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Recent Developments in the Isolation, Synthesis, and Bioactivities of Bispyrroloquinone Alkaloids of Marine Origin

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Abstract

The ocean continues to provide a plethora of unique scaffolds capable of remarkable biological applications. A large number of pyrroloiminoquinone alkaloids, including discorhabdins, epinardins, batzellines, makaluvamines, and veitamine have already been isolated from marine organisms. A class of pyrroloiminoquinone-related alkaloids known as bispyrroloquinones is the focus of this review. This family of marine alkaloids, which contain an aryl substituted bispyrroloquinone ring system, includes three subclasses of alkaloids namely, wakayin, tsitsikammamines A-B and zyzzyanones A-D. Both wakayin and the tsitsikammamines contain a tetracyclic fused bispyrroloiminoquinone ring system, while zyzzyanones contain a fused tricyclic bispyrroloquinone ring system.

The unique chemical structures of these marine natural products and their diverse biological properties, including antifungal and antimicrobial activity, as well as the potent, albeit generally nonspecific and universal cytotoxicities, have attracted great interest of synthetic chemists over the past three decades. Tsitsikammamines, wakayin, and several of their analogues show inhibition of topoisomerases. One additional possible mechanism of anticancer activity of tsitsikammamines analogues that was discovered recently is through the inhibition of indoleamine 2,3-dioxygenase, an enzyme involved in tumoral immune resistance. This review discusses the isolation, synthesis, and bioactivities of bispyrroloquinone alkaloids and their analogues.

Keywords

Bispyrroloquinones; tsitsikammamines; wakayin; zyzzyanones; topoisomerase; anticancer and antimicrobial

1. Introduction

Since the olden ages, humanity has relied on nature to provide therapeutics for a plethora of deadly diseases [1]. Nearly fifty percent of new drugs introduced in the past four decades have been natural products, their mimics, or their synthetic derivatives [2]. Of these compounds, those that are derived from marine sources are believed to be superior to terrestrial natural products in terms of their chemical novelty and their ability to induce

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potent bioactivities. A contributing factor for this observation stems from the fact that greater than 70% of earth's surface is covered by oceans and thus marine ecosystems exhibit a higher biological diversity [3-6]. Moreover, the sedentary lifestyle adopted by majority of the marine organisms has necessitated the evolution of highly efficient chemical agents for their defense mechanisms.

These chemical substances, which are produced within their system, are called 'marine natural products.' Usually existing as secondary metabolites in marine invertebrates such as sponges, bryozoans, tunicates, and ascidians, marine natural products help these organisms by deterring their predators or paralyzing their prey [7, 8]. Upon the release of these compounds into their surroundings, they are usually rapidly diluted by water. Hence, in order to be effective against their predators or prey, the compounds need to exhibit high potency. For this reason, marine natural products usually demonstrate extremely potent biological activities and continue to be a rich source of a vast array of such medicinally valuable compounds [7, 9-13]

As a result of the potential for new drug discovery scaffolds, marine natural products have attracted the attention of scientists from various disciplines, including organic chemistry, bioorganic chemistry, medicinal chemistry, pharmacology, and biology. With the improvement in the deep-sea sample collection technology and the large scale drug production through aqua culture, there has been an increase in the number of biologically active natural products isolated from marine sources in the last decade [14].

1.a. Marine derived drugs

More than a dozen of these marine alkaloids are currently undergoing various phases of human clinical trials for treatment of different types of cancers. Compounds such as trabectedin, bryostatins, plitidepsin, and eribulin shown in Fig. 1 capture the pharmacological value of marine natural products.

Trabectedin (ET-743, Yondelis®) was originally isolated from the Caribbean sea squirt, *Ecteinascidia turbinate*. It is the first anticancer marine-derived drug that was approved by the European Union for the treatment of patients with advanced soft tissue sarcoma after failure of anthracyclines and ifosfamide. Trabectedin is also in phase II trials for prostate, breast and pediatric cancer [15-17].

Bryostatins represent an important group of pharmaceutically promising substances that are produced by commensal microorganisms naturally occurring in marine invertebrates [18]. So far, twenty bryostatins have been discovered, which elicit a remarkable range of biological activities, including antineoplastic activity, synergistic chemotherapeutic activity, and cognition memory enhancement [19]. The most extensively studied compound, bryostatin 1, selectively modulates the function of various individual protein kinase C isozymes. It has been proposed for phase I and phase II clinical trials for the treatment against Alzheimer's disease [18, 20, 21].

Similarly, plitidepsin was originally isolated from the marine tunicate *Plitidepsin*. This compound exhibits antitumor, antiviral, and immunosuppressive activities and has especially

shown promise in shrinking tumors in pancreatic, stomach, bladder, and prostate cancers [22]. It was granted orphan drug status by the European Medicines Agency for treating acute lymphoblastic leukemia. It is currently in phase II clinical trials for solid and hematological malignant neoplasias like T cell lymphoma and in phase III clinical trials for multiple myeloma [23].

Finally, eribulin is U.S. Food and Drug Administration approved anticancer drug under the trade name halaven, which is used to treat patients with metastatic breast cancer who have received at least two prior chemotherapy regimens for late-stage disease [24, 25]. Eribulin is also being investigated by Eisai Co. for use in a variety of other solid tumors, including non-small cell lung cancer, prostate cancer and sarcoma.

1.b. Marine Sponges

Of the various sources of marine natural products, marine sponges are a particularly fertile field for the discovery of bioactive scaffolds. Several reviews concerning the sponge-derived bioactive marine alkaloids with future pharmaceutical applications have been published in the last decades [3, 26-31]. Sponges produce a plethora of chemical compounds with widely varying carbon skeletons. Most bioactive compounds from sponges which have been isolated in sufficient quantities exhibited a variety of activities such as anti-inflammatory, antitumor, immunosuppressive, neurosuppressive, antiviral, antimalarial, and antibiotic activities [1, 26, 27, 32, 33]. Unfortunately, many alkaloids with unique structures are available only in minute quantities, precluding their thorough biological evaluations. The only practical way to access these alkaloids and their analogues in larger quantities is through synthetic means.

Marine sponges of the genera *Latrunculia*, *Batzella*, *Prianos* and *Zyzzya* are a rich source of alkaloids bearing a pyrroloiminoquinone ring system [29, 34]. This series of alkaloids comprise of more than 60 metabolites including discorhabdins [35], epinardins [36], batzellines [37], isobatzellines [37], makaluvamines [33, 38-42], and veitamine [43]. These alkaloids have shown a variety of biological activities such as inhibition of topoisomerase I [38] and II, [33] cytotoxicity against different tumor cell lines, [33, 44] antifungal [38] and antimicrobial activities [32].

A related family of marine alkaloids containing an aryl substituted bispyrroloquinone ring system is of considerable significance to both organic chemists and biologists. This class includes three subclasses of alkaloids wakayin, tsitsikammamines A-B, and zyzzyanones A-D (Fig. 2). Both wakayin and the tsitsikammamines contain a tetracyclic fused bispyrroloiminoquinone ring system and while zyzzyanones contains a fused tricyclic bispyrroloquinone ring system.

The potent bioactivities resulting from these unique ring structures have made them attractive targets for organic synthesis and drug discovery. However, the bioactivities of these compounds are yet to be tapped for medicinal applications. One of the reasons for this slow progress in the development of this class of compounds is the lack of good synthetic methods to make these compounds and their analogues in large scale. Recently, there has been a rapid growth of interest in the synthesis and biological evaluation of this class of

compounds and their analogues. Quite a few syntheses and synthetic approaches have been reported for this class of compounds. This review focuses on the isolation, characterization, synthesis and biological activities of wakayin, tsitsikammamines, and zyzzyanones and their analogues.

2. Isolation

Many industrial and academic groups are accessing marine environments using advanced technology platforms. Liquid-solid chromatographic techniques such as HPLC or partition chromatographic methods are still the major tools for isolating pure compounds. A summary of bispyrroloquinone alkaloids, and the years of their isolation can be found below in Table 1.

2.a. Wakayin

Wakayin (**2**) is the first pyrroloiminoquinone alkaloid that was isolated from the ascidian *Clavelina* species, reported by Ireland and Copp's group in 1991 [49]. This was the first indication that pyrroloiminoquinone metabolites are not confined to marine sponges, tentatively suggesting a possible symbiont source for these bispyrroloiminoquinone metabolites. The MeOH-CHCl₃ extract of the ascidian was crudely partitioned by reverse phase flash chromatography using MeOH-aqueous trifluoroacetic acid solvent mixtures. Biologically active fractions were combined and purified by repeated elution through sephadex LH-20 affording the respective compound as a dark blue trifluoroacetate salt (0.005% wet weight). Structure elucidation of wakayin employed HRFABMS, UV analysis, and DQCOSY, HMQC, and HMBC NMR.

2.b. Tsitsikammamines A-B

In 1996, two bispyrroloiminoquinone alkaloids were isolated from a sponge collected from the Tsitsikamma Marine Reserve, South Africa. Coetzee *et al* [45] performed the initial separation of the alkaloids from a MeOH : CHCl₃ extract using a -18 Sep-Pak cartridge with a solvent elution gradient from water to MeOH. Further chromatography of the mixture on a variety of reverse phase HPLC columns, relying on a combination of antibiotic bioassays and ¹H NMR spectroscopy yielded tsitsikammamine A (**1a**, 0.04%) and tsitsikammamine B (**1b**, 0.045%) as dark green oils. Structure elucidation of these compounds employed ¹H-¹H COSY NMR, UV analysis, and HRFABMS. These structural compositions incorporated features of both the discorhabdins and makaluvamines. Years later in 2004, their corresponding N-18 oxime derivatives were obtained from *T. favus* [46]. Natural products containing either N-oxide or oxime moieties are rare in the marine environment. The reverse phase HPLC purification of these analogues required aqueous MeOH eluents containing 0.05% trifluoroacetic acid. Compounds (**1c**) and (**1d**) are considered to be isolation artifacts of the naturally occurring N-oxides of tsitsikammamines A and B.

2.c. Zyzzyanones A-D

Zyzzyanone A (**3a**), a bispyrroloquinone, was isolated along with a few makaluvamines and damirones from the Australian marine sponge *Z. fuliginosa* by Utkina's group in 2004 [47]. Often, this compound is referred as a biosynthetic *seco*-analogue of tsitsikammamines and is

the first representative of a new class of marine alkaloids containing a pyrrolo[3,2-f]indol-4,8(1H,7H)-dione skeleton. Briefly, the freeze-dried sponge (125 g) was extracted with 50% EtOH at room temperature. The extract was concentrated under reduced pressure to yield a dark red residue. This residue was subjected to column chromatography on a Polychrome-1 (powder Teflon) column with a solvent elution gradient from water to EtOH. A brownish-green fraction eluted with 50% EtOH was chromatographed on a Sephadex LH-20 column using CHCl₃–EtOH–TFA (3:1:0.1%) to yield zyzzyanone A (0.006% on the dry weight of the sponge). Zyzzyanone A can be seen to have a plausible interrelationship with the makaluvamines and with the tsitsikammamines.

One year later, continuous investigation on the same sponge in an effort to obtain more zyzzyanone A, the same group isolated three new minor bispyrroloquinones: zyzzyanone B (**3b**), an N1-demethyl analogue of zyzzyanone A; zyzzyanone C (**3c**), an N11-formyl analogue of zyzzyanone A; and zyzzyanone D (**3d**), an N11-formyl analogue of zyzzyanone B [48]. These scaffolds can also be seen to have a plausible interrelationship with the makaluvamines and with the tsitsikammamines.

3. Synthetic studies

A diverse library of bispyrroloquinone containing scaffolds is required for the understanding of the mechanism of bispyrroloquinone's biological activities. This opportunity has attracted synthetic chemists over the last few decades. Table 2 and the subsequent sections below summarize the striking work on the synthesis of wakayin, tsitsikammamines, zyzzyanones and their related compounds.

3.a. Synthesis of wakayin and its analogues

3.a.i. Oxidative formation of the pyrrole ring D of wakayin—Owing to its unique structure and potent antitumor activities, wakayin became an interesting target for the synthetic studies since its first report of isolation in 1991. The synthetic attempts have turned out to be quite challenging because of its highly hindered fused ring structure. So far, no successful synthesis of wakayin has been reported in the literature. However, synthetic attempts and synthesis of different analogues of wakayin have been reported. Cava *et al* reported the first attempt at the synthesis of wakayin analogues in 1998 [49] which describes a model reaction of oxidative formation of the critical pyrrole ring D of wakayin as shown in Scheme 1. The N-methyltryptamine (**4**) was reacted with 2-methoxynaphthoquinone (**5**) in refluxing EtOH to afford 2-(indol-3-ylethylamino)-naphthoquinone (**6**) in 91% yield. Further, oxidation of compound **6** using DDQ in HOAc afforded the desired model compound **7** in 78% yield.

3.a.ii. Formation of bispyrroloquinone ring system of wakayin—The second attempt at the synthesis of bispyrroloquinone ring system present in wakayin was reported by Barret *et al* in 1999 [50]. Bispyrroloquinone ring system was constructed in a single step starting from oxotryptamine (**8**) and N-tosylindole-4,7-quinone (**9**) as shown in Scheme 2. Addition of indole-4,7-quinone to the alcoholic solution of compound **8** goes through amination and oxidation to afford the intermediate **10** which then cyclizes to afford a single

regioisomer of the compound **11**. Tosyl group present in the compound **11** was removed by hydrolysis using NaOH in MeOH to afford compound **12**.

3.a.iii. Pyrazolic analogues of wakayin—In 2006, Delfourne *et al* synthesized several analogues of wakayin and tsitsikammamines in which one of the two pyrrole rings was replaced with a pyrazole ring [51]. A novel synthetic strategy was employed for making these analogues, which involved [3 + 2] cycloaddition of 3-ethylamine-indole-4,7-dione derivative (**19**) with different substituted diazo compounds. The quinone **19** was synthesized as shown in Scheme 3. The synthesis started with 4,7-dimethoxyindole (**13**), which in turn was prepared from 2,5-dimethoxybenzaldehyde according to a five-step procedure previously published [57]. Compound **13** was formylated using POCl₃ in DMF to afford dimethoxyindole-3-carboxaldehyde **14**. The Henry reaction of **14**, by treatment with CH₃NO₂ in the presence of NH₄OAc in HOAc, afforded the nitro compound **15**. The indole nitrogen of compound **15** was tosylated by treatment with *p*-toluene sulfonyl chloride in the presence of KOH. The resulting compound **16** was reduced using LiAlH₄ to the corresponding amino derivative **17**. The side chain amino group of **17** was then protected as the Boc derivative **18** by treatment with NaH and di-*tert*-butyl dicarbonate in THF. Oxidation of **18** by ceric ammonium nitrate (CAN) gave rise to quinone **19**.

The conversion of the quinone **19** to pyrazolic analogues is outlined in Scheme 4. [3+2] cycloaddition of adequately substituted diazo compounds (**20**) with the quinone **19** in THF gave two regioisomeric products **21a-c** and **24a-c**. Boc group deprotection of the side chain amino group using TFA in CH₂Cl₂ gave the corresponding amines as trifluoroacetate salts in good yields. Further, refluxing in EtOH in the presence of both 4 Å molecular sieves and NaHCO₃ afforded the cyclized compounds **22a-c** and **25a-c**. Finally, removal of tosyl group was performed using NaOH in dioxane to afford the pyrazolic analogues **23a-c** and **26a-c**.

3.a.iv. Diimidazole analogues of wakayin—In 2008, Skibbo *et al* succeeded in preparing the previously unreported diimidazolo[1,5,4-*de*, 1,5,4-*h*]-quinoxaline ring system as a structural analogue of wakayin. This synthesis permits the convenient incorporation of various R groups for the analogue development. The synthesis of diimidazole analogues is described in the Scheme 5. The first part of the synthesis is the preparation of the key intermediate **30** from which various substituted analogues were made. The synthesis started with the nitration of *m*-chlorophenol followed by methylation of the phenolic OH to yield 3-chloro-4-nitroanisole **27** [52]. Treatment of compound **27** with ethanolamine resulted in nucleophilic aromatic substitution to afford compound **28**. Catalytic reduction of **28** resulted in an *ortho* diamine that was immediately treated with HOAc to afford the Phillips cyclization product, **29**. *O*-Acetylation of compound **29** followed by nitration using fuming HNO₃ afforded the compound **30**.

Treatment of **30** with various primary alkyl amines in DMF resulted in nucleophilic aromatic substitution of the methoxy group along with deacetylation to afford the compounds **31a-d**. The alcoholic group in **31a-d** was converted to corresponding mesylates **32a-d** by treatment with MsCl and pyridine. Compounds **32a-d** were then converted to the tetrahydroquinoxoline intermediates **33a-d** by catalytic reduction of the nitro group followed

by internal nucleophilic displacement of mesylate. As these cyclization products are electron rich and susceptible to aerobic oxidation, **33a-d** were immediately cyclized to **34a-d** in the presence of acetic anhydride and HBr. Oxidation of compounds **34a-d** using Fremy's salt afforded the final products **35a-d** as orange yellow solids.

3.a.v. Open analogues of wakayin—Open analogues of wakayin were synthesized by Besson *et al* in 2000 [53] using a previously reported tricyclic pyrroloiminoquinone derivative **44** [58]. This synthesis used a quinoline derivative, 6,7-Dimethoxy-4-methylquinoline (**38**) as the starting material, which in turn was prepared by the reaction between 3,4-dimethoxyaniline (**36**) and methyl vinyl ketone (**37**) in refluxing HOAc in the presence of FeCl₃. Nitration of **38** led to the 5- nitro compound **39** in a good yield. Oxidation of the methyl group of **39** to aldehyde by Vismara's method was achieved by treatment with iodine, TFA, tert-butyl iodide and FeCl₂ in DMSO to obtain the compound **40**.

The aldehyde group in compound **40** was then protected as a dimethyl acetal to give **41**. The pyridine ring and the nitro group were simultaneously reduced by an excess of NaBH₄ in MeOH with NiCl₂ to afford diamine **42** in a very good yield. Heating of compound **42** at 80°C in aqueous HCl and THF led to the cyclization of pyrrole ring resulting in compound **43**. Amino indole **43** was treated with CAN in aqueous acetonitrile to generate the quinone **44**. C-7 methoxy group present in compound **44** was substituted with various amines *in situ*, without isolating the intermediate to afford the open chain analogues of wakayin **45a-e**.

3.b. Synthesis of tsitsikammamine and its analogues

3.b.i. Synthesis of tsitsikammamine A—A concise route to the marine bispyrroloiminoquinone alkaloid tsitsikammamine A (**1a**) and its regioisomeric analogue (**58**) was developed by Delfourne *et al* in 2009 as outlined in Scheme 7. Reaction of compound **46** with indole-4,7-quinone derivative **47** in absolute EtOH gave two regioisomeric aminated products, **48** and **49**. The formation of the second five-membered nitrogen ring in **48** was achieved by direct cyclization in TFA and CH₂Cl₂, and was concomitant with the cleavage of the Boc protecting group to give compound **50**. MnO₂ oxidation of compound **50** gave the bispyrroloquinone derivative **51**, which was subsequently cyclized into the corresponding iminoquinone **52**. The tosyl group of **52** was then removed by a reaction with 1N NaOH in dioxane followed by demethylation using BBr₃ affording tsitsikammamine A (**1a**).

Following same set of synthetic reactions the same paper converted compound **49** to a regioisomeric analogue (**58**) of tsitsikammamine A as shown in Scheme 8.

3.b.ii. Aza analogues of wakayin and tsitsikammamines—Two classes of aza analogues of tsitsikammamines have been synthesized [55]. The strategy used was based on the 1,3-dipolar cycloaddition reaction between indole 4,7-dione and a diazoaminopropane derivative. The cycloaddition reaction involving N-tosylindole-4,7-dione (**59**) and diazo-Moz-aminopropane (**60**) gave a mixture of two regioisomers **61a/b**. The mixture of cycloadducts **61a/b** was tosylated prior to the cleavage of the Moz protecting group to give

compounds **62a/b**. The Moz-protective group in **62a/b** was cleaved quantitatively by TFA in the presence of m-cresol to give the corresponding salts **63a/b**, which were cyclized into the tetracyclic derivatives **64a/b** as a mixture using 4 Å molecular sieves in EtOH. Compounds **64a** and **64b** were then separated by flash chromatography. Finally, the two tosyl groups present in **64a** and **64b** were cleaved by treatment with NaOH in dioxane to afford the target aza analogues **65a** and **65b**.

3.c. Synthesis of zyzzyanones

3.c.i. Zyzzyanone A—The first total synthesis reported for zyzzyanones A-D was published by the Velu group in 2013 [56]. Scheme 10 outlines the synthesis of zyzzyanone A (**3a**), which begins with the previously known [59] *N*-tosyl-6-(methoxy)-1*H*-indole-4,7-dione derivative containing a (N-Boc)ethyl amine side chain (**66**).

Addition of benzyl amine to **66** in a mixture of MeOH and THF (1:1) at room temperature produced the aminated compound **67** with a 78 % yield. Next, in the presence of Mn(OAc)₃ in acetonitrile at 80°C, treatment of benzyloxyphenyl acetaldehyde diethyl acetal (**68**) resulted in the formation of bispyrroloquinone derivative **69** in 72 % yield. Methylation of compound **69** was carried out using MeI in the presence of NaH in anhydrous DMF at room temperature. The expected monomethylated compound **70**, along with the unexpected dimethylated compound **71** was produced in 46 % and 34 % yields. Compound **71** is presumably formed by the removal of N-tosyl group from **70** followed by a second N-methylation, both steps occurring in the same reaction. Debenzylation of compound **71** was carried out with Pd black in the presence of HCOONH₄ in anhydrous EtOH to afford compound **72** in 55 % yield. Finally, removal of the N-Boc group present in compound **72** was carried out through the use of TFA in CH₂Cl₂ to afford zyzzyanone A (**3a**) as a TFA salt in 81 % yield.

3.c.ii. Zyzzyanone B—The synthesis of zyzzyanone B (**3b**) is outlined in Scheme 11. To begin, detosylation of **70** by treatment with NaN₃ in anhydrous DMF for 4 hours resulted in compound **73** with a 75 % yield. Next, debenzylation occurred using Pd black in the presence of HCOONH₄ in anhydrous EtOH, which resulted in the removal of both O- and N- benzyl groups to afford compound **74** in 53 % yield. Removal of the Boc group from compound **74** using 1:1 mixture of TFA and CH₂Cl₂ at room temperature resulted in the formation of zyzzyanone B (**3b**) as a TFA salt in 85 % yield.

3.c.iii. Zyzzyanone C—Zyzzyanone C (**3c**) was synthesized as outlined in Scheme 12. Removal of the Boc group from compound **71** and immediate formylation of the crude product using HCOOEt and Et₃N at reflux conditions produced the N-formylated intermediate **75** in 72 % yield. Next, Pd black was used to cleave both O- and N- benzyl groups present in compound **75** in the presence of HCOONH₄ in refluxing EtOH to afford zyzzyanone C (**3c**) in 55 % yield.

3.c.iv. Zyzzyanone D—Scheme 13 demonstrates the conversion of compound **72** to zyzzyanone D (**3d**). 1:1 mixture of TFA and CH₂Cl₂ was used to remove the Boc group to obtain the amino compound which was immediately subjected to formylation using

HCOOEt in the presence of Et₃N under reflux conditions to afford compound **76** in 64 % yield. The expected formylation along with detosylation were observed. Both N-benzyl and O-benzyl groups present in compound **76** were removed by treatment with Pd black in the presence of HCOONH₄ in EtOH under reflux conditions to afford zyzzyanone D (**3d**) in 58 % yield.

4. Bioactivities

Bispyrroloquinone alkaloids have gained considerable attention within the past two decades not only due to their intriguing structures but also due to their encouraging pharmacological bioactivities, namely their cytotoxicity and antitumor activity.

Novel emerging natural products that show cytotoxicity and antitumor activity are often evaluated for their effects on topoisomerases because they are a viable target for the development of cancer drugs. These enzymes regulate the overwinding or underwinding of supercoiled DNA strands during DNA replication, transcription, and various other stages of normal cellular functioning. The two types, I and II, differ in their structures, mechanisms, and cellular functions. Type I enzyme exists as a monomer and causes transient single strand breaks in duplex DNA whereas the type II isozyme is dimeric and causes cuts in both strands of duplex DNA. In most of the evaluations, the clinical drug camptothecin is often used as a positive control for topoisomerase I inhibition while etoposide is used as a positive control for topoisomerase II inhibition [60-62].

Since marine alkaloids have shown a considerable affinity to both isozymes [42, 46, 63-65], many research groups have evaluated topoisomerase inhibition of wakayin and tsitsikammamine analogues. The sections below will provide a brief summary of the key contributions made in this context as well as other bioactivities reported for wakayin, tsitsikammamines, zyzzyanones, and their analogues.

4.a. Biological activities of wakayin and tsitsikammamines

In 1991, along with the isolation and structure elucidation of wakayin, Ireland *et al* also reported its *in vitro* cytotoxicity against the human colon tumor cell line, HCT116. Antimicrobial activity against *Bacillus subtilis* MIC 0.3 µg/mL was also observed. Its 3-fold differential toxicity toward the Chinese hamster ovary cell line EM9 (sensitive to DNA damaging genotoxic agents) versus BRI (resistant to carmustine) provided preliminary evidence that wakayin exhibits cytotoxicity by interfering with or damaging DNA. Evaluation of its inhibitory effects on topoisomerase I and II resulted in IC₅₀ values of 10 and 250 µM, respectively. Thus, wakayin is an inhibitor of topoisomerases and is also shown to be a strong DNA binder.

Similarly, along with the details of the isolation of Tsitsikammamines in 1996, Coetzee *et al* reported both compounds exhibited antimicrobial activity against *Bacillus subtilis*. Initial preliminary studies have shown that while tsitsikammamines are cytotoxic and exhibit anti-fungal activity, they do not inhibit either topoisomerase I or II. In addition, cell growth inhibitory activities against the HCT-116 cell line were reported to be 1.4 and 2.4 µM for tsitsikammamines A and B, respectively.

Following this, when the novel N-18 oxime analogues of tsitsikammamines A and B were first isolated from *T. favus* extracts in 2004, Coleman *et al* compared the *in vitro* activity of wakayin and all four tsitsikammamines compounds against HCT-116 (Table 5). The N-oxime analogues of tsitsikammamines A and B were shown to be less active compared to the original scaffolds [46].

These four compounds were also evaluated for their topoisomerase inhibition, as a possible target of the observed cytotoxicity. Authors believed that due to the structural homology between wakayin and the tsitsikammamines, tsitsikammamines might exhibit analogous topoisomerase I inhibition and DNA intercalation, even though the preliminary studies in 1996 did not observe topoisomerase inhibition.

Results of their study demonstrated tsitsikammamines to exhibit similar topoisomerase I inhibition to wakayin [66], which required 10-100-fold the molar concentrations that camptothecin requires to elicit DNA cleavage through topoisomerase I inhibition. The micromolar concentration (K_s) of a compound that decreases DNA-bound ethidium bromide fluorescence by 50% is routinely used to quantify DNA intercalation. The strong intercalating ability of wakayin is reflected in a K_s value of 20 μ M. Of the four tsitsikammamines, (**1a**) and its N- oxime (**1c**) exhibited similar DNA binding to wakayin (**2**), while (**1b**) and its N-oxime (**1d**) displayed a weaker DNA binding in comparison to wakayin.

4.a.i Pyrazolic analogues of wakayin and tsitsikammamine—Encouraged by the results obtained from the natural compounds, pyrazolic analogues of the marine alkaloids wakayin and tsitsikammamines A and B that were synthesized using [3 + 2] cycloaddition reactions (Scheme 4) were evaluated *in vitro* for antiproliferative activity against five distinct cancer cell lines and for their inhibitory effect on topoisomerases I and II [51]. Only a few of them exhibited cytotoxic activity with IC_{50} values in the micromolar range. A subset of the compounds showed inhibition of topoisomerase I and/or II catalyzed relaxation of supercoiled DNA at a concentration comparable to the drugs camptothecin and etoposide. Table 4 summarizes the key compounds exhibiting good bioactivities.

Compounds **22b** and **25b** structurally parallel tsitsikammamine A, in which one pyrrole ring is modified to a pyrazole ring. These two most active analogues demonstrated a 3- and 10-fold improved activity in topoisomerase I inhibition respectively. **25b** was the sole scaffold to also inhibit human topoisomerase II activity at a concentration comparable to that of the control drug etoposide (100 μ M).

Three human cancer cell lines (HCT15 and HT29, colon; PC3, prostate) and two murine cell lines (CT-26, colon; Renca, kidney) were used for further *in vitro* evaluation of their antiproliferative activities. Compounds **22a**, **25a**, and **25c** demonstrated antiproliferative effect, blockage of cell proliferation in the micromolar range, but these compounds showed no relevant inhibition of topoisomerases. Thus, these analogues use another mechanism for their cytotoxic activity against cancer cells. Despite the promising topoisomerase inhibition observed by **22b** and **25b**, these scaffolds did not inhibit the cell growth of tested tumoral

cell lines. Author hypothesize that this could be a result of poor cell penetration as their log P values of 3.6 show they are fairly lipophilic.

4.a.ii. Diimidazole analogues of wakayin—The four analogues of wakayin based on diimidazo[1,5,4-de, 1,5,4-h]-quinoxaline (**35a-d**) that were described in Scheme 5 were subjected to an extensive investigation for their cytostatic and cytotoxic activity using a 60 human cancer cell-line panel consisting of the major histological tissue types [52]. The concentrations of drug required for 50% growth inhibition (GI₅₀), total growth inhibition (TGI), and the concentration required for 50% cell death (LC₅₀) were reported for each compound. Results showed that the cyclopropyl derivative **35c** was inactive against all cell lines. However, the methyl and phenethyl derivatives, **35a** and **35b**, displayed a degree of cytostatic activity against some histological cancer types. Compounds **35a** and **35b** were most active against the ovarian and melanoma cancer panels, respectively. However, **35d** performed with the highest activity against leukemia and renal histological cancer types. This compound fared the strongest in the renal panel because of its cytostatic activity against the renal cancer cell line A498. The 10,000-fold increase in cytostatic activity accompanying the change from methyl to methoxyethyl indicates that such oxygen-containing side chains should be the subject of further development.

4.a.iii. Open analogues of wakayin—Besson *et al* evaluated five analogues (**42a-42e**) described in Scheme 6 for their effects on cell proliferation and cell cycle progression on a murine L1210 leukemia cell line. [53]. IC₅₀ values are reported in the Table 5. Compound **42a** represents a strict open analogue of wakayin. This compound is the least active of the four tested compounds on the L1210 cell line. As a result, the authors suggest that the enhanced flexibility of the substituent at C-7 may not be favorable for interaction with biological targets. However, compounds **42b-e** all inhibited the growth of L1210 cells at concentrations less than 0.5 μM. This difference in activity could be attributed to the compounds' water solubility due to the substitution by a primary amino or an amino alkyl group. Despite interesting antiproliferative properties, none of the pyrroloiminoquinone derivatives induce significant modification of the L1210 cell cycle, proving that the pyrroloiminoquinone skeleton on its own is not able to exert a specific cytotoxic action.

4.a.iv. Aza analogues of tsitsikammamine—Several aza-analogues of natural products have been reported to have better antitumor activity compared to those of the corresponding natural products. In 2006, Delfourne group synthesized compounds **65a** and **65b** (Scheme 9) in which the pyrrole ring of the pyrroloquinone moiety has been replaced by a pyrazole ring to represent aza analogues of both wakayin and tsitsikammamine [55]. These scaffolds were subjected to a cell-free assay to study their ability to inhibit the DNA cleavage activities of human topoisomerases I and II. Unfortunately, no inhibition of topoisomerase II activity was observed at the maximum tested concentration, limited by final DMSO concentration in the assay mixture by both **65a** and **65b**. Additionally, **65b** produced no effect on topoisomerase I also. A partial activity was observed at 100 μM for **65a** against topoisomerase I. Surprisingly, the precursor tricyclic analogue mixture of **61a/b** inhibited the catalytic activity of topoisomerase I, with potency comparable to that of camptothecin.

4.a.v. Enzymatic IDO1 and MTT results of various cancer cell lines exhibited by tsitsikammamine analogues—

Tsitsikammamines analogues and the corresponding synthetic intermediates that were described in scheme 7 and 8 were evaluated in an *in vitro* antiproliferative assay against the U373 glioblastoma, A549 non-small-cell lung cancer, and PC-3 prostate cancer cell lines as well as two human normal fibroblast cell lines (NHDF and Wi38) [54]. These cell lines were specifically chosen because they display distinct sensitivities to various cytotoxic compounds. For example both U373 and A549 cell lines are resistant to apoptosis, while PC-3 cells are sensitive to apoptosis. Likewise, U373 is sensitive to autophagy-related cell death and A549 is sensitive to lysosomal membrane permeabilization related cell death. Thus, this combination of cell lines serves to elucidate the mechanism of action of the tsitsikammamines analogues for their antiproliferative activity. The result of this study is summarized in Table 6.

Compounds **48** and **49** displayed significant anti-tumor activity against PC-3 prostate cancer cells while having minimal effect against U373 GBM and A549 NSCLC cells, suggesting activation of apoptosis. In contrast, compounds **56** and **52** may employ non-apoptotic cell death processes for anti-tumor activity.

Compound's regiochemistry consistent with the natural product tsitsikammamine A usually appeared to display higher *in vitro* antitumor activity than their regioisomers (eg: **58**, **1a**, **51-55**). Compound **51** emerged to be the most active; it was the only analogue to achieve single digit μM range growth inhibitory IC_{50} *in vitro* for the three cell lines under study. Authors concluded that **51** and its derivatives represent interesting novel anti-cancer agents whose effects could be associated with a specific antiproliferative activity.

Three years later, the same group revisited this series of analogues to elucidate the mechanism of cell toxicity and have shown these compounds exert inhibitory activity on indoleamine 2,3-dioxygenase, an enzyme involved in tumoral immune resistance [67]. Indoleamine 2,3-dioxygenase (IDO1) is an enzyme that exerts its immunosuppressive effects on T-lymphocytes in a twofold manner: by depleting Trp locally and by action of toxic Trp catabolites. Tryptophan 2,3-dioxygenase (TDO) has also recently been shown to be expressed constitutively in human glioblastomas where it promotes tumor progression by action of a downstream tryptophan catabolite kynurenine which acts as an endogenous ligand of the aryl hydrocarbon receptor. It has been shown that TDO inhibition reverses tumoral immune resistance *in vivo*. Thus the recent years witnessed many attempts to discover and develop IDO and TDO inhibitors [68-70].

The key results of the inhibitory study of tsitsikammamine derivatives and synthetic intermediates on IDO1 and TDO are summarized in the Table 6. Consistent with the *in vitro* activity in the MTT colorimetric assay, the regioisomer leading to the natural geometry of the final tetracyclic product, **1a**, was at least twice as active on IDO1 in the enzymatic assay than the corresponding regioisomer (eg: **55-51**, **54-50**, **56-52**). Secondly, deletion of the conjugated double bond within the anisyl moiety in the tricyclic series was found to improve the bioactivity in the enzymatic assays (eg: **55-54**, **51-50**). Compounds bearing this structural modification produced greater than 40% IDO1 inhibition at $10\mu\text{M}$. Lastly, the tetracyclic iminioquinone ring enhances the cellular toxicity (eg: **55-56**, **51-52**). It should be

noted that no inhibition on TDO was observed, establishing the selectivity of these compounds for IDO1. Thus, these series of compounds show potential for future development in the field of anticancer immunotherapy.

4.b Bioactivities of Zyzzyanones

Initial biological evaluation of zyzzyanones revealed that they exhibit moderate cytotoxic activity against mouse Ehrlich carcinoma cells (IC₅₀, 25 µg/mL) for all four natural products. Zyzzyanone A inhibited the cell division of fertilized sea urchin eggs at concentration of 25µg/mL and also exhibited UV-A and UV-B absorbing activity. These data support the earlier suggestion that the intact pyrroloiminoquinone moiety enhances cytotoxicity. No new reports have been made following these results.

5. Conclusions

Oceans account for a major proportion of the earth's surface. Marine natural products are continuously isolated from the wide range of invertebrate species found in oceans such as sponges, mollusks, ascidians, corals, and algae. A class of structural scaffolds known as bispyrroloquinones is present in three subclasses of biologically active marine alkaloids: wakayin, tsitsikammamines, and zyzzyanones. Since their first isolation and preliminary biological evaluation was published, several reports of their total synthesis and the synthesis of their analogues have emerged. Both tsitsikammamines and zyzzyanones have been synthesized to its final structure. Though several efforts have been made by different groups to accomplish total synthesis of wakayin, none have been successfully developed till date. However, aza and pyrazolic analogues as well as open analogues of wakayin have been reported.

Several derivatives of tsitsikammamines and wakayin have previously been shown to be potent anticancer agents. Mechanism of action of tsitsikammamines is reported to be through inhibition of topoisomerases. One additional possible mechanism of anticancer activity of tsitsikammamines analogues stems from their inhibition on indoleamine 2,3-dioxygenase, an enzyme involved in tumoral immune resistance. However, further investigation on the exact mechanism for their bioactivity is still required for many analogues, which will be beneficial for the ongoing development and lead optimization of this class of compounds accomplished by several groups. Nevertheless, bispyrroloquinones have established their potential for future development as anticancer agents and in our opinion, this class of molecules deserve more scientific attention and interdisciplinary research to see a transition into a number of new treatments for cancer in the years to come.

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List of acronyms

BBr₃	Boron tribromide
Boc	<i>tert</i> -Butoxycarbonyl
CH₂Cl₂	Methylene chloride
CHCl₃	Chloroform
CH₃NO₂	Nitromethane
COSY	Correlation spectroscopy
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxy ribonucleic acid
DQCOSY	Double quantum filtered COSY
EtOH	Ethanol
FeCl₃	Ferric chloride
FeCl₂	Ferrous chloride
HBr	Hydrobromic acid
HCl	Hydrochloric acid
HMBC	Heteronuclear multiple bond coherence
HMQC	Heteronuclear multiple quantum coherence
HNO₃	Nitric acid
HOAc	Acetic acid
HPLC	High performance liquid chromatography
HRFABMS	High resolution fast atom bombardment mass spectroscopy
IC₅₀	Inhibitory concentration at 50%

KOH	Potassium hydroxide
LiAlH₄	Lithium aluminum hydride
MeI	Methyl iodide
MeOH	Methanol
MIC	Minimum inhibitory concentration
MnO₂	Manganese dioxide
MsCl	Methane sulfonyl chloride
NaH	Sodium hydride
NaHCO₃	Sodium bicarbonate
NaN₃	Sodium azide
NaOH	Sodium hydroxide
NH₄OAc	Ammonium acetate
NiCl₂	Nickel chloride
NMR	Nuclear magnetic resonance
Pd	Palladium
POCl₃	Phosphoryl chloride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
UV	Ultraviolet

Figure 1. Marine derived drugs

Figure 2. Representative bispyrroloquinone alkaloids isolated from marine sources

Scheme 1. Oxidative formation of the pyrrole ring D of wakayin

Scheme 2. Formation of bispyrroloquinone ring system of wakayin

Scheme 3. Synthesis of 3-ethylamine-indole- 4,7-dione derivative

Scheme 4. Synthesis of pyrazolic analogues of wakayin

Scheme 5. Synthesis of diimidazole analogues of wakayin

Scheme 6. Synthesis of an open analogue of wakayin

Scheme 7. Synthesis of tsitsikammamine A

Scheme 8. Synthesis of regioisomer of tsitsikammamine A

Scheme 9. Synthesis of aza analogues of tsitsikammamines

Scheme 10. Synthesis of zyzzyanone A

Scheme 11. Synthesis of zyzzyanone B

Scheme 12. Synthesis of zyzzyanone C

Scheme 13. Synthesis of zyzzyanone D

Table 1
Source and year of discovery of Bispyrroloquinone alkaloids

Natural product	Year	Marine source	Locality	Authors
Wakayin	1991	<i>Ascidia clavelina</i>	N/A	Ireland <i>et al</i>
Tsitsikammamines A & B	1996	<i>Tsitsikamma favus</i>	Algoa Bay, South Africa	Coetzee and Davies-Coleman <i>et al</i>
N-18oxime tsitsikammamines analogue	2004	<i>Tsitsikamma favus</i>	Southeastern coast of South Africa	Davies-Coleman <i>et al</i>
Zyzzyanone A	2004	<i>Zyzzya fuliginosa</i>	Australia	Utkina <i>et al</i>
Zyzzyanone B-D	2005	<i>Zyzzya fuliginosa</i>	Australia	Utkina <i>et al</i>

Table 2
Summary of synthesis of bispyrroloquinone natural products and their analogues

Author	Year	Description of synthetic contribution	Scheme	Reference
Cava <i>et al</i>	1998	Model reaction: oxidative formation of pyrrole ring of wakayin	1	[49]
Barret <i>et al</i>	1999	Bispyrroloquinone ring system of wakayin	2	[50]
Delfourne <i>et al</i>	2006	Several pyrazolic analogues of wakayin and tsitsikammamine	3,4	[51]
Skibbo <i>et al</i>	2008	Diimidazole structural analogues of wakayin	5	[52]
Besson <i>et al</i>	2000	Open analogues of wakayin	6	[53]
Delfourne <i>et al</i>	2009	Total synthesis of tsitsikammamine A-B	7,8	[54]
Delfourne <i>et al</i>	2006	Aza analogues of wakayin and tsitsikammamine	9	[55]
Velu <i>et al</i>	2013	Total synthesis of Zyzzyanones A-D	10-13	[56]

Table 3
Cytotoxicity activities against the human colon tumor cell line and topoisomerase I inhibition of tsitsikammamines and wakayin

Structure	Compound ID	HCT-116 (IC ₅₀ , μ M)	K _s (μ M)
Tsitsikammamine A	1a	1.4	15
Tsitsikammamine B	1b	2.4	45
Tsitsikammamine A N-Oxime	1c	128.2	20
Tsitsikammamine B N-Oxime	1d	16.5	30
Wakayin	2	1.5	20

Table 4
Bioactivity of pyrazolic analogues of wakayin and tsitsikammamines

Compd ID	Structure	Topoisomerase Inhibition (IC ₅₀ , μ M)		Inhibition of Cancer cell proliferation (IC ₅₀ , μ M) or % inhibition @ 10 μ M				
		<i>hTopoI</i>	<i>hTopoII</i>	<i>HCT15</i>	<i>HT29</i>	<i>PC3</i>	<i>CT26</i>	<i>Renca</i>
22a		300	ND	6.5	3.1	4.9	5.3	0.14
22b		30	>100	0%	23%	0%	0%	0%
25a		>100	>100	5	1.9	5.8	4.9	4.6
25b		10	100	0%	41%	0%	0%	0%

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Compd ID	Structure	Topoisomerase Inhibition (IC ₅₀ , μM)		Inhibition of Cancer cell proliferation (IC ₅₀ , μM) or % inhibition @ 10 μM					
		<i>hTopoI</i>	<i>hTopoII</i>	<i>HCT15</i>	<i>HT29</i>	<i>PC3</i>	<i>CT26</i>	<i>Renca</i>	
25c		>100	ND	1.6	2.9	5.2	2.9	1.9	
Etop		ND	100	4.5	6.6	7.8	0.2	2.9	
Camp		100	ND	0.018	0.030	0.052	0.034	0.304	

Etop: etoposide, Camp: camptothecin, ND: not determined.

Table 5
Bioactivity of open analogues of wakayin against L1210

Compound ID	Structure ^a	IC ₅₀ (μM)
42a		5.3
42b		0.3
42c		0.38
42d		2.1
42e		0.25

^a All compounds are isolated as TFA salts.

Table 6
Activities of tsitsikammamine analogues against enzymatic IDO1 and MTT results on various cancer cell lines

Compd ID	Structure	U373 GBM IC ₅₀ (μM)	A549 NSCLC IC ₅₀ (μM)	PC-3 IC ₅₀ (μM)	Mean IC ₅₀ (μM) enzymatic assay	mIDO1 inh. @ 10 μM (%)	mTDO1 inh. @ 10 μM (%)	Cell viability @ 10 μM (%)
48		>100	>100	9.5	26.9	31.3	NI	>90
49		>100	>100	9.7	10.1	17.4	NI	>90
50		48.5	52.4	33.7	2.8	26.1	NI	83
51		3.2	4.1	4.2	5.4	15.6	NI	80
52		57.4	15.4	>100	2.2	32.0	3.1	>90

Compd ID	Structure	U373 GBM IC ₅₀ (μM)	A549 NSCLC IC ₅₀ (μM)	PC-3 IC ₅₀ (μM)	Mean IC ₅₀ (μM) enzymatic assay	mIDO1 inh. @ 10 μM (%)	mTDO1 inh. @ 10 μM (%)	Cell viability@ 10 μM (%)
53		>100	>100	>3	0.9	19.4	NI	>90
54		28.3	35.7	26.1	5.6	40.2	4.7	>90
55		8.5	11.2	31.3	11.9	26.0	NI	>90
56		41.4	3.1	>100	4.2	ND	ND	<50
57		4.1	38.1	55.7	7.1	ND	ND	<50

Compd ID	Structure	U373 GBM IC ₅₀ (μM)	A549 NSCLC IC ₅₀ (μM)	PC-3 IC ₅₀ (μM)	Mean IC ₅₀ (μM) enzymatic assay	mIDO1 inh. @ 10 μM (%)	mTDO1 inh. @ 10 μM (%)	Cell viability@ 10 μM (%)
58		62.2	35.2	63.7	7.0	5.3	NI	>90
1a		18.1	15.6	23.0	NA	NA	NA	NA

NA: not available; NI: no inhibition; ND: not determined.