on the previous literature reports, we propose that the acetal 43 first gets converted to the aldehyde 42.$^{40}$ The
aldehyde 42 undergoes a single electron transfer in the presence of Mn(OAc)₃ to form the free radical 45. This radical undergoes an addition to the aminoquinone (21a) resulting in the formation of second radical 46, which loses another electron in presence of Mn(OAc)₃ to form cationic intermediate 47. Loss of proton from 47 regenerates the quinone 48. The lone pair on N-atom in 48 attacks the aldehyde carbon resulting into cyclized intermediate 49. A proton transfer occurs in 49 to form 50, which loses a water molecule to afford the product 44a.

A second mechanistic pathway bypassing the formation of enolate can also be proposed as shown in Figure 10.

**Figure 10:** Alternate reaction mechanism proposed for Mn(OAc)₃ mediated oxidative free radical cyclization of aminoquinone 21a with acetal 43.
This pathway involves the formation of the radical from the acetal instead of the aldehyde. According to this mechanism the acetal 43 loses an electron to Mn(OAc)$_3$ to form a radical intermediate 51, which adds to the quinone 21a to form radical intermediate 52. Intermediate radical 52 loses another electron to Mn(OAc)$_3$ to form cationic intermediate 53. The quinone intermediate 54 is formed by the loss of proton from 53. The lone pair on N-atom in 54 attacks the acetal carbon resulting in the formation of cyclized intermediate 55. Intermediate 55 loses a proton to form 56. Compound 56 undergoes elimination of an EtOH molecule to form the product 44a.

After the successful completion of the cyclization reaction, we wanted to demonstrate that the protecting groups present in the product 44a could be removed without affecting the quinone moiety. This is essential because such deprotection reactions will be needed during the total synthesis of zyzzyanones. There are three protecting groups in this molecule namely, N-tosyl, N-benzyl and O-benzyl.

<table>
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<th>Exp No</th>
<th>Reagent (equiv)</th>
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<th>Time</th>
<th>Temp</th>
<th>Yield</th>
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<td>MeOH</td>
<td>1 h</td>
<td>25°C</td>
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<tr>
<td>2</td>
<td>NaN$_3$ (1.2 eq)</td>
<td>DMF</td>
<td>4 h</td>
<td>25°C</td>
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**Figure 11:** Detosylation of compound 44a
N-Tosyl group present in compound 44a was removed first by treatment with NaN₃ and DMF at room temperature for 4 hours to afford the product 57 in 75% yield. Alternatively, the detosylation was carried out using NaOMe in MeOH at room temperature for an hour resulted in the formation of 57 in a similar yield. These reactions are outlined in Figure 11.

Both N-benzyl and O-benzyl groups present in 57 were removed in one step. Debenzylation reaction of compound 57 was first attempted using 10% Pd/C and HCOONH₄ in EtOH under reflux conditions. This debenzylation reaction did not go to completion and resulted only in O-debenzylation. In order to achieve debenzylation of both O- and N-benzyl groups a stronger catalyst such as Pd black was needed. So, we attempted this transfer hydrogenolysis of compound 57 using Pd black and HCOONH₄ in EtOH. This reaction worked well and resulted in the removal of both O- and N-benzyl groups in one pot to afford the compound 58 in 50% yield as shown in Figure 12.

![Figure 12: Debenzylation of compound 57](image)

After working out the model reaction conditions we were ready to apply this methodology to synthesize zyzzyanone A. Since zyzzyanone A contains a 2-(N-methyl)amino ethyl side chain, it is appropriate to start our synthesis with an aminoquinone resembling compound 34 described in the retrosynthetic scheme of
zyzzyanone A (Figure 4). One such aminoquinone 63 was synthesized from 4,6,7-trimethoxyindole (8) as outlined in Figure 13.

![Chemical reaction diagram]

**Figure 13**: Synthesis of aminoquinone 63

The synthesis begins with treatment of previously reported 4,6,7-trimethoxyindole (8) with oxalyl chloride in anhydrous ether at 0°C followed by the treatment with N-methylbenzylamine in ether at room temperature to form oxoacetamide 59 in 75% yield. The reduction of carbonyl groups present in compound 59 using LiAlH₄ in anhydrous ether under reflux conditions resulted in the formation of tryptamine derivative 60 in 95% yield. The indole N-atom of the compound 60 was then protected with a tosyl group by treatment with Ts₂O in the presence of KH in anhydrous THF to afford the N-tosyl derivative 61 in 69% yield. Compound 61 was then oxidized to corresponding quinone 62 by treatment with CAN in the presence of Bu₄NHSO₄ water at
room temperature in 80% yield. The quinone 62 was aminated with benzyl amine in MeOH and THF (1:1) to furnish the aminoquinone intermediate 63 in 75% yield.

With the aminoquinone 63 in hand we were ready to attempt the cyclization reaction with acetal expecting the formation of the zyzzyanone A ring system. So, compound 63 was reacted with the acetal 43 in the presence of Mn(OAc)$_3$ in CH$_3$CN under reflux conditions as shown in Figure 14. Progress of the reaction was monitored by TLC which showed a number of overlapping spots. To our surprise, we discovered that the expected cyclization did not occur at all.

![Figure 14: Attempted oxidative cyclization of compound 63 with acetal 43](image)

Work up of the reaction and attempted purification of the product did not result in any single product in pure form. The failure of this reaction could be attributed to the reactive aminoalkyl side chain that is present in compound 63. This side chain amine is less sterically hindered and is more nucleophilic compared to the benzylamino NH group. For this reason, the side amine could be reacting with the acetal preferentially forming unexpected side products in this reaction. So, we decided to modify the quinone precursor of this reaction with a side chain containing less reactive carbamate group (compound 66). Compound 66 was prepared from 6-methoxy-3-(2-methylaminoethyl)-1H-indole-4,7-dione (14), which in turn can be synthesized from 4,6,7-trimethoxy indole (8).
Synthesis of compound $14$ is reported previously and described in detail in Chapter 1 of this dissertation. Conversion of compound $14$ to compound $66$ and its oxidative cyclization with acetal $43$ is given in Figure 15.

![Synthesis diagram]

**Figure 15**: Synthesis of compound $66$

Compound $14$ was reacted with benzyl amine in THF and MeOH (1:1) at room temperature to yield the aminated compound $65$ in $78\%$ yield. Reaction of compound $65$ with acetal $43$ in the presence of Mn(OAc)$_3$ in CH$_3$CN under reflux conditions worked well to afford the cyclized product $66$ in $74\%$ yield. This reaction was a major breakthrough in zyzyyanone synthesis as it allowed us to introduce both the aromatic ring and the aminoethyl side chain to the bispyrroloquinone ring system in one step. In addition, this allowed us to prepare all four zyzyyanones from this intermediate $66$ in a few steps.
Zyzzyanones A and B contain a N-Me group in the side chain. So it is apparent that we introduce an N-Me group in the side chain of compound 66. With this objective, we attempted N-methylation reaction on compound 66 under different conditions. Our first attempt of N-methylation was by using moist CH₃I in the presence of Ag₂O, following a literature procedure for similar methylation. This reaction did not work and resulted only in the recovery of starting materials even after stirring at room temperature for 20 hours. Higher temperatures resulted in the formation of complex mixtures. Next attempt of methylation of compound 66 was by using CH₃I in the presence of NaH in THF. This reaction also did not work well and resulted in formation of complex mixtures at higher temperatures. Then we attempted the methylation using MeI in the presence of NaH in DMF at room temperature for 24 hours as shown in Figure 16. This reaction yielded a mixture of two products 67 and 68 in 46 % and 24 % yields, respectively. The major product 67 was the expected side chain N-methylated compound and the unexpected minor product 68 is a dimethyl derivative. The formation of the intermediate 68 can be attributed to the use of excess NaH and absence of absolutely anhydrous conditions during the reaction. During the prolonged reaction times, the expected product 67 formed first underwent detosylation and a second methylation occurred on the pyrrole N-atom to form the product 68. OH or I⁻ can both act as nucleophiles for removal of tosyl group. Even though, compound 68 was an unexpected side product of the reaction, it was a useful compound for our synthesis as it served as a precursor for the synthesis of zyzzyanone A. Compound 67 served as a precursor for the synthesis of zyzzyanone B.
Figure 16: N-Methylation of compound 66

Conversion of compound 68 to zyzzyanone A

Conversion of compound 68 to zyzzyanone A is outlined in Figure 17. In order to convert compound 68 to zyzzyanone A, we needed to remove N-benzyl, O-benzyl and N-BOC protecting groups from 68. Treatment of compound 68 with HCOONH$_4$ and Pd-black in EtOH resulted in the removal of both O- and N-benzyl groups in one step to form the compound 69 in 45% yield. Deprotection of the Boc group from compound 69 by treatment with a 1:1 mixture of TFA and CH$_2$Cl$_2$ for 2 hours resulted in zyzzyanone A in 80% yield. The spectral data of synthetic zyzzyanone A was found to be in good agreement to the values reported for this natural product.$^1$
Figure 17: Conversion of compound 68 to zyzzyanone A

Conversion of compound 67 to zyzzyanone B

The next attempt was to synthesize zyzzyanone B from compound 67. The chemistry is outlined in Figure 18. In order to convert compound 67 to zyzzyanone B, we needed to remove N-benzyl, O-benzyl, N-tosyl and N-Boc protecting groups from the compound 67. Compound 67 was detosylated by treatment with NaN₃ in DMF for 4 hours to obtain detosylated product 70 in 74% yield. Compound 70 was then debenzylated using Pd black, HCOONH₄ in EtOH to obtain debenzylated compound 71 in 47% yield. Removal of the Boc group from compound 71 using 1:1 mixture of TFA and CH₂Cl₂ at room temperature for 2 hours resulted in the formation of zyzzyanone B in 87% yield. The spectral data of synthetic zyzzyanone B was found to be exactly identical to the values reported for this natural product.²
Next objective in the synthesis was to synthesize zyzzyanones C and D starting from compounds 67 and 68. Both these alkaloids contained N-formyl groups in the side chain. So, it was important to standardize conditions for the N-formylation of the side chain N-atom of compounds 67 and 68. Both compounds 67 and 68 contained N-Boc groups in the side chain. So, the Boc groups were removed from these compounds before the formylation reactions were carried out. Compound 68 was first treated with TFA/CH$_2$Cl$_2$ to remove the Boc group and the crude product thus obtained was subjected to formylation reactions without further purification. N-formylations have been reported in literature using HCOOH/Ac$_2$O. Following this procedure, we first attempted N-formylation of compound 68 after the removal of the Boc group using HCOOH and Ac$_2$O.
at room temperature and under reflux conditions. At room temperature, reaction for 36 hours mostly resulted in the recovery of starting material. However, the same reaction at reflux condition for 15 hours resulted in a number of side reactions along with about 5% of expected product in isolated yield. Further literature search revealed an alternate condition using trifluoroethyl formate, Et$_3$N and ether for N-formylation.\textsuperscript{43} The formylation of compound 68 after the removal of Boc group, using trifluoroethyl formate, Et$_3$N resulted in an improved yield to 40% of the product. Reactions in which the use of HCOOEt as a formylation agent have also been reported in literature.\textsuperscript{44} This is what has worked well in our hands. Formylation of compound 68 after the removal of Boc group using HCOOEt and Et$_3$N at reflux conditions resulted in the formation of N-formylated product 73 in 72% yield (Figure 19).

Conversion of compound 68 to zyzzyanone C

Conversion of compound 68 to zyzzyanone C is given in Figure 19. The Boc protecting group present in the side chain of compound 68 was first removed by the treatment with a 1:1 mixture of TFA and CH$_2$Cl$_2$ to afford the crude product 72 which was subjected to formylation using HCOOEt in the presence of Et$_3$N under reflux conditions for 24 hours to afford the N-formyl compound 73 in 72% yield. Removal of both O- and N-benzyl groups present in compound 73 using Pd black and HCOONH$_4$ in refluxing EtOH afforded zyzzyanone C in 30% yield. The spectral data of synthetic zyzzyanone C was found to be in good agreement to the values reported for this natural product.\textsuperscript{2}
Figure 19: Synthesis of zyzyanone C.

Conversion of compound 67 to zyzyanone D

Conversion of compound 67 to zyzyanone D is shown in Figure 20. As in the synthesis of zyzyanone C, compound 67 was treated with a 1:1 mixture of TFA and CH$_2$Cl$_2$ to remove the Boc group to obtain compound 74. The crude product 74, thus obtained was subjected to formylation using HCOOEt in the presence of Et$_3$N under reflux conditions. This reaction resulted in the expected formylation along with detosylation to afford the compound 75 in 67 % yield. Since HCOOEt was used as a formylating agent the ethoxide ion is the leaving group in the formylation reaction. Ethoxide ion is also an excellent reagent for N-detosylation. This could possibly be the
reason for the detosylation observed in this reaction. Both N-benzyl and O-benzyl groups present in compound 75 were removed by treatment with Pd-black and HCOONH$_4$ in EtOH under reflux conditions to afford zyzzyanone D in 42% yield. The spectral data of synthetic zyzzyanone D was found to be a good match to the values reported for this natural product.$^2$

Figure 20: Synthesis of zyzzyanone D

Conclusions

In conclusion, the first total synthesis of zyzzyanones A-D starting from a 6-benzylamino indole-4,7-quinone derivative is accomplished. The key step of the synthesis is the construction of a pyrrole ring by a Mn(OAc)$_3$ mediated oxidative free radical cyclization of 6-benzylamino indole-4,7-quinone derivative with 4-
benzyloxyphenyl acetaldehyde diethyl acetal in CH$_3$CN. All of the intermediate compounds and the final natural products were characterized by $^1$H-NMR, $^{13}$C-NMR, MS and HRMS data. The spectral data of synthetic zyzzyanones were found to be matching well to the values reported for these natural products. In addition to synthesizing zyzzyanones, this synthetic methodology will be useful for the synthesis of other marine alkaloids such as tsitsikammamines and wakayin. This methodology will also be useful in the synthesis of various analogs of these alkaloids with specific substitution patterns. This will eventually assist in the structure activity relationship studies and optimization of the lead molecules derived from these natural products.

**Experimental**

**General Considerations:** Solvent evaporations were carried out *in vacuo* with rotary evaporator. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator (Whatmann, silica gel, UV254, 25 μm plates). Spots were visualized by UV light (254 and 365 nm). Purification by column and flash chromatography was carried out using ‘Dynamic Adsorbents’ silica gel (18-32 μM) in the solvent systems indicated. The amount (weight) of silica gel for column chromatography was in the range of 50-100 times the amount (weight) of the crude compounds being separated. Proton nuclear magnetic resonance ($^1$H-NMR) and carbon nuclear magnetic resonance ($^{13}$C-NMR) spectra were recorded on a Bruker ARX 400 or Bruker DPX-300 spectrometer using TMS as internal standard. The values of chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. The chemical shift values are reported as parts per million (ppm) relative to tetramethylsilane as internal standard. Mass spectra were recorded on
Micromass Platform LCC instrument. Anhydrous solvents used for reactions were purchased in Sure-Seal™ bottles from Aldrich Chemical Company. Other reagents were purchased from Aldrich, Acros or Fisher chemical companies and used as received. Spectral data with extra signals in parentheses are indicative of inseparable geometric isomers, observed in compounds (59), (74), (75), zyzzyanones C and D. Zyzzyanones A-B were isolated as TFA salts.

2-[4-(Benzyloxy)phenyl]ethanol (41)

To a solution of 2-(4-hydroxyphenyl) ethanol, 40 (5.00 g, 36.2 mmol) in anhydrous DMF (20 mL), K₂CO₃ (15.0 g, 109 mmol) was added and the reaction mixture was stirred at room temperature for 30 minutes. Benzyl bromide (6.24 g, 36.5 mmol) was added to this reaction mixture and stirred for 4 hours at room temperature. The TLC examination (EtOAc / hexanes, 1:3) showed the completion of the reaction. The reaction mixture was quenched with water (200 mL) and EtOAc (200 mL) was added. The organic layer was separated and the aqueous layer was extracted with EtOAc (100 mL). The combined EtOAc extract was washed with water (3 × 100 mL), brine (1 × 100 mL) and dried over anhydrous Na₂SO₄. The drying agent was filtered off and solvent was evaporated off under reduced pressure to obtain the compound 41 as a white solid (8.00 g, 97%). The ¹H and ¹³C NMR data matched well with those reported in literature.⁴⁵ ¹H NMR (CDCl₃) δ 2.78 (t, 2H, J = 6.4 Hz), 3.78 (t, 2H, J = 6.4 Hz), 5.03 (s, 2H), 6.92 (d, 2H, J = 8.4 Hz), 7.12 (d, 2H, J = 8.4 Hz), 7.35-7.45 (m, 5H); ¹³C NMR δ 38.7, 64.2, 70.5, 115.4(2C), 127.9(2C), 128.4, 129.0(2C), 130.5(2C), 131.2, 137.5, 157.9 and MS (ES⁺) m/z 227 (M-H).
2-[4-(Benzyloxy)phenyl]ethanal (42)

To a solution of compound 41 (7.0 g, 31 mmol) in DMSO (15 mL), IBX (13 g, 46 mmol) was added and suspension was stirred for 3 hours at room temperature. TLC examination (EtOAc / hexanes, 1:3) indicated completion of the reaction. EtOAc (350 mL) was added to the reaction mixture. The insolubles were filtered off under suction. The EtOAc layer was washed with water (3 × 150 mL), brine (1 × 150 mL) and dried over anhydrous Na₂SO₄. After the removal of the drying agent, the solvent was evaporated under reduced pressure to obtain the compound 42 (6.6 g, 95%) was characterized by proton and ¹³C NMR. The proton NMR matched well with the one reported in literature.³⁸ ¹H NMR (CDCl₃) δ 3.63 (d, 2H, J = 2.1 Hz), 5.06 (s, 2H), 6.98 (d, 2H, J = 8.4 Hz), 7.13 (d, 2H, J = 8.4 Hz), 7.30-7.60 (m, 5H), 9.72(t, 1H, J = 2.1 Hz); ¹³C NMR δ 49.7, 70.0, 115.3(2C), 124.0, 127.5(2C), 128.0, 128.6(2C), 130.7(2C), 136.8, 158.1, 199.8 and MS (ES⁺) m/z 265 (M+H), 263 (M-H).

1-[4-(2,2-Diethoxyethyl)phenoxy]methylbenzene (43)

To a solution of compound 42 (6.00 g, 26.4 mmol) in anhydrous EtOH (100 mL), conc. H₂SO₄ (0.100 g, 1.02 mmol) was added and the reaction mixture was stirred for 3 hours. The TLC examination (EtOAc / hexanes, 1:1) showed completion of reaction. The reaction mixture was neutralized with saturated NaHCO₃. The solvent was evaporated under reduced pressure and the residual slurry was partitioned between EtOAc (250 mL) and water (250 mL). The aqueous layer was drained off and the organic layer was washed with water (3 × 100 mL), brine (1 × 100 mL) and dried over anhydrous Na₂SO₄. The drying agent was removed and the solvent was evaporated off to obtain the crude.
product, which was purified by flash column chromatography over Si gel using EtOAc / hexanes (1:10) to obtain pure acetal 43 (7.18 g, 90%); \(^1\)H NMR (CDCl\(_3\)) δ 1.23 (t, 6H, J = 7.2 Hz), 2.92 (d, 2H, J = 5.7 Hz), 3.45-3.55 (m, 2H), 3.70-3.80 (m, 2H), 4.64 (t, 1H, J = 5.7 Hz), 5.09 (s, 2H), 6.96 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.4 Hz), 7.30-7.70 (m, 5H); \(^1^3\)C NMR δ 15.3( 2C), 40.0, 61.9(2C), 70.0, 104.0, 114.6(2C), 127.5 (2C), 127.9, 128.6(2C), 129.7, 130.6 (2C), 137.2, 157.4; and MS (ES\(^+\)) m/z 323 (M + Na).

1-Benzyl-3-(4-benzylxyphenyl)-7-tosyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (44a).

To a solution of N-tosyl-6-(benzylamino)-1H-indole-4,7-dione, 21a (0.100 g, 0.240 mmol) in CH\(_3\)CN (50 mL), 4-benzylxyphenyl acetaldehyde diethyl acetal, 43 (0.500 g, 1.80 mmol) and Mn(OAc)\(_3\) (0.462 g, 1.80 mmol) were added and the reaction mixture was refluxed for 40 hours. TLC examination (EtOAc / hexanes, 1:1) revealed that the reaction was complete. The reaction mixture was then allowed to attain room temperature and the solvent was evaporated off in vacuo. The residue obtained was then dissolved in EtOAc (50 mL), washed with a saturated solution of NaHSO\(_3\) (1 × 20 mL), water (2 × 20 mL), brine (1 × 20 mL) and dried over anhydrous Na\(_2\)SO\(_4\). Removal of solvent from the dried extract afforded the crude product which was purified by column chromatography over Si gel using EtOAc/hexanes (1:3) as eluent to afford the pure product 44a as a red solid (0.120 g, 80%); Mp 164-165°C; \(^1\)H NMR (CDCl\(_3\)) δ 2.42 (s, 3H), 5.07 (s, 2H), 5.57 (s, 2H), 6.72 (d, 1H, J = 3.3 Hz), 6.80 (s, 1H), 6.96 (d, 2H, J = 7.8 Hz), 7.15 -7.26 (m ,2H), 7.27 – 7.48 (m, 10H), 7.54 (d, 2H, J = 7.8 Hz), 7.71 (d, 1H, J = 3.3 Hz), 7.98 (d, 2H, J = 8.4 Hz); \(^1^3\)C NMR (CDCl\(_3\)) δ 21.8, 52.4, 70.1, 108.4, 114.5(2C), 122.7, 125.1, 126.6, 127.5(2C), 128.0(2C), 128.2, 128.3(2C), 128.7(2C), 150
128.8(2C), 129.1(2C), 129.5(2C), 130.1(2C), 130.5, 130.6, 132.4, 133.6, 134.4, 136.2, 137.1, 145.8, 158.7, 167.3, 178.7; and HRMS calcd for C_{37}H_{28}N_{2}O_{5}S, [M]^{+}: 612.1719, found 612.1702.

3-(4-Benzylxoyophenyl)-1-(4-methoxybenzyl)-7-tosyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (44b).

Following a procedure similar to synthesis of 44a, the aminoquinone 21b (0.160 g, 0.370 mmol) was reacted with acetal 43 (0.771 g, 2.51 mmol) and Mn(OAc)$_3$ (0.688 g, 2.51 mmol) in CH$_3$CN (80 mL) to obtain compound 44b (0.168 g, 70%); $^1$H NMR (CDCl$_3$) $\delta$ 2.43 (s, 3H), 3.79 (s, 3H), 5.07 (s, 2H), 5.48 (s, 2H), 6.74 (s, 1H), 6.77 (s, 1H), 6.81 (d, 2H, $J = 7.8$ Hz), 6.95 (d, 2H, $J = 7.8$ Hz), 7.18 (d, 2H, $J = 7.8$ Hz), 7.20 – 7.50 (m, 7H), 7.53 (d, 2H, $J = 8.1$ Hz), 7.71 (s, 1H), 8.01 (d, 2H, $J = 7.8$ Hz); $^{13}$C NMR (CDCl$_3$) $\delta$ 21.8, 52.0, 55.3, 70.1, 108.3, 114.5(2C), 122.7, 125.2, 126.5, 127.5(2C), 128.0(2C), 128.2(2C), 128.6(2C), 129.1(2C), 129.4, 129.5(2C), 129.7(2C) 130.0(2C), 130.4, 130.6, 133.5, 134.5, 136.2, 137.1, 145.8, 158.6, 159.6, 167.3, 178.7 and HRMS calcd for C$_{38}$H$_{30}$N$_2$O$_6$S, [M]$^+$: 642.1825, found 642.1812.

1-Benzyl-3-(4-benzylxoyophenyl)-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (57)

**Method A:** To a solution of compound 44a (0.05 g, 0.08 mmol) in anhydrous MeOH (5 mL), NaOMe (0.044 g, 0.17 mmol) was added and the reaction mixture was stirred at room temperature for 45 min. TLC examination (CHCl$_3$ / EtOAc, 9:1) revealed that the reaction is complete. The reaction mixture was then quenched with water (5 mL), and the solvent was evaporated off in vacuo. The resulting aqueous layer was extracted.
with EtOAc (2 × 10 mL). The EtOAc extract was washed with water (3 × 5 mL), brine (1 × 5 mL) and dried over anhydrous Na₂SO₄. Removal of solvent from the dried extract afforded the crude product which was purified by column chromatography over Si gel using EtOAc/hexanes (1:4) as eluent to yield the pure product, 57 (0.028 g, 75%).

**Method B:** Alternatively, NaN₃ (0.0040 g, 0.050 mmol) was added to compound 44a (0.025 g, 0.040 mmol) in DMF (1 mL), and the reaction mixture was stirred for 4 hours at room temperature. The reaction was quenched with water and extracted with EtOAc (2 × 10 mL). The extract was washed with water (3 × 5 mL), brine (1 × 5 mL) and dried over anhydrous Na₂SO₄. The drying agent was filtered off and solvent was evaporated to afford the crude product which was purified by column chromatography on Si gel using EtOAc / hexanes (1:4) to yield compound 57. (0.015 g, 74%); Mp 236-237°C; ¹H NMR (CDCl₃) δ 5.10 (s, 2H), 5.64 (s, 2H), 6.62 (t, 1H, J = 2.7 Hz), 6.87 (s, 1H), 6.97 (d, 2H, J = 9.0 Hz), 7.30-7.50 (m, 11H), 7.60 (d, 2H, J = 9.0 Hz), 9.53 (bs, 1H); ¹³C NMR (CDCl₃) δ 52.3, 70.1, 108.8, 114.4(2C), 124.3, 124.4, 125.4, 127.3, 127.4(2C), 127.5(2C), 128.0, 128.1(2C), 128.4, 128.6(2C), 128.9(2C), 130.2(2C), 130.3, 132.1, 136.4, 137.1, 158.5, 169.6, 179.6; MS (ES⁺) m/z 459 (M+H); and HRMS calcd for C₃₀H₂₂N₂O₃ [M⁺]: 458.1630, found 458.1646.

**3-(4-Hydroxyphenyl)-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (58)**

To a solution of compound 57 (0.015 g, 0.020 mmol) in anhydrous EtOH (15 mL), HCOONH₄ (0.050 g, 0.80 mmol) and Pd black (0.030 g, 0.30 mmol) were added and the reaction mixture was refluxed for 15 hours. TLC examination (CHCl₃ / MeOH, 20:1) revealed that the reaction was complete. The reaction mixture was then allowed to
attain room temperature and filtered through a pad of celite 545 and washed with EtOAc/CHCl₃ (1:1). The combined filtrate and washings were evaporated in vacuo. The residue obtained was dissolved in EtOAc (10 mL) and washed with water (2 × 5 mL), brine (1 × 5 mL) and dried over anhydrous Na₂SO₄. The drying agent was removed by filtration and the solvent was evaporated off in vacuo to obtain the crude product which was purified by column chromatography over Si gel using MeOH/CHCl₃ (1:25) as eluent to furnish the pure product 58 (0.0040 g, 50%); ¹H NMR (CD₃OD) δ 6.54 (d, 1H, J = 2.7 Hz), 6.79 (d, 2H, J = 8.7 Hz), 7.01 (d, 1H, J = 2.7 Hz), 7.04 (s, 1H), 7.55 (d, 2H, J = 8.7 Hz); ¹³C NMR (CD₃OD) δ 109.3, 115.6(2C), 123.3, 124.6, 126.1, 126.2, 129.2, 130.0, 131.1(2C), 132.9, 134.9, 157.9, 169.8, 182.5; MS (ES⁺) m/z 279 (M + H), 277 (M-H); and HRMS calcd for C₁₆H₁₀N₂O₃ [M⁺] 278.0691, found 278.0700.

**N-Benzyl-2-(4,6,7-trimethoxy-1H-indol-3-yl)-N-methyl-2-oxoacetamide (59)**

A solution of 4,6,7-trimethoxyindole, 8 (2.00 g, 9.70 mmol) in anhydrous ether (50 mL) under N₂ atmosphere was cooled to 0°C. A solution of oxalyl chloride (1.84 g, 14.5 mmol) in the same solvent (25 mL) was added to this over a period of 1 hour. After being stirred for 1 hour at 0 °C, the reaction mixture was warmed to room temperature and stirred for further 3 hours. N-methylbenzylamine (4.09 g, 33.8 mmol) was added to this over a period of 40 minutes, and the resulting mixture was stirred for an additional 15 hours at 25°C. TLC (EtOAc / hexanes, 1:1) was examined which revealed completion of the reaction. The precipitated product was filtered off and washed with ether (100 mL). The residue was redissolved in CHCl₃ (300 mL), washed with 2N HCl (3 × 100 mL), water (2 × 100 mL), brine (1 × 100 mL) and dried over anhydrous Na₂SO₄. Drying agent
was filtered off and solvent was evaporated under reduced pressure to obtain the crude product, which was purified by column chromatography over Si gel using EtOAc/ CHCl₃ (1:4) to obtain pure compound 59 as a bright yellow solid. (2.80 g, 75%); ¹H NMR (CDCl₃) δ 2.97 (s, 3H), 3.87(3.89) (s, 3H), 3.93 (s, 3H), 3.94(s, 3H), 4.52(4.72) (s, 2H), 6.42 (s, 1H), 7.25–7.40 (m, 5H), 7.86(7.94) (d, 1H, J = 2.8 Hz) and 8.90 (bs, 1H); ¹³C NMR (CDCl₃) δ 31.4(35.0), 50.1(53.9), 56.5(56.53), 57.41(57.43), 61.3, 93.7(93.8), 110.1(110.2), 116.2(116.3), 127.6, 127.9, 128.2(128.3)(2C), 128.6(128.8)(2C), 128.9(129.0), 132.51(132.54), 133.9(134.1), 135.8, 136.4, 148.3(148.4), 149.8(150.0), 168.8(169.0), 186.2(186.5) and MS (ES⁺) m/z 383 (M + H).

N-Benzyl-2-(4,6,7-trimethoxy-1H-indol-3-yl)-N-methylethanamine (60)

To a solution of glyoxamide 59 (1.32 g, 3.50 mmol) in anhydrous THF (150 mL) maintained under N₂, LiAlH₄ (1.31 g, 34.5 mmol) was slowly added and stirred at room temperature for about 20 minutes. The reaction mixture was then refluxed for 18 hours. TLC examination (EtOAc / CHCl₃, 1:1) indicated completion of the reaction. Reaction mixture was cooled to 0°C using an ice bath, following which a saturated Na₂SO₄ solution was slowly added to destroy excess LiAlH₄. Inorganic salts were allowed to settle down and removed by filtration through celite 585. The filtrate was concentrated under reduced pressure, and the residue obtained was dissolved in CH₂Cl₂ (100 mL), washed with water (3 × 50 mL), brine (1 × 50 mL) and dried over anhydrous Na₂SO₄. Removal of solvent from the dried and filtered extract furnished the pure product 60 as thick oil (1.10 g, 91%); ¹H NMR (CDCl₃) δ 2.20(2.32) (s, 3H), 2.65–2.79 (m, 2H), 2.98–3.10 (m, 2H), 3.50 (3.59) (s, 2H), 3.80 (s, 3H), 3.89 (s, 3H), 3.91 (s, 3H), 6.20 (s, 1H),
6.77 (s, 1H), 7.15–7.40 (m, 5H) and 8.13 (bs, 1H); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 24.6, 42.3, 55.3, 58.0, 59.3, 61.0, 62.4, 90.2, 113.8, 115.4, 120.2, 126.9, 128.2(2C), 129.2(2C), 131.9, 139.4, 146.9 and 150.3 and MS (ES\(^{+}\)) \(m/z\) 355 (M + H).

**N-Benzyl-2-(4,6,7-trimethoxy-1-tosyl-1H-indol-3-yl)-N-methylethanamine (61)**

A solution of compound 60 (0.480 g, 1.35 mmol) in anhydrous THF (25 mL) was added to a suspension of KH (30% weight dispersion in mineral oil) (0.757 g, 137 mmol) in the same solvent (25 mL) maintained under N\(_2\) dropwise over a period of 45 minutes. The mixture was stirred for 90 minutes at room temperature and then cooled to 0 °C. A solution of Ts\(_2\)O (0.644 g, 1.97 mmol) in THF (50 mL) was slowly added to this, and the reaction mixture was further stirred for 1 hour at 0°C and an additional 18 hours at room temperature. TLC examination (EtOAc / CHCl\(_3\), 1:1) at this stage revealed completion of the reaction. Excess KH was destroyed by very slow addition of water at 0 °C. The solvent was then completely removed and the residue was partitioned between water (250 mL) and CH\(_2\)Cl\(_2\) (250 mL). The CH\(_2\)Cl\(_2\) layer was washed with water (3 × 100 mL), brine (1 × 100 mL) and dried over anhydrous Na\(_2\)SO\(_4\). The solvent was evaporated off and the residue obtained was purified by column chromatography over Si gel using EtOAc/CHCl\(_3\) (1:10) as eluent to furnish compound 61 (0.600 g, 86%); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 2.28 (s, 3H), 2.33 (s, 3H), 2.71 (t, 2H, \(J = 7.2\) Hz), 3.00 (t, 2H, \(J = 7.2\) Hz), 3.57 (s, 2H), 3.72 (s, 3H), 3.74 (s, 3H), 3.81 (s, 3H), 6.29 (s, 1H), 7.16 (d, 2H, \(J = 8.4\) Hz), 7.20–7.35 (m, 5H), 7.42 (s, 1H) and 7.72 (d, 2H, \(J = 8.4\) Hz); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 21.4, 24.6, 42.1, 55.3, 56.8, 57.8, 60.5, 62.3, 92.6, 116.3, 118.9, 123.8, 126.8, 127.1(2C), 139.4,
128.1(2C), 128.9, 129.4, 129.7, 130.8, 136.8, 139.1, 143.8, 149.6 and 150.4 and MS (ES$^+$) m/z 509 (M + H).

3-(2-(N-Benzyl-N-methylamino)ethyl)-6-methoxy-1-tosyl-1H-indole-4,7-dione (62)

To a solution of compound 61 (0.420 g, 0.820 mmol) in CH$_2$Cl$_2$ (50 mL), Bu$_4$NHSO$_4$ (0.559 g, 1.64 mmol) was added and the resulting mixture was stirred for 5 min. CAN (0.904 g, 1.64 mmol) was added, and the suspension was stirred for 5 minutes. One drop of water was added, and the reaction mixture was stirred for an additional 5 min. After every 5 minutes a drop of water was added, and the progress of the reaction was monitored by TLC (MeOH / CHCl$_3$, 1:20). It required four drops of water and 60 minutes of stirring for the reaction to go to completion. The reaction mass was diluted with water (100 mL) and CH$_2$Cl$_2$ (150 mL). The water layer was separated and the organic layer was washed with water (2 × 50 ml), brine (1 × 50 mL) and dried over anhydrous Na$_2$SO$_4$. The solvent was evaporated off after filtering off the drying agent and the residue obtained was purified using column chromatography over Si gel using MeOH / CHCl$_3$ (1:100 ) to yield compound 62 as an oil (0.290 g, 74%); $^1$H NMR (CDCl$_3$) δ 2.32 (s, 3H), 2.42 (s, 3H), 2.69 (t, 2H, $J = 6.9$ Hz), 2.99 (t, 2H, $J = 6.9$ Hz), 3.59 (s, 2H), 3.77 (s, 3H), 5.66 (s, 1H), 7.18-7.30 (m, 5H), 7.34 (d, 2H, $J = 8.4$ Hz), 7.73 (s, 1H), 8.05 (d, 2H, $J = 8.4$ Hz); $^{13}$C NMR (CDCl$_3$) δ 21.8, 23.1, 42.0, 56.1, 56.7, 62.2, 106.7, 124.3, 127.1, 127.4, 128.3(2C), 128.7, 129.1 (2C), 129.3(2C),129.5, 129.6, 129.7(2C), 133.9, 146.1, 159.5, 169.1, 183.9 and MS (ES$^+$) m/z 479 (M+H).
3-(2-(N-Benzyl-N-methylamino)ethyl)-6-(benzylamino)-1-tosyl-1H-indole-4,7-dione (63)

Quinone 62 (0.28 g, 0.58 mmol) was dissolved in MeOH and THF (1:1, 50 mL). A solution of benzyl amine (0.093 g, 0.88 mmol) in MeOH was added and the reaction mixture was stirred at room temperature for 23 hours. TLC examination (MeOH / CHCl₃, 1:10) revealed completion of the reaction. The solvent was evaporated off under reduced pressure. The residue obtained was purified by column chromatography over Si gel using MeOH/CHCl₃ (1:30) as eluent to afford compound 63 (0.25 g, 78%); ¹H NMR (CDCl₃) δ 2.28 (s, 3H), 2.43 (s, 3H), 2.68 (t, 2H, J = 7.2 Hz), 3.01 (t, 2H, J = 7.2 Hz), 3.56 (s, 2H), 4.22 (d, 2H, J = 5.4 Hz), 5.26 (s, 1H), 5.97 (bt, 1H), 7.10-7.40 (m, 12H), 7.38 (s, 1H), 7.72 (s, 1H), 7.97 (d, 2H, J = 8.4 Hz); ¹³C NMR (CDCl₃) δ 21.8, 23.1, 42.3, 47.2, 56.4, 62.2, 98.2, 125.1, 127.0, 127.9 (2C), 128.0, 128.2, 128.4(2C), 129.0(2C), 129.1(2C), 129.2(2C), 129.8(2C), 130.3, 130.8, 134.4, 136.0, 139.3, 146.0, 147.5, 170.3, 183.3 and MS (ES⁺) m/z 554 (M+H).

tert-Butyl-2-(6-(benzylamino)-4,7-dihydro-4,7-dioxo-1-tosyl-1H-indol-3-yl)ethylcarbamate (65)

To a solution of quinone, 14 (0.77 g, 1.6 mmol) in MeOH/THF (1:1, 150 mL), benzyl amine (0.26 g, 2.5 mmol) was added and the reaction mixture was stirred at room temperature for 20 hours. TLC examination (EtOAc / hexanes, 1:1) revealed that the reaction was complete. The solvent was evaporated off in vacuo and the residue obtained was purified by column chromatography over Si gel using EtOAc / hexanes (1:4) as eluent to afford the pure aminated product 65 (0.7 g, 78%); Mp. 154-155°C; ¹H NMR
(CDCl$_3$) $\delta$ 1.41 (s, 9H), 2.43 (s, 3H), 2.98 (t, 2H, $J = 6.4$ Hz), 3.39 (q, 2H, $J = 6.4$ Hz),
4.23 (d, 2H, $J = 5.6$ Hz), 4.87 (bs, 1H), 5.29(s, 1H), 6.02 (bt, 1H, $J = 5.6$ Hz), 7.24 (d, 2H, $J = 8.4$ Hz), 7.30-7.40 (m, 5H), 7.67 (s, 1H), 7.97 (d, 2H, $J = 8.4$ Hz); $^{13}$C NMR (CDCl$_3$) $\delta$ 21.8, 25.7, 28.4 (3C), 40.5, 47.2, 79.1, 98.0, 123.6, 127.3, 127.7(2C), 128.1(2C), 128.9(2C), 129.7(3C), 130.1, 130.6, 133.9, 135.8, 146.1, 147.4, 156.0, 170.2, 183.2; MS (ES$^+$) m/z 550 (M+H) and HRMS calcd for C$_{25}$H$_{22}$N$_3$O$_6$S [M-C$_4$H$_9$]$^+$ 492.1229, found 492.1227.

2-[7-Benzyl-5-(4-benzyloxyphenyl)-4,8-dioxo-1-tosyl-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indol-3-yl]-ethyl-carbamic acid tert-butyl ester (66)

To a solution of quinone 65 (0.700 g, 1.27 mmol) and acetal, 43 (2.66 g, 8.90 mmol) in CH$_3$CN (100 mL), Mn(OAc)$_3$ (2.4 g, 8.9 mmol) was added and the reaction mixture was refluxed for 40 hours. TLC examination (EtOAc / hexanes, 1:1) revealed that the reaction was complete. The reaction mixture was then allowed to attain room temperature and the solvent was evaporated off in vacuo. The residue obtained was dissolved in EtOAc (100 mL), washed with saturated NaHSO$_3$ (2 × 50 mL), water (2 × 50 mL), brine (1 × 50 mL) and dried over anhydrous Na$_2$SO$_4$. The drying agent was removed by filtration and the solvent was removed to obtain the crude product which was purified by column chromatography over Si gel using EtOAc / hexanes (1:3) as eluent to furnish the compound 66 as an orange solid (0.690 g, 72%); Mp 193-194°C; $^1$H NMR (CDCl$_3$) $\delta$ 1.38 (s, 9H), 2.42 (s, 3H), 2.98 (t, 2H, $J = 6.4$ Hz), 3.38 (q, 2H, $J = 6.4$ Hz), 4.76 (bs, 1H), 5.08(s, 2H), 5.55 (s, 2H ), 6.78 (s, 1H ), 6.97(d, 2H, $J = 8.8$ Hz), 7.15-7.25 (m, 2H), 7.20-7.45(m, 10H), 7.50(d, 2H, $J = 8.4$ Hz), 7.57(s. 1H), 7.97(d, 2H, $J = 8.4$ Hz)
Hz); $^{13}$C NMR (CDCl$_3$) δ 21.8, 25.8, 28.4(3C), 40.6, 52.2, 70.0, 79.1, 114.4(2C), 122.8, 123.3, 125.1, 126.4, 127.5(2C), 127.9(2C), 128.0(2C), 128.4, 128.6(3C), 128.7(2C), 129.0(2C), 129.4(2C), 129.9(3C), 130.0, 131.1, 134.2, 136.2, 137.0, 145.5, 156.0, 158.5, 167.0, 180.4; MS (ES$^+$) m/z 756 (M+H) and HRMS calcd for C$_{39}$H$_{32}$N$_3$O$_5$S [M-C$_4$H$_9$CO$_2$]$^+$ 654.2063, found 654.2061.

2-[7-Benzyl-5-(4-benzyloxyphenyl)-4,8-dioxo-1-tosyl(toluene-4-sulfonyl)-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indol-3-yl]-ethyl-methyl-carbamic acid tert-butylester (67) and 2-(7-Benzyl-5-(4-benzyloxy-phenyl)-1-methyl-4,8-dioxo-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indol-3-yl]-ethyl-methyl-carbamic acid tert-butyl ester (68)

To a solution of compound 66 (0.300 g, 0.400 mmol) in anhydrous DMF (3 mL) NaH (0.0630 g, 1.60 mmol, 60% dispersion in mineral oil) was added at 0°C. The reaction mixture was stirred for 30 minutes followed by addition of CH$_3$I (0.250 g, 1.75 mmol). The temperature was gradually increased to room temperature and stirred for 30 hours. TLC examination (EtOAc / hexanes, 1:1) revealed that the reaction was complete. The reaction mixture was quenched with saturated solution of NH$_4$Cl and extracted with EtOAc (100 mL). The EtOAc extract was further washed with water (3 × 40 mL), brine (1 × 40 mL) and dried over Na$_2$SO$_4$. The drying agent was filtered and the solvent was evaporated to obtain the crude product which was purified by column chromatography over Si gel using EtOAc / hexanes (1:1) as eluent to afford pure compounds 67 (0.141 g, 46%) and 68 (0.0600 g, 24%) as orange and yellow solids. Compound 67: Mp 165-166°C (dec); $^1$H NMR (CDCl$_3$) δ 1.30-1.50 (m, 9H), 2.41 (s, 3H), 2.83 (s, 3H), 2.90-3.10 (m, 2H), 3.5(t, 2H, $J = 7.2$ Hz), 5.08 (s, 2H), 5.56 (s, 2H), 6.78 (s, 1H), 6.97 (d, 2H, $J = 8.7$ Hz).
Hz), 7.10-7.20 (m, 2H), 7.20-7.50 (m, 10H), 7.52 (d, 2H, J = 8.7 Hz), 7.96 (d, 2H, J = 8.4 Hz); $^{13}$C NMR (CDCl$_3$) δ 21.7, 24.2, 28.4(3C), 34.7, 48.4, 52.3, 70.0, 79.3, 114.5 (2C), 122.9, 123.4, 125.2, 126.4, 127.4(2C), 127.9(2C), 128.0(2C), 128.4, 128.6(3C), 128.7(2C), 129.0(3C), 129.4(2C), 130.0(3C), 131.1, 134.4, 136.3, 137.0, 145.5, 155.7, 158.5, 167.1, 180.1; MS (ES$^+$) m/z 770 (M+H) and HRMS calcd for C$_{40}$H$_{34}$N$_3$O$_5$S [M-C$_4$H$_9$CO$_2$]$^+$ 668.2219, found 668.2215.

Compound 68: Mp 120-122°C (dec); $^1$H NMR (CDCl$_3$) δ 1.30-1.50 (m, 9H), 2.79 (s, 3H), 2.90-3.10 (m, 2H), 3.46 (t, 2H, J = 6.9 Hz), 3.89 (s, 3H), 5.09 (s, 2H), 5.65(s, 2H), 6.77 (s, 1H), 6.98(d, 2H, J = 8.7 Hz), 7.21-7.45 (m, 11H), 7.60 (d, 2H, J = 8.7 Hz); $^{13}$C NMR (CDCl$_3$) δ 24.2, 28.4(3C), 34.3, 36.4, 48.9, 52.1, 70.1, 79.0, 114.4(2C), 123.1, 124.0, 125.7,125.8, 126.3, 127.3, 127.5 (2C), 127.9(2C), 128.1,128.6(3C), 128.8(2C), 129.4, 130.1(2C), 130.3, 130.7, 136.7, 137.1, 155.8, 158.3, 170.6, 180.5; MS (ES$^+$) m/z 630 (M+H) and HRMS calcd for C$_{39}$H$_{39}$N$_3$O$_5$ 629.2890, found 629.2884.

2-[5-(4-Hydroxyphenyl)-1-methyl-4,8-dioxo-1,4,7,8-tetrahydro-pyrrolo[3,2-f]-indol-3-yl]-ethyl-methyl-carbamic acid tert-butyl ester (69)

To a solution of compound 68 (0.014 g, 0.020 mmol) in anhydrous EtOH (10 mL), HCOONH$_4$ (0.050 g, 0.80 mmol) and Pd black (0.021 g, 0.20 mmol) were added and the reaction mixture was refluxed for 15 hours. TLC examination (CHCl$_3$ / MeOH, 20:1) revealed that the reaction was complete. The reaction mixture was allowed to attain room temperature and filtered through a pad of celite 585 and washed with EtOAc:CHCl$_3$ (1:1). The filtrate and washings were combined and the solvent was evaporated off in vacuo. The residue obtained was dissolved in EtOAc (20 mL) and washed with water (2
× 10 mL), brine (1 × 10 mL) and dried over anhydrous Na₂SO₄. The drying agent was filtered and the solvent was evaporated to obtain the crude product which was purified by column chromatography over Si gel using MeOH/CHCl₃ (1:25) as eluent to furnish the pure product 69 (0.0040 g, 45%); ¹H NMR (CD₃OD) δ 1.20-1.60 (m, 9H), 2.88 (s, 3H), 2.94 (t, 2H, J = 6.0 Hz), 3.40-3.65 (m, 2H), 3.95 (s, 3H), 6.76 (s, 1H), 6.81 (dd, 2H, J₁ = 6.6 Hz, J₂ = 2.1Hz), 7.04 (s, 1H), 7.58 (dd, 2H, J₁ = 6.6 Hz, J₂ = 2.1Hz); ¹³C NMR (CD₃OD) δ 25.0, 28.5(3C), 30.7, 34.2, 36.3, 80.6, 115.7(2C), 123.3, 124.5(2C), 126.3, 127.7, 128.8, 131.1(2C), 131.3, 131.4, 131.5, 135.0, 157.9, 170.6, 182.7; MS (ES⁺) m/z 448 (M-H); and HRMS calcd for C₂₀H₁₈N₃O₃ [M-C₄H₉CO₂]⁺ 348.1348, found 348.1341.

5-(4-Hydroxyphenyl)-1-methyl-3-(2-methylamino-ethyl)-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (Zyzyananone A)

To a suspension of compound 69 (0.005 g, 0.01 mmol) in CH₂Cl₂ (1 mL) was added a 1:1 mixture of TFA and CH₂Cl₂ (0.2 mL) dropwise at room temperature. The reaction mixture was stirred at room temperature for 2 hours following which the TLC (MeOH / CHCl₃, 1:25) indicated the completion of reaction. The solvent was evaporated off and co-evaporated with CHCl₃ (3 × 3 mL) to yield pure zyzyananone A (0.003 g, 80%); ¹H NMR (CD₃OD) δ 2.69 (s, 3H), 3.10 (t, 2H, J = 7.2 Hz), 3.24 (t, 2H, J = 7.2 Hz), 3.97 (s, 3H), 6.78 (d, 2H, J = 8.7 Hz), 6.89 (s, 1H), 7.05 (s, 1H), 7.55 (d, 2H, J = 8.7 Hz); ¹³C NMR (CD₃OD) δ 23.7, 33.7, 36.5, 50.7, 115.7(2C), 120.9, 123.0, 124.7, 126.1, 127.4, 128.9, 131.1(2C), 131.3, 131.9, 135.0, 158.0, 170.5, 182.9; MS (ES⁺) m/z 350 (M+H); and HRMS calcd for C₂₀H₁₉N₃O₃ 349.1426, found 349.1429.
2-[7-Benzyl-5-(4-benzoxoxyphenyl)-4,8-dioxo-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indol-3-yl]-ethyl-methyl-carbamic acid tert-butyl ester (70)

To a solution of compound 67 (0.067 g, 0.087 mmol) in DMF (1 mL), NaN₃ (0.012 g, 0.17 mmol) was added and stirred for 4 hours at room temperature following which the TLC (EtOAc / hexanes, 1:1) indicated the completion of reaction. The reaction mixture was quenched with water and extracted with EtOAc (2 × 20 mL). The EtOAc extract was further washed with water (3 × 20 mL), brine (1 × 20 mL) and dried over anhydrous Na₂SO₄. The drying agent was filtered off and the solvent was evaporated off to obtain the crude product which was purified by column chromatography over Si gel using EtOAc/hexanes (1:10) as eluent to furnish the pure product 70. (0.040 g, 75%); ¹H NMR (CDCl₃) δ 1.30-1.50 (m, 9H), 2.82(s, 3H), 2.90-3.15(m, 2H), 3.48(t, 2H, J = 6.9 Hz), 5.10(s, 2H), 5.64(s, 2H), 6.83(s, 1H), 7.00(d, 2H, J = 7.2 Hz), 7.20-7.50(m, 11H), 7.59(d, 2H, J = 7.2 Hz), 9.48(bs, 1H); ¹³C NMR δ 25.4, 28.5(3C), 34.8, 49.0, 52.4, 70.2, 79.2, 114.4(2C), 123.4, 124.5, 124.7, 125.6, 127.1, 127.4, 127.5(3C), 127.9, 128.1, 128.6(3C), 128.9(3C), 129.7,130.2(2C), 132.5, 136.5, 137.1, 155.9, 158.5, 169.3, 180.7; MS (ES⁺) m/z 614 (M-H), 516 (M+H-C₆H₉CO₂) and HRMS: calcd for C₃₈H₃₇N₃O₅ 615.2733, found 615.2742.

2-[5-(4-Hydroxyphenyl)-4,8-dioxo-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indol-3-yl]-ethyl-methyl-carbamic acid tert-butyl ester (71)

To a solution of 70 (0.038 g, 0.060 mmol) in anhydrous EtOH (10 mL), HCOONH₄ (0.15 g, 2.40 mmol) and Pd black (0.070 g, 0.66 mmol) were added and refluxed for 15 hours. TLC examination (CHCl₃ / MeOH, 20:1) revealed that the reaction
was complete. The reaction mixture was allowed to attain room temperature and filtered through a pad of celite 585 and washed with EtOAc / CHCl₃ (1:1). The combined filtrate and washings was evaporated off *in vacuo*. The residue obtained was dissolved in EtOAc (25 mL) and washed with water (2 × 10 mL), brine (1 × 10 mL) and dried over anhydrous Na₂SO₄. The drying agent was removed by filtration and the solvent was evaporated off *in vacuo* to obtain the crude product which was purified by column chromatography over Si gel using MeOH / CHCl₃ (1:25) as eluent to furnish the pure compound 71 (0.010 g, 47%); ¹H NMR (CD₃OD) δ 1.30-1.50 (m, 9H), 2.84(s, 3H), 2.94(t, 2H, J = 6.3 Hz), 3.40-3.55(m, 2H), 6.60-6.80 (m, 3H), 7.01(s, 1H), 7.55(dd, 2H, J₁= 6.9 Hz , J₂= 2.1 Hz); ¹³C NMR δ 25.1, 28.6(3C), 30.7, 34.2, 80.6, 115.7(2C), 123.9, 124.7, 125.3, 125.4, 125.6, 126.3, 126.7, 129.1, 131.1(2C), 133.3, 134.5, 157.9, 169.8, 183.2; MS (ES⁺)m/z 434 (M-H), 336 (M+H-C₄H₉CO₂) and HRMS: calcd for C₂₄H₂₅N₃O₄ 435.1794, found 435.1796.

3-(4-Hydroxyphenyl)-5-(2-methylamino-ethyl)-1H,7H-pyrrolo[3,2-f]indole-4,8-dione (Zyzzyanone B)

To a suspension of compound 71 (0.007 g, 0.02 mmol) in CH₂Cl₂ (1 mL) a 1:1 mixture of TFA and CH₂Cl₂ (0.2 mL) was added drop wise at room temperature. The reaction mixture was stirred at room temperature for 2 hours following which the TLC (MeOH / CHCl₃, 1:25) indicated the completion of reaction. The solvent was evaporated off and co-evaporated with CHCl₃ (3 × 3 mL) to yield pure zyzzyanone B (0.005 g, 87%); ¹H NMR (CD₃OD) δ 2.70(s, 3H), 3.09(t, 2H, J = 7.2 Hz), 3.26(t, 2H, J = 7.2 Hz), 6.77 (d, 2H, J = 8.7 Hz), 6.95(s, 1H), 7.03 (s, 1H), 7.54(d, 2H, J = 8.7 Hz); ¹³C NMR δ 23.9, 33.7, 50.8, 115.6(2C), 121.6, 123.6, 124.9, 125.7, 126.1, 126.4, 129.2, 131.2(2C),
N-2-[7-Benzyl-5-(4-benzyloxyphenyl)-1-methyl-4,8-dioxo-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indol-3-yl]-ethyl-N-methyl-formamide (73)

To a solution of compound 68 (0.040 g, 0.064 mmol) in CH₂Cl₂ (4 mL), a 1:1 mixture of TFA and CH₂Cl₂ (1 mL) was added drop wise at room temperature. The reaction mixture was stirred for 2 hours following which TLC (MeOH / CHCl₃, 1:25) indicated the removal of the BOC group. The solvent was evaporated off and the residue co-evaporated with CHCl₃ (3 × 10 mL). To this residue in HCOOEt (15 mL), Et₃N (1 mL) was added and refluxed for 24 hours. TLC analysis (CHCl₃ / MeOH, 20:1) indicated completion of the reaction. The excess reagents were evaporated off and the residue was purified by column chromatography over Si gel using CHCl₃ as eluent to obtain compound 73 (0.029 g, 72%); ¹H NMR (CDCl₃) δ 2.89 (2.92) (s, 3H), 2.90-3.10 (m, 2H), 3.50-3.65 (m, 2H), 3.92 (s, 3H), 5.1 (s, 2H), 5.66 (s, 2H), 6.48 (6.65) (s, 1H), 6.80 (6.81) (s, 1H), 7.00 (d, 2H, J = 9.0 Hz), 7.25-7.50 (m, 11H), 7.56-7.64 (m, 2H), 7.82 (8.01) (s, 1H); ¹³C NMR (CDCl₃) δ 23.1 (24.8), 29.8 (34.9), 36.6, 44.0 (49.6), 52.2, 70.1, 114.49(114.52) (2C), 121.4(122.4), 123.95 (124.01), 125.5, 125.7, 125.76 (125.79), 126.4(126.5), 127.5(2C), 127.6(2C), 128.02(128.08), 128.12(128.19), 128.4, 128.7(2C), 128.96(128.98) (2C), 129.50 (129.56), 130.2 (2C), 130.5(130.7), 130.8(130.9), 136.6 (136.7), 158.46 (158.51), 162.7(163.1), 170.57 (170.62), 180.6(180.7); MS (ES⁺) m/z 558 (M+H); and HRMS: calcd for C₃₅H₃₁N₃O₄ 557.2315, found 557.2328.
N-2-[5-(4-Benzylxylophenyl)-1-methyl-4,8-dioxo-1,4,7,8 tetrahydropyrrolo[3,2-f]indol-3-yl]-ethyl-N-methyl-formamide (Zyzzyanone C)

To a solution of compound 73 (0.035 g, 0.060 mmol) in anhydrous EtOH (10 mL), HCOONH₄ (0.15 g, 2.5 mmol) and Pd black (0.070 g, 0.65 mmol) were added and refluxed for 15 hours. TLC examination (CHCl₃/MeOH, 20:1) revealed that the reaction was complete. The reaction mixture was allowed to attain room temperature and filtered through a pad of celite 585 and washed with EtOAc/CHCl₃ (1:1). The filtrate and washings were combined and the solvent was evaporated off in vacuo. The residue obtained was dissolved in EtOAc (25 mL) and washed with water (2 × 10 mL), brine (1 × 10 mL) and dried over anhydrous Na₂SO₄. The drying agent was filtered and the solvent was evaporated off to obtain the crude product which was purified by column chromatography over Si gel using MeOH/CHCl₃ (1:25) as eluent to furnish zyzzyanone C (0.0070 g, 30%); ¹H NMR (DMSO-d₆) δ 2.76(2.88) (s, 3H), 2.83-2.93 (m, 2H), 3.40-3.50 (m, 2H), 3.89 (s, 3H), 6.75(d, 2H, J = 8.7 Hz), 6.99(7.01) (s, 1H), 7.20(s, 1H), 7.56(d, 2H, J = 8.7 Hz), 7.77(7.95) (s, 1H), 9.44(bs. 1H), 12.62(bs, 1H); ¹³C NMR (DMSO-d₆) δ 22.7 (24.2), 29.0(34.1), 35.9, 43.2(48.6), 114.6 (2C), 121.4, 121.5(122.2), 123.9, 124.1, 125.4, 126.5(126.6), 129.3(129.4), 129.8(2C), 130.1(130.5), 133.4, 156.7, 162.3, 168.7, 180.5; MS (ES⁺) m/z 378 (M+H); and HRMS: calcd for C₂₁H₁₉N₃O₄ 377.1376, found 377.1383.
N-2-[7-Benzyl-5-(4-benzyloxyphenyl)-4,8-dioxo-1,4,7,8-tetrahydropyrrolo [3,2-f]indol-3-yl]-ethyl-N-methyl-formamide (75)

To a solution of compound 67 (0.055 g, 0.071 mmol) in CH$_2$Cl$_2$ (4 mL), a 1:1 mixture of TFA and CH$_2$Cl$_2$ (1 mL) was added dropwise at room temperature. The reaction mixture was stirred for 2 hours. TLC (MeOH / CHCl$_3$, 1:25) indicated completion of the removal of BOC group. The solvent was evaporated off and the residue was co-evaporated with CHCl$_3$ (3 × 10 mL). To this residue in HCOOEt (15 mL) and Et$_3$N (1 mL) were added and refluxed for 24 hours following which TLC (CHCl$_3$ / MeOH, 20:1) indicated completion of the reaction. The excess reagents were evaporated off and the residue obtained was purified by column chromatography over Si gel using MeOH / CHCl$_3$ (1:50) as eluent to obtain compound 75 (0.025 g, 64%); $^1$H NMR (CDCl$_3$) δ 2.89(2.91) (s, 3H), 2.90-3.10 (m, 2H), 3.50-3.70 (m, 2H), 5.1 (s, 2H), 5.60(5.62) (s, 2H), 6.58(6.75) (s, 1H), 6.82 (s, 1H), 7.00 (d, 2H, $J = 9.0$ Hz), 7.20-7.50 (m, 11H), 7.58 (d, 2H, $J = 9.0$ Hz), 7.83(8.01) (s, 1H), 9.75(9.97) (bs, 1H); $^{13}$C NMR (CDCl$_3$) δ 22.9 (24.6), 29.6(34.8), 43.9(49.5), 52.1, 69.9, 114.3(114.34) (2C), 122.4, 123.5(123.8), 124.2(124.3), 124.4(124.5), 125.3(125.4), 127.0(127.1), 127.2(2C), 127.3(2C), 127.8, 128.0,128.3 128.5(2C), 128.8(2C), 130.0(2C), 130.1, 132.4(132.9), 136.28(136.34), 136.9, 158.3(158.4), 162.7(163.2), 169.16(169.22), 180.6(180.7); MS (ES$^+$) m/z 544 (M+H); and HRMS: calcd for C$_{34}$H$_{29}$N$_3$O$_4$ 543.2158, found 543.2180.
N-2-[5-(4-Hydroxyphenyl)-4,8-dioxo-1,4,7,8-tetrahydropyrrolo[3,2-f]indol-3-yl]-
ethyl-N-methyl-formamide (Zyzzyanone D)

To a solution of compound 75 (0.023 g, 0.040 mmol) in anhydrous EtOH (10 mL), HCOONH$_4$ (0.1 g, 1.6 mmol) and Pd black (0.050 g, 0.47 mmol) were added and refluxed for 15 hours. TLC examination (CHCl$_3$ / MeOH, 20:1) revealed that the reaction was complete. The reaction mixture was allowed to attain room temperature and filtered through a pad of celite 545 and washed with EtOAc / CHCl$_3$ (1:1). The filtrate and washings were combined and the solvent was evaporated off in vacuo. The residue obtained was dissolved in EtOAc (25 mL), washed with water (2 × 10 mL), brine (1 × 10 mL) and dried over anhydrous Na$_2$SO$_4$. The drying agent was filtered and the solvent was evaporated off to obtain the crude product which was purified by column chromatography over Si gel using MeOH / CHCl$_3$ (1:25) as eluent to furnish zyzzyanone D (0.0060 g, 42%); $^1$H NMR (DMSO-d$_6$) δ 2.75 (2.85) (s, 3H), 2.87-2.95 (m, 2H), 3.42-3.52 (m, 2H), 6.75 (d, 2H, $J = 8.7$ Hz), 6.95 (s, 1H), 7.17 (s, 1H), 7.55 (d, 2H, $J = 8.7$ Hz), 7.72 (7.94) (s, 1H), 9.45 (bs, 1H), 12.43 (bs, 1H), 12.60 (bs, 1H); $^{13}$C NMR (DMSO-d$_6$) δ 22.7 (24.2), 29.0 (34.0), 43.2 (48.7), 114.6 (2C), 121.8, 122.4 (123.1), 123.9 (124.0), 124.1, 124.4 (124.5), 124.6 (125.1), 126.8 (126.9), 129.9 (2C), 131.5 (131.7), 133.0, 156.6, 162.27 (162.3), 168.0 (168.1), 180.4 (181.0); MS (ES$^+$) m/z 364(M+H), 362(M-H) and HRMS: calcd for C$_{20}$H$_{17}$N$_3$O$_4$ 363.1219, found 363.1225.

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SUMMARY

Greater than 70% of earth’s surface is covered by oceans with a vast biological diversity due to multitudes of marine ecosystems. As a result of their sedentary existence, nature has endowed marine organisms with chemical means for their defense. These defense mechanisms manifest as chemical substances called marine natural products and usually exist as secondary metabolites in marine invertebrates such as sponges, bryozoans, tunicates, ascidians and marine bacteria. Exploration of global marine resources have greatly increased in the past few decades. This is attributed to the improvement in the deep-sea sample collection technology. These explorations have demonstrated the ocean to be a treasure house of medicinally useful compounds. In the recent past, there has been an increase in the number of biologically active alkaloids isolated from marine sources. Scientists from various disciplines, such as organic chemistry, bioorganic chemistry, medicinal chemistry, pharmacology and biology are involved in research and investigations to realize their objective of drug-discovery based on these bioactive marine compounds. Pharmacological value of the marine alkaloids is further reinforced by the fact that about a dozen of marine alkaloids are currently undergoing various phases of human clinical trials for treatment of different cancers. The largest number of bioactive marine alkaloids with novel structures have been isolated from marine sponges.
Marine sponges of the genera *Latrunculia*, *Batzella*, *Prianos* and *Zyzzya* are a rich source of alkaloids bearing a pyrrolo[4,3,2-de]quinoline skeleton. This series of alkaloids comprise of about 60 metabolites including discorhabdins, epinardins, batzellines, isobatzellines, makaluvamines, veiutamine, tsitsikammamines and wakayin. Pyrrolo[4,3,2-de]quinoline alkaloids have shown a variety of biological activities such as inhibition of topoisomerase I and II, cytotoxicity against different tumor cell lines, antifungal and antimicrobial activities. Pyrrolo[4,3,2-de]quinoline and closely related class of alkaloids have recently received increasing attention as a source of new anticancer drugs. Their unique fused ring skeletons carrying interesting biological properties have made them targets for several synthetic and biological studies. Several reviews have been published on the chemistry and bioactivity of this class of compounds.

This PhD dissertation is focused on the synthesis and biological evaluation of marine alkaloids and their analogs that contain a pyrroloquinoline or a bispyrroloquinone skeleton.

Chapter 1 of this dissertation culminated with the successful synthesis of makaluvamine analogs and evaluation of their anticancer activity. Makaluvamines are among the potent natural products belonging to the pyrroloquinoline class of alkaloids. We were able to synthesize, characterize and evaluate several analogs of makaluvamines. While improving the yield of the synthesis of makaluvamine analogs we have developed a new detosylation method for the removal of tosyl group from N-tosyl pyrroloiminoquinones. In this method, we have used NaN₃ as a detosylating agent in polar solvents such as DMF or DMSO. *In vitro* evaluation of several of these analogs against a number of cancer cell lines such as breast cancer, lung cancer, prostate cancer,
pancreatic cancer, gliomas and ovarian cancer proved their effectiveness as potential anticancer agents. Mechanistic studies on some of the most active compounds attributed their cytotoxic effects to inhibition of cell cycle progression and induction of apoptosis via both p53-dependent and p53-independent pathways. It is also possible that these compounds activate the DNA damage response and inhibit topoisomerase II, although the effects on apoptosis and the cell cycle appear to be the most important mechanisms of action for this class of compounds. Two of the most potent benzylamino analogs of makaluvamines demonstrated potent in vivo activity in mouse xenograft models of two types (ER+ and ER−) of breast cancers and also in a model of ovarian cancer. The synthetic and biological results obtained in this chapter of the dissertation have increased our understanding about the synthesis of makaluvamine analogs, their anticancer activity as well as the mechanism by which they produce anticancer activity.

Chapter 2 of this dissertation accomplished the development of a new CAN mediated oxidative free radical cyclization reaction between 6-benzylamionindolole-4,7-quinones and 1,3-dicarbonyl compounds to form a tricyclic bispyrroloquinone ring system. Bispyrroloquinone and bispyrroloiminoquinone are two common polycyclic structural scaffolds present in a number of biologically active marine alkaloids such as zyzzyanones, tsitsikammamines and wakayin. So, this methodology will be useful in the synthesis of these alkaloids and/or their analogs. A plausible mechanism for this transformation is proposed based on the previous literature reports of similar reactions. In order to demonstrate the generality of this synthetic methodology, the oxidative free radical reaction was extended to different combinations of aminoquinones and β-dicarbonyl compounds. The methodology was also extended to explore the use of β-
ketosulfides, β-ketosulfoxides and β-ketosulfones in the place of β-dicarbonyl compounds. The reaction worked in the case of β-ketosulfones. However, it failed to give expected products in the case of β-ketosulfides and β-ketosulfoxide compounds.

Synthetic procedures for the removal of protecting groups such as N-benzyl O-benzyl and N-tosyl from the bispyrroloquinone ring system have also been standardized.

Chapter 3 of this dissertation was focused on the synthesis of four marine alkaloids, zyzzyanones A-D. This is a continuation of the research idea conceived in chapter 2 of this dissertation. Our first objective was to apply the CAN mediated oxidative cyclization for the synthesis of zyzzyanones A-D. As zyzzyanones contained a phenolic ring as a substituent on the bispyrroloquinone ring, we had to modify the oxidative cyclization developed in chapter 2. This is mainly because CAN mediated oxidative cyclization reaction using 6-benzylamnioindole-4,7-quinone with 1,3-dicarbonyl compounds was not a suitable reaction for introducing an aromatic rings on the bispyrroloquinone ring. Our efforts to overcome this problem resulted in the identification of Mn(OAc)$_3$ as an alternative oxidizing agent and use of acetal as an alternative reagent for 1,3-dicarbonyl compound. We prepared 4-benzyloxyphenyl acetaldehyde acetal and demonstrated that the oxidative cyclization of reaction of 6-benzylamnioindole-4,7-quinone with this acetal in the presence of Mn(OAc)$_3$ worked well in a model system. However, when the reaction was attempted with the quinone containing an aminoethyl side chain as required for zyzzyanones, the reaction did not work. This essentially drew our attention to the reactivity of the tertiary amino group in the side chain which may have contributed to the failure of the reaction. We solved this problem by using a Boc-protected aminoethyl side chain instead of a tertiary aminoethyl.
side chain. This reaction worked very well to afford a key intermediate compound from which all four zyzzyanones A-D were prepared in a few steps.

**Future directions:** We have accomplished quite a lot of work in this dissertation. However, a lot remains to be done. Based on the synthetic methodology developed in this thesis, we have additional objectives that we plan to pursue in the immediate future. This includes the synthesis of other similar but, more complex marine alkaloids, tsitsikammamines and wakayin. Both these alkaloids will be synthesized utilizing the synthetic methodology developed for zyzzyanone synthesis.

Tsitsikammamines A and B were isolated in 1996 from the South African Latrunculid sponge *Tsitsikamma favus*.\(^{109}\) Reinvestigation of extracts of this species searching for minor pyrroloiminoquinone metabolites yielded N-18 oxime analogues of tsitsikammamine A and B. Tsitsikammamines A and B exhibited cytotoxicity against HCT-116 with IC\(_{50}\) values of 1.4 µM and 2.4 µM respectively. Mechanism of action of tsitsikammamines is reported to be inhibition of topoisomerase I enzyme.\(^{109}\)

![Tsitsikammamine](image)

*Figure 1:* Structures of Tsitsikammamines.

Wakayin was isolated by Ireland *et al* in 1991 from an ascidian Clavelina species. It has exhibited cytotoxic activity which is attributed to its inhibition of enzyme
topoisomerase I. Wakayin exhibited *in vitro* cytotoxicity against HCT116 with an IC\textsubscript{50} value of 0.5 µg/mL.\textsuperscript{110} Inhibition of topoisomerase II enzyme (250 µM) and the observation of a 3-fold differential toxicity toward the CHO cell line EM9 (sensitive to DNA-damaging genotoxic agents) versus BR16 (resistant to BCNU) provided preliminary evidence that wakayin exhibits its cytotoxicity by interfering with or damaging DNA. Antimicrobial activity of wakayin against *Bacillus subtilis* (MIC = 0.3 µg/ml) has also been observed.\textsuperscript{110}

![Wakayin](image)

**Figure 2:** Structure of marine alkaloid Wakayin.

Bispyrrolloiminoquinone is the structural feature present in marine natural products such as wakayin and tsitsikammamines. Several efforts have been made by different groups to accomplish total synthesis of wakayin. No total synthesis has been published till date. However, two models were designed using methods for construction of pyrrole ring bearing the indole moiety on the indole-4,7-quinone or naphthoquinone. The first attempt was by Zhang *et al* by condensation of N-methyl tryptamine with 2-methoxynapthoquinone and subsequent oxidative cyclization of the resultant adduct by DDQ to obtain 3-(1H-indol-3-yl)-1-methyl-1H-benzo[f]indole-4,9-dione.\textsuperscript{111} The second analog was published by Barret and Roue by reaction of an oxotryptamine on indole 4,7-
quinone to obtain 3-(1H-indol-3-yl)-1H-7-tosyl-pyrrolo[3,2-f]indole-4,8-dione which was further detosylated to form 1H,7H-pyrrolo[3,2-f]indole-4,8-dione. Aza and pyrazolic analogs of these natural products have also been reported. The first and only total synthesis of tsitsikammamine A was reported by Rives et al. Our group has published method involving formation of the bispyrroloiminoquinone skeleton present in tsitsikammamine and wakayin. This method involved the treatment of N-tosylpyrroloiminoquinone derivative and ethyl acetoacetate in the presence of CAN in MeOH. We propose to extend the synthetic methodology developed for the synthesis of zyzzyanones described in detail in chapter 3 of this dissertation, for the synthesis of tsitsikammamines and wakayin and their analogs. These compounds and their analogs will be subjected to biological evaluation with the goal of identifying new and potent leads for anticancer drug discovery.

**Proposed synthesis of tsitsikammamine A**

Our proposed synthetic strategy for the synthesis of tsitsikammamine is an extension of the synthesis of zyzzyanones. The proposed strategy is shown in the reaction scheme in Figure 3. According to this Scheme, we propose to start our synthesis with the previously synthesized intermediate 66. This compound was obtained by the Mn(OAc)$_3$ mediated oxidative cyclization reaction of the 6-benzylaminoindole-4,7-quinone and 4-benzyloxyphenyl acetaldehyde acetal as described in Chapter 3 of this dissertation. The BOC group present in compound 66 will be removed by treatment with TFA in CH$_2$Cl$_2$ to afford the free amine 76. Similar to the cyclization reaction we have used in the synthesis of compound 8, we will reflux the compound 66 in CHCl$_3$ to form the cyclized
compound 77. The compound 77 will be debenzylated using transfer hydrogenolysis conditions to obtain compound 78 which upon detosylation using NaN₃ in DMF will afford tsitsikammamine A. One drawback of our synthesis has been low yields for the debenzylation reaction. We will be attempting to improve the yield of the debenzylation by using transfer hydrogenolysis reagents such as cyclohexene.

**Figure 3**: Proposed synthesis of tsitsikammamine A.
Proposed synthesis of wakayin

The synthesis of wakayin is another goal we look forward to accomplishing. The retrosynthesis of wakayin shows that the synthetic strategy has to be similar to that used in the synthesis of tsitsikammamine A, as this synthesis also involves construction of the tetracyclic iminoquinone system. Synthesis of wakayin is outlined in Figure 4.

Figure 4: Proposed synthesis of Wakayin.

The important step in this synthesis is construction of the bispyrroloquinone system with the side chain and the indole group incorporated at the 5– position of the indoloquinone. We believe this may be possible using the Mn(OAc)$_3$ mediated oxidative cyclization between the aminoquinone 65 and indole-3-acetaldehyde acetal 79. This
acetal will be reacted with Boc-protected benzylamino quinone intermediate 65 in the presence of Mn(OAc)$_3$ to obtain the compound 80. The BOC group present in 80 will be removed by treatment with TFA in CH$_2$Cl$_2$ to form compound 81, which will be cyclized by refluxing in CHCl$_3$ to afford compound 82. The N-tosyl group present in 82 will be removed by treatment with NaN$_3$ in DMF to obtain compound 83, which will be subjected to debenzylation using Pd black to afford wakayin.
LIST OF GENERAL REFERENCES


100. El-Naggar, M.; Capon, R. J. Discorhabdins Revisited: Cytotoxic Alkaloids from Southern Australian Marine Sponges of the Genera Higginsia and Spongisorites†. *Journal of Natural Products* 2009, 72, 460-464.


APPENDIX A

SPECTRA SUPPORTING FIRST ARTICLE
$^1$H NMR of Compound 1a
$^{13}$C NMR of Compound 1a
$^1$H NMR of Compound 1b
$^{13}$C NMR of Compound 1b
$^1$H NMR of Compound 1c
$^{13}$C NMR of Compound 1c
$^1$H NMR of Compound 1d
$^{13}$C NMR of Compound 1d
$^1$H NMR of Compound 1e
$^{13}$C NMR of Compound 1e
$^{1}H$ NMR of Compound 1f
$^{13}$C NMR of Compound 1f
$^1$H NMR of Compound 2a

ppm
$^{13}$C NMR of Compound 2a
$^1$H NMR of Compound $2b$
$^{13}$C NMR of Compound 2b
$^1$H NMR of Compound 2d
$^{13}$C NMR of Compound 2d
$^1$H NMR of Compound 2e
$^{13}$C NMR of Compound 2e
$^1$H NMR of Compound 2f
$^{13}$C NMR of Compound 2f
$^1$H NMR of Compound 2m
$^{13}$C NMR of Compound 2m
$^{1}H$ NMR of Compound 2n
$^{13}$C NMR of Compound 2n
$^1$H NMR of Compound 2o
$^{13}$C NMR of Compound 20
$^1$H NMR of Compound 2p
$^{13}$C NMR of Compound 2p
$^{1}$H NMR of Compound 2q
$^{13}$C NMR of Compound 2q
$^1$H NMR of Compound 2r
$^1$H NMR of Compound 2s
$^{13}$C NMR of Compound 2s
$^{1}$H NMR of Compound 2t
\[ ^{13} \text{C NMR of Compound 2t} \]
$^1$H NMR of Compound 2u
$^{13}$C NMR of Compound 2u
\[^1\text{H} \text{NMR of Compound } 2v\]
$^{13}$C NMR of Compound 2v
APPENDIX B

SPECTRA SUPPORTING SECOND ARTICLE
$^1$H NMR of Compound 21a
$^{13}$C NMR of Compound 21a
$^1$H NMR of Compound 21b
$^{13}$C NMR of Compound 21b
$^1$H NMR of Compound 21c
$^{13}$C NMR of Compound 21c
$^1$H NMR of Compound 21d
$^{13}$C NMR of Compound 21d
$^1$H NMR of Compound 25a
$^{13}$C NMR of Compound 25a
$^{1}$H NMR of Compound 25b
$^{13}$C NMR of Compound 25b
$^1$H NMR of Compound 25c
$^{13}$C NMR of Compound 25c
$^1$H NMR of Compound 25d
$^{13}$C NMR of Compound 25d
$^1$H NMR of Compound 25e
$^{13}$C NMR of Compound 25e
$^{13}$C NMR of Compound 25f
\(^1\)H NMR of Compound 25g
$^{13}$C NMR of Compound 25g
$^1$H NMR of Compound 25h
$^{13}$C NMR of Compound 25h
$^1$H NMR of Compound 25i
$^1$H NMR of Compound 25i
$^1$H NMR of Compound 25j
$^{13}$C NMR of Compound 25j
$^1$H NMR of Compound 25k
$^{13}$C NMR of Compound 25k
$^1$H NMR of Compound 251
$^{13}$C NMR of Compound 251
$^1$H NMR of Compound 25m
$^{13}$C NMR of Compound 25m
$^1$H NMR of Compound 25n
$^{13}$C NMR of Compound 25n
$^1$H NMR of Compound 250
$^{13}$C NMR of Compound 25o
$^1$H NMR of Compound 25p
$^{13}$C NMR of Compound 25p
$^1$H NMR of Compound 31a
$^{13}$C NMR of Compound 31a
$^1$H NMR of Compound 31h
$^{13}$C NMR of Compound $31h$
$^1$H NMR of Compound 32a
$^{13}$C NMR of Compound 32a
$^1$H NMR of Compound 32h
$^{13}$C NMR of Compound 32h
APPENDIX C

SPECTRA SUPPORTING THIRD ARTICLE
$^1$H NMR of Compound 41
$^{13}$C NMR of Compound 41
\[ ^1H \text{NMR of Compound 42} \]
$^{13}$C NMR of Compound 42
$^1$H NMR of Compound 43

[Graph depicting NMR spectrum]
$^{13}$C NMR of Compound 43
$^1$H NMR of Compound 44a
$^{13}$C NMR of Compound 44a
$^1$H NMR of Compound 44b
$^{13}$C NMR of Compound 44b
$^1$H NMR of Compound 57
$^{13}$C NMR of Compound 57
$^1$H NMR of Compound 58
$^{13}$C NMR of Compound 58
$^1$H NMR of Compound 59
$^{13}$C NMR of Compound 59
$^1$H NMR of Compound 60
$^{13}$C NMR of Compound 60
$^1$H NMR of Compound 61
$^{13}$C NMR of Compound 61
$^1$H NMR of Compound 62
$^{13}$C NMR of Compound 62
$^1$H NMR of Compound 63
$^{13}$C NMR of Compound 63
$^1$H NMR of Compound 65
$^{13}$C NMR of Compound 65
$^1$H NMR of Compound 66
$^{13}$C NMR of Compound 66
$^1$H NMR of Compound 67
$^{13}$C NMR of Compound 67
$^1$H NMR of Compound 68
$^{13}$C NMR of Compound 68
$^{13}$C NMR of zyzzyzynone A
$^1$H NMR of Compound 69
$^{13}$C NMR of Compound 69
$^1$H NMR of Compound 70
$^{13}$C NMR of Compound 70
$^1$H NMR of zyzzyanone B
$^{13}$C NMR of zyzzyanone B
$^1$H NMR of Compound 73
$^{13}$C NMR of Compound 73
$^1$H NMR of zyzzyanone C
$^{13}$C NMR of zyzzyanone C
\(^1\)H NMR of Compound 75
$^{13}$C NMR of Compound 75
$^1$H NMR of zyzzyanone D
$^{13}$C NMR of zyzzyanone D