THE TAMBJAMINES: PYRROLYLPYRROMETHENE-CONTAINING ALKALOIDS WITH DIVERSE BIOLOGICAL PROFILES

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Abstract. *A group of yellow-pigmented natural products often derived from marine sources and collectively known as the tambjamines incorporate a methoxy-substituted pyrrolylpyrromethene core and vary in the nature of the alkyl groups attached to a third and exocyclic nitrogen. These fascinating alkaloids are both structurally and biogentically related to the better-known prodigiosin class of natural product and, like them, the tambjamines display a remarkable array of potentially useful biological properties. As such they are attracting increasing attention as possible leads for the development of a range of new therapeutic agents. This review covers the relevant scientific literature on the tambjamines up to mid-2023.*

Contents

1. Introduction

The title akaloids, most of which are derived from marine sources, take the general form **1** (Figure 1) and so embody a methoxy-substituted pyrrolylpyrromethene core. They vary in the nature of the R group attached to the exocyclic nitrogen and can also incorporate one or two bromines on the pyrrolic ring. They are structurally and biogentically related to the better-known, tripyrrole-containing prodigiosins. The most immediately striking feature of these compounds are their colors, the tambjamines being yellow while the prodigiosins, including the eponymous **2**, are blood-red. On the other hand, the "dimeric" or dipyrrolyldipyrromethane **3** (which has been isolated, like **2**, and as its hydrochloride salt, from, *inter alia*, *Serratia marcescens*) is blue in color.[1] Beyond being sought for their potential as natural colorants,[2] such compounds display a fascinating range of biological properties and have thus been the subjects of considerable research, especially in more recent times. In contrast to the much more extensively studied prodigiosins,[3] the tambjamines are only now being recognised as warranting detailed investigation. The purpose of this chapter, then, is to provide the reader with what we believe to be the first comprehensive review[4] of the tambjamines in the hope that this stimulates further research on these fascinating compounds.

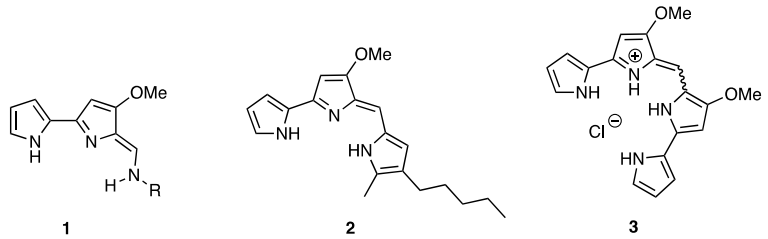
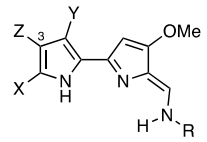


Figure 1. The core structure **1** of the tambjamines, the structure of prodigiosin **2** and that of the "dimeric" dipyrrolyldipyrromethene **3** (shown as the hydrochloride salt).

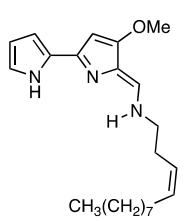
The material presented herein is based upon searching the scientific literature, using both Google (including Google Scholar) and SciFinder, in late July 2023.

2. Isolation, structural elucidation, ecological roles and distribution of the producing organisms

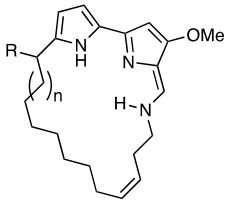
While the prodigiosins have been, by virue of their spectacular coloring, known for millennia[3b] (and the structure of the parent system **2** correctly assigned[5] and then synthesised[6] some seventy years ago), the first four members of the tambjamine family, *viz*. congeners A-D **4**-**7**, respectively, (Figure 2) were reported by Carte´and Faulkner in 1983.[7] These were isolated from the nembrothid nudibranchs (molluscs) *Tambje abdere*, *T. eliora* and *Roboastra tigris* collected in the Gulf of California. Structural elucidation was facilitated by their ready hydrolysis, involving cleavage of the exocyclic imine residue, to the corresponding dipyrrole carboxaldehyes, the non-brominated form of which was known from studies of the prodigiosins. Various ecological observations[7,8] established that these tambjamines are derived from the bryozoan *Sessibugula translucens*, a food source for the nudibranchs. Clearly, while the tambjamines are seen as defensive chemicals produced by the bryozoan they do not deter *Tambje abdere* that appears to have assimilated these compounds for their own defence against the carnivorous *Roboastra tigris*.



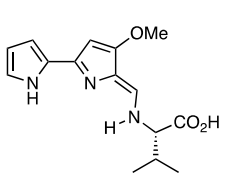
4 (R=X=Y=Z=H) [tambjamine A]
5 (R=Y=Z=H, X=Br) [tambjamine B]
6 [R=$CH_2CH(CH_3)_2$, X=Y=Z=H] [tambjamine C]
7 [R=$CH_2CH(CH_3)_2$, X=Z=H, Y=Br] [[tambjamine D]
8 (R=CH_2CH_3, X=Y=Z=H) [tambjamine E]
9 (CH_2CH_2Ph, X=Y=Z=H) [tambjamine F]
10 (R=CH_2CH_3, X=Br, Y=Z=H) [tambjamine G]
11 (R=$CH_2CH_2CH_3$, X=Br, Y=Z=H) [tambjamine H]
12 [R=$CH_2CH(CH_3)_2$, X=Br, Y=Z=H] [tambjamine I]
13 [R=$CH_2CH(CH_3)CH_2CH_3$, X=Br, Y=Z=H] [tambjamine J]
14 [R=$CH_2CH(CH_3)CH_2CH_3$, X=Z=H, Y=Br] [tambjamine J1]
15 [R=$CH_2CH_2CH(CH_3)_2$, X=Y=Z=H] [tambjamine K]
16 (R=CH_2CH_3, X=Y=H, Z=Br) [tambjamine L]
17 [R=$(CH_2)_{11}CH_3$], X=Y=Z=H [BE-18591]



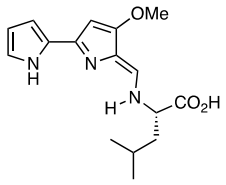
18 [tambjamine YP1]



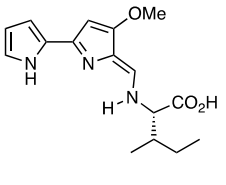
19 (R=H, n=1) [tambjamine MYP1]
20 (R=CH_3, n=2) [paucibactin A]



21 [tambjamine M]



22 [tambjamine N]



23 [tambjamine O]

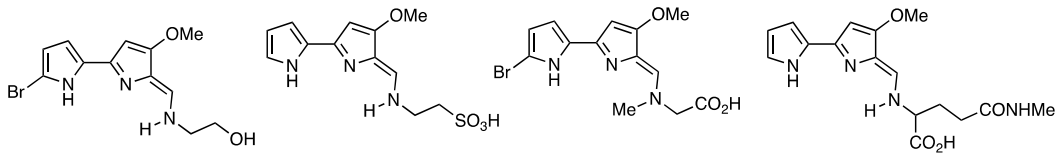
Figure 2. The established structures **4**-**23** of all the tambjamine-type natural products reported to date.

In 1990, Fenical and co-workers reported the isolation of non-brominated tambjamines A **4**, C **6**, E **8**, and F **9** (the latter pair being new ones) as well as the corresponding tambjamine aldehyde and the tetrapyrrole **3** from the nudibranch predators to the marine ascidian (sea squirt) *Atapozoa* sp. collected in the Western Pacific.[9] Various studies led to the conclusion, at that time, that this sea squirt was capable of the *de novo* biosynthesis of these compounds.[9] A 1994 study[10] of the alkaloids isolated from samples of the marine bryozoan *Bugula dentata* collected in Southern Tasmania resulted in the identification of four new

tambjamines, namely congeners G **10**, H **11**, I **12**, and J **13**, as well as the previously reported compounds C **6**, and E **8**. Berlinck and co-workers[11] subsequently isolated, from a specimen of *B. dentata* collected in Brazilian waters, an isomer of tambjamine J **13** which they denoted as J1 and to which they assigned structure **14**. Accompanying this alkaloid were congeners A **4**, C **6**, D **7**, and tambjamine K **15**. The last of these compounds had previously been isolated from the Azorean nudibranch *Tambja ceutae* that feeds on *B. dentata*.[12] The latter organism was also found to contain tambjamines A **4** and C **6** as well as the by now ubiquitous co-isolate **3**. Tambjamine L **16** was, on the other hand, isolated from both the nudibranch *Tambja capensis* and its bryozoan food source *B. dentata*, specimens of these having been being collected in temperate South African waters.[13] The structure of metabolite **16** is notable in that it is the first and thus far only tambjamine to be obtained that incorporates a bromine at the C3 position on the pyrrole ring. Accompanying compound **16** were congeners A **4**, E **8**, and K **15** but the dominant co-isolate was, once again, tetrapyrrole **3**.

The first example of a tambjamine to be isolated from terrestrial sources was BE-18591 **17**, this "yellowish green" compound being reported by Suda *et al*[14] who obtained it, through fermentation, from the *Streptomyces* strain BA18591 found associated with a plant sample collected in Hamamatsu, Shizuoka Prefecture, Japan. The structure of alkaloid **17** is notable in that it is also the first tambjamine to be found that embodies a fatty amine residue (*viz*. 1-dodecylamine). Interestingly, more than a decade later Kumar and co-workers reported[15] the isolation and structural elucidation of an unsaturated analogue, **18**, of compound **17** that was subsequently dubbed tambjamine YP1.[16] This yellow pigment was originally isolated from the marine bacterium *Pseudoalteromonas tunicata*. Biosynthetic considerations and analyses then led, in 2019, Ross and co-workers to report[17] on the isolation of a macrocyclic analogue of alkaloid **18**, namely tambjamine MYP1 **19**, which was derived from the marine bacterium *Pseudoalteromonas citrea*. The structure of compound **19** was confirmed by single-crystal X-ray analysis. Very recently, Ahn *et al* have described[18] the homologous and methylated tambjamine macrocycle paucibactin A **20**, obtained from the marine bacterium *Paucibacter aquatile* DH15 found in the Daechung Reservoir, South Korea.

In a further variation on the more common tambjamine structures (as represented by compounds **4**-**16**), the Berlinck group have reported,[19] by using metabolomic techniques, the isolation and then the structural elucidation of congeners M **21**, N **22**, and O **23** (Figure 2) that embody α-amino acid residues and that are likely derived from these. Samples of the producing organism, namely the marine invertebrate *Roboasta ernsti*, were collected in waters off the Southeastern coast of Brazil while the structures of compounds **21**-**23** were confirmed by total synthesis (see below). Four more minor metabolites were also identified and structures **24**-**27** (Figure 3) tentatively assigned to them. Once again, tambjamines A **4**, C **6**, and D **7** proved to be the major metabolites obtained from *R. ernsti* and these are believed to be the key defensive chemicals produced by the organism.



24 **25** **26** **27**

Figure 3. The suggested structures **24**-**27** of tambjamines that have yet to be rigorously established.

The foregoing commentary together with the report[20] that tambjamine A **4** has been isolated from the Antarctic bryozoan *Bugula longissima* testifies to the remarkable distribution of these alkaloids around the globe. Furthermore, the recognition that the tambjamines, like the prodigiosins,[3b] are secondary metabolites with the former most likely arising from surface-associated microbial symbionts of the above-mentioned bryozoans and nudibranches has prompted searches for the biosynthetic gene clusters (BGCs) encoding for their *in vivo* production. Such studies[21] have, *inter alia*, highlighted the diversity and distribution of the bacteria producing the tambjamines.

3. Biogenesis

Unsurprisingly, the significant structural overlap between the heterocyclic cores of the prodigiosins and the tambjamines reflects the similarities in their biogenesis. It is clear from a range of studies that in the closing stages of the formation of both of these natural product classes that 4-methoxy-2,2'-bipyrrole-5-carboxaldehyde (MBC) **28** is the common intermediate and that this condenses, in enzyme-mediated processes and in the presence of ATP, with either 2-methyl-3-amylpyrrole (MAP) **29** or, for example, the fatty acid-derived amine **30** so as to deliver the corresponding natural products, namely alkaloids **2** and **17** respectively (Figure 4).

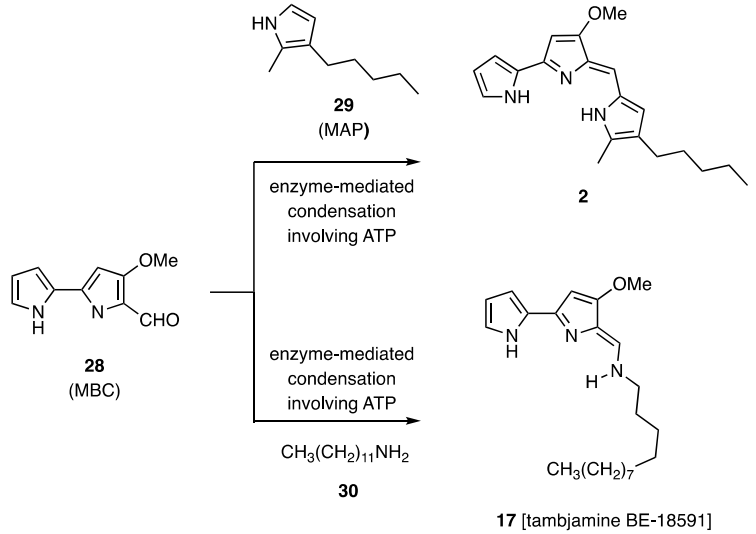


Figure 4. The closing stages in the biosyntheses of prodigiosin **2** and tambjamine BE-18591 **17**.

The biogenesis of MBC has been the subject of extensive study and it is clear that, in both terrestrially and marine-derived bacteria, L-proline is the progenitor of the mono-substituted pyrrole ring while its tri-substituted counterpart is, at least in the former case, derived from L-serine.[22] The *in vivo* production of MAP has also been the subject of intense investigation and this is now believed[23] to start with a 2-octenoyl thioester rather than, as previously thought, 2-octenal. Functional genomics have been used to identify a gene cluster encoding for the biosynthesis of tambjamine YP1 **18**[24] and so supporting a pathway of the same general form for the production of this natural product. Detailed studies of the origins of the fatty amine tail in compound **18** have resulted in the identification of a responsible enzyme (TamA) in the producing organism (*Pseudoalteromonas tunicata*) that, *inter alia*, controls the chain length of this fragment.[25] TamA is a didomain enzyme comprising a catalytic adenylation and an acyl carrier protein domain and this acts, in concert with two other enzymes, namely TamT and Tam H, to convert lauric acid into the requisite unsaturated, C12 and primary amine[26] for condensation with MBC. While the *Streptomyces*-derived tambjamine BE-18591 **17** is structurally very similar to tambjamine YP1 **18**, Ross and co-workers have recently shown[27] that the origins of the amine tail in the latter are quite distinct.

It has been recognised for quite some time[28] that dedicated enzymes are involved in the condensation step, as shown in Figure 4, leading to prodigiosin **2**. More recently, attention has been paid to exploiting (successfully) such catalysts in condensing MBC with a range of non-natural nucleophiles (*ie* replacements for the natural substrate MAP) and so forming novel analogues of the natural product.[29-31] The corresponding condensing enzymes associated with the formation of the tambjamines have also been identified, purified and kinetically characterized[32,33] on the basis that these, too, could be exploited in making non-natural systems. Interestingly, one such enzyme[32] has been co-opted in the production of prodiginine derivatives despite this being a homologue of the natural system PigC. Genetic engineering of PigC has resulted in the identification of more effective or promiscuous variants that enable enhanced

production of prodiginine and/or the formation of analogues.[34] As such, it seems entirely reasonable to assume that analogous manipulations of the enzymes associated with the biosynthesis of the tambjamines will follow.

The Berlinck group's recent report[19] on the isolation of tambjamines N-O **21**-**23**, respectively, bearing *S*-configured carboxylic acid side-chains, clearly suggests that MBC condenses with at least some naturally occurring α-amino acids and it seems reasonable to suppose that enzymes exist that facilitate such conversions. The *in vivo* decarboxylation of compounds **21** and **22**, which may represent a detoxification mechanism,[19] would afford tambjamines D **7** and K **15**, respectively. An equivalent process involving congener **23** would deliver the non-brominated counterpart to tambjamines J and J1 **13** and **14**, respectively, although this remains to be isolated from natural sources.

As is clearly the case with the prodiginines,[35] post-condensation events (especially oxidations) are also operative in the biogenesis of certain tambjamines. So, for example, it seems likely that tambjamine YP1 **18** is the biosynthetic precursor to its macrocyclic counterpart MYP1 **19** and that the *in vivo* conversion of the former into the latter is mediated by a Rieske-type oxygenase (TamC).[17]

The almost routine occurrence of the bright blue[1] to purple coloured[36] dipyrrolyldipyrromethane **3** as a co-isolate of the tambjamines has prompted speculation about its mode of formation. It seems clear that this arises from condensation of MBC with its non-formylated counterpart in much the same way as prodiginine **2** itself is formed. The latter condensation partner is probably formed by decarbonylation of MBC but whether product **3** is an artifact of the isolation process and/or a true natural product remains to be established. Certainly, a number of the organisms from which this pigment has been derived are themselves conspicuously blue in color.[13]

4. Biological and related activities

Attending the majority of the studies concerned with the isolation of the tambjamines has been at least some preliminary evaluations of their biological activities. Original efforts in this regard were prompted by their structural resemblance to the prodigiosins, compounds long-recognised[3] for their remarkable array of biological effects including immunosuppressive ones.

In their paper detailing the isolation and structure elucidation of tambjamines A-D, Carté and Faulkner[7] not only delineated the ecological roles of these compounds (as anti-feedants)[8,9a] but also established that they exerted some "moderate" anti-microbial activity while the corresponding aldehydes, including MBC, that are the hydrolysis products derived from the natural products, did not. All of the compounds (the aldehydes included) proved to be inhibitors of cell division in a fertilized sea urchin egg assay. The same was true for tambjamines E **8**, G **10**, and I **12** with the last member of this trio causing notable mortality at 2.6 x 10^{-5} mmol/mL concentrations.[10] Tambjamine K **14** proved significantly cytotoxic toward CaCo-2 tumour cells while co-isolate **3** was more active than **14** against C6 glioma cells. BE-18591 **17**, the *Streptomyces*-derived tambjamine, significantly enhanced, at 20 mg/kg dosages, the survival rates of mice transplanted with Ehrlich ascites tumour cells. This compound also proved active against a range of both Gram-positive and -negative bacteria while, additionally, being shown to inhibit immunoproliferation (in mice) and gastritis (in rabbits).[37] The unsaturated analogue of BE-18591 **17**, namely tambjamine YP1 **18**, and produced by the marine bacterium *Pseudoalteromonas tunicata* D2, is responsible for the slow-killing of the nematode *Caenorhabditis elegans*. On the other hand, the macrocyclic tambjamine paucibactin **20** has been shown to exert cyanocidal activities and may thus be capable of managing *Microcystis* and *Anabaena* (*Dolichospermum*) algal blooms provided, *inter alia*, the photo-sensistivity of the compound could be reduced.

Other, more focused studies of the biological properties of the tambjamines have served to reinforce the notion that these compounds have significant biological activity. An early and particularly notable body of work was concerned with the binding of the tambjamines, notably congener E **8**, to DNA and the resulting capacities for cleaving a supercoiled, single-stranded variant in the presence of copper and oxygen.[38] It appears from energy-transfer measurements, for example,[39] that tambjamine E **8**, like prodigiosin **2**, binds to DNA *via* a minor-groove intercalation mode and with a preference for AT sites. Since MBC **28** is not an effective DNA binder, the enamine residue within compound **8** must be playing a significant role in the selective binding event. Interestingly, prodigiosin **2** is more potent than tambjamine E **8** in copper-mediated DNA cleavages and can act on the double-stranded form,[40] as can tetrapyrrole **3**.[41] The superior copper-nuclease activity of compounds **2** and **3** over tambjamine E **8** correlate with their cytotoxicities as

determined in an assay using the HL-60 leukemia cell line[41,42] and so suggesting the biological activities of these compounds might derive from their capacities to target DNA. Various studies, including computational ones,[43] have yet to provide an adequate explanation as to why tambjamine E **8** is an AT-specific binder.

Brazilian-led groups have continued to evaluate to the cytotoxic and antimicrobial properties of both synthetically-derived and naturally-sourced tambjamines[44-46] and the outcomes of certain such studies, as shown in Table 1, reveal that they are broadly active against a range of cancer cell lines. Unfortunately, they also show similar toxicities towards certain normal (healthy) cell lines meaning their therapeutic indices are rather poor. Such issues could presumably be addressed by using, in their possible development as anti-cancer agents, targeted delivery techniques.[46]

Given the earlier studies of Manderville and co-workers,[38-42] the genotoxic effects of synthetically-derived tambjamine D **7** were evaluated, using an alkaline version of the comet assay as well as a micronucleus test, in order to establish the degree to which it could damage DNA. This revealed that the compound effected significant DNA-strand breaks and that, relative to the negative control (DMSO) and at all concentrations tested, it also increased the number of micro-nucleated V79 cells. This same alkaloid also induced apoptotic cell death that probably follows, at least in part, from its pro-oxidant properties (as revealed in a nitrite to nitrate conversion assay).[45] Such results further highlight the anticancer potential of this class of natural product.

The same panel of natural products, *viz*. compounds **4**, **6-13**, **17** and **18**, were evaluated as anti-microbials[46] and most of these were strongly active against the fungus *Malassezia furfur* (this being associated with certain dermatological conditions) and, in fact, more effective than the positive control amphotericin B.

Given the presence of the three potentially coordinating nitrogens associated with all of the tambjamines, they might, at least in their s-*cis*/*Z*-configured forms, serve as tridentate ligands and thus complex with certain metal ions and/or serve as anion transporters. Indeed, it is has been recognised that such properties are likely contributing to the observed biological effects.[17,30] Notably, tambjamines C **6**, E **8**, F **9**, and BE-18591 **17** as well as some synthetic analogues are potent transmembrane anion transporters as evidenced by their capacities to effect the biologically relevant bicarbonate/chloride exchange in model phospholipid liposomes and with the efficiency of such processes being influenced by the lipophilicity of the aliphatic tail of the "ligand".[47]

A further intriguing biological property of the tambjamines is that *Caenorhabditis elegans* exhibit an inate aversion to tambjamine YP1 **18**.[48]

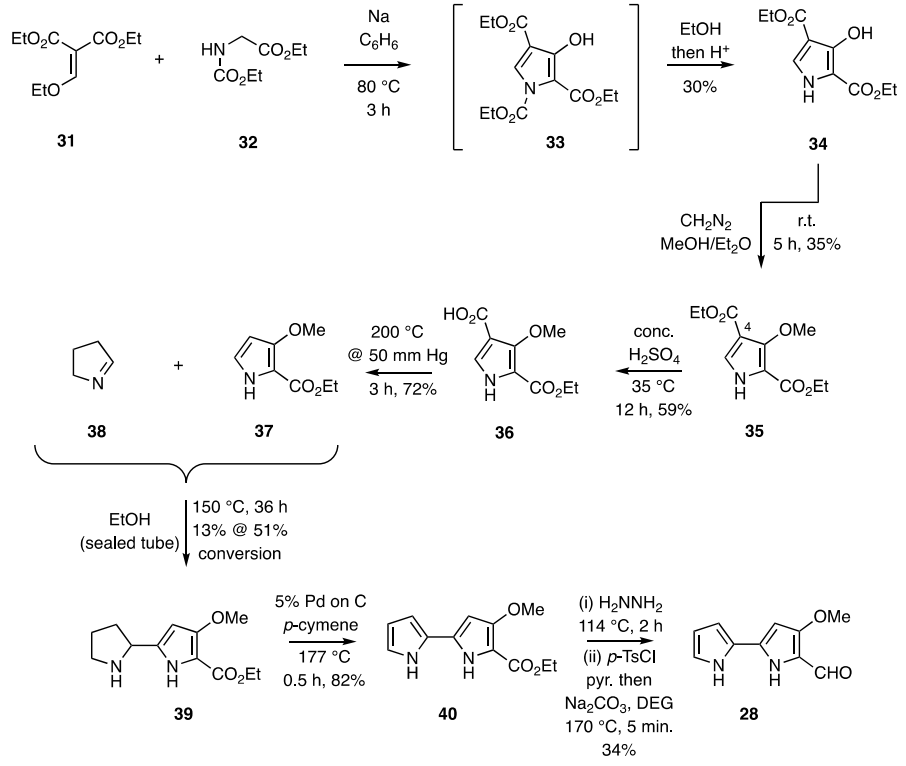
Table 1. Cytotoxic effects of tambjamines **4**, **6-13**, **17** and **18** against a range of cancer and other cell lines.[a,b]

	Cell Line								
	CEM[c]	HL60[d]	MCF-7[e]	HCT-8[f]	B16[g]	MDA-MB435[h]	SF-295[i]	PBM[j]	V79[k]
Tambjamine									
A **4**	inact.[l]	inact.	inact.	inact.	inact.	NR[m]	NR	NR	NR
C **6**	0.7	NR	NR	1.4	NR	3.4	1.6	2.53	NR
D **7**	12.2	13.2	13.2	10.1	6.7	NR	NR	NR	1.2
E **8**	NR	>25	NR	>25	NR	>25	>25	>25	NR
F **9**	NR	0.8	NR	1.0	NR	2.3	1.1	3.3	NR
G **10**	NR	0.8	NR	0.7	NR	1.2	0.6	2.1	NR
H **11**	NR	2.1	NR	2.5	NR	2.6	1.3	4.2	NR
I **12**	NR	0.6	NR	0.5	NR	0.8	0.4	1.1	NR
J **13**	NR	0.5	NR	0.4	NR	0.6	0.4	0.8	NR
BE-18591 **17**	NR	0.2	NR	0.5	NR	0.5	0.4	0.5	NR
YP1 **18**	NR	0.7	NR	1.0	NR	1.1	0.8	0.6	NR
Doxorubicin[n]	NR	0.02	NR	0.2	NR	0.5	0.04	0.2	NR

[a]Data are presented as IC_{50} values (μg/mL) but confidence intervals have been ommitted for clarity; [b]data taken from ref. 44, 45 or 46; [c]a T lymphoblast leukemia cell line; [d]a promyeoblast leukemia cell line; [e]a breast cancer cell line;[f]a adenocarcinoma cell line; [g]a murine melanoma cancer cell line; [h]a human breast cancer cell line; [i]a human glioblastoma brain cancer cell line; [j]a (normal) human peripheral blood mononuclear cell line; [k]a murine derived cell line exhibiting fibroblast morphology; [l]inact.=inactive; [m]NR=not reported; [n]doxorubicin used a positive control.

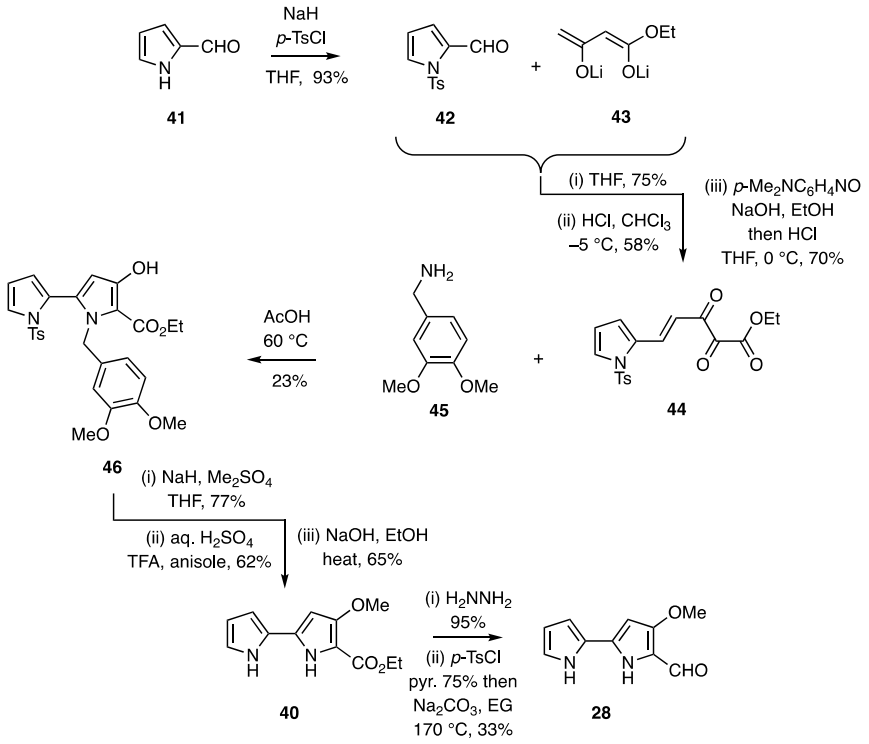
5. Total syntheses of the tambjamines

Given the pathway operating in the biogenesis of the tambjamines (Figure 4) and the successful mimicking of the analogous one involved in producing the prodigiosins and related compounds,[3,49] the most obvious means for preparing the title alkaloids would seem to involve condensing MBC **28** with the relevant amine. The viability of such an approach was first demonstrated by Davis, Carroll and Quinn[50] who were able to generate samples of MBC through base-promoted hydrolysis of a mixture of tambjamines C **6**, E **8**, and F **9** isolated from the Great Barrier Reef ascidian *Sigillina signifera*. Thereafter, the purified aldehyde **28** was reacted with a series of primary amines in the presence of acetic acid and so affording a small combinatorial library of analogues of the natural products. Accordingly, any total synthesis of the tambjamines could be realized by preparing MBC **28** *de novo* but early efforts to do so involved rather lengthy reaction sequences. The first route to this aldehyde, due to Rapoport and Holden,[6] is shown in Scheme 1. So, reaction of diethyl ethoxymethylenemalonate **31** with the sodium salt of ethyl *N*-ethoxycarbonylglycinate **32** afforded pyrrole **33** that was immediately treated with ethanol to afford its *N*-unsubstituted counterpart **34** in 30% overall yield. Reaction of this last compound with diazomethane then afforded *O*-methyl ether **35** and this was accompanied by the corresponding and chromatographically separable *N*-,*O*-dimethylated congener. Selective cleavage of the C4 ester moiety within compound **35** could be achieved using concentrated sulfuric acid and product **36** then subjected to thermally-induced decarboxylation to form the 2,3-di-substituted pyrrole **37** in 59% yield. Reaction of an ethanolic solution of product **37** with 3,4-dihydro-2*H*-pyrrole **38** in a sealed tube at elevated temperatures then afforded the tetrahydro-dipyrrole **39** albeit in just 13% yield (at 51% conversion). Dehydrogenation of compound **38** under standard conditions than gave ester **40** (82%) and subjection of this last compound to a MacFayden-Stevens (M-S) reduction sequence, using diethylglycol (DEG) as solvent in the last step, finally gave MBC **28** in 34% yield.



Scheme 1. Rapoport's synthesis of MBC **28**.

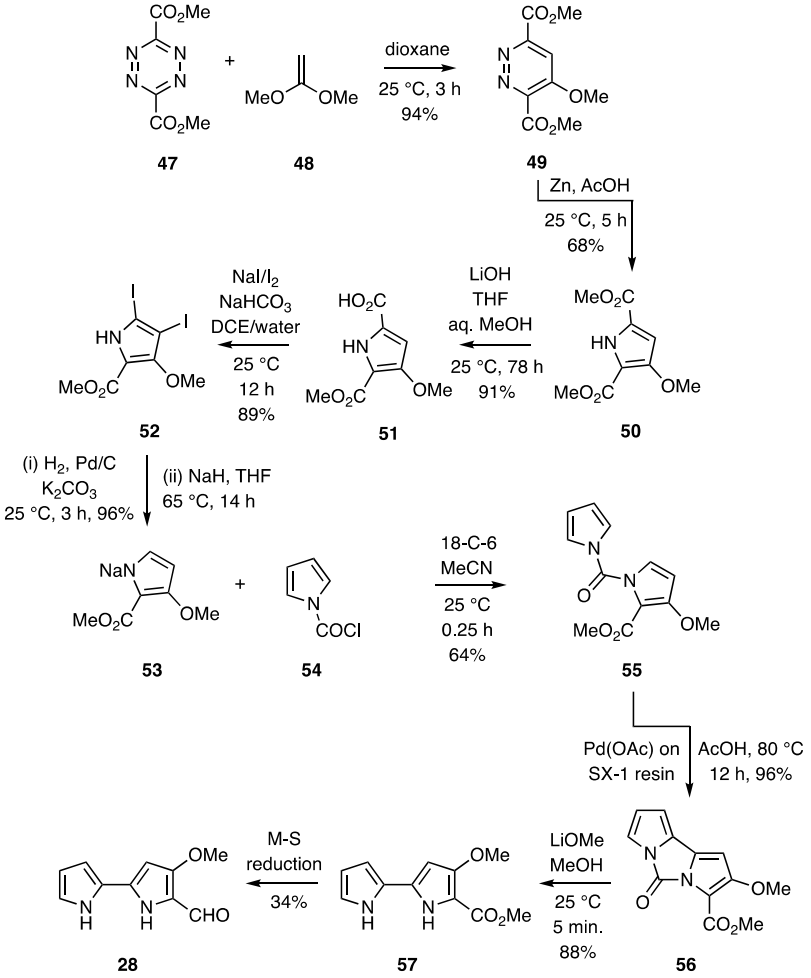
Another approach to MBC of similar length was reported by Wasserman and Lombardo,[51] some details of which are presented in Scheme 2. So, *N*-tosylation of pyrrole-2-carboxaldehyde **41** afforded sulfonamide **42** (93%) that was reacted with the dianion **43** derived from ethyl acetoacetate and the product alcohol (75%) subjected to an acid-catalysed dehydration to afford the corresponding α,β-unsaturated ketone (58%). The active methylene residue of the last compound was subject, *via* nitroso-group transfer under alkaline conditions, to oxidation and so affording the vicinal tricarbonyl compound **44** in 70% yield. Reaction of product **44** with the dimethoxylated benzylamine **45** then afforded bipyrrole **46** and *O*-methylation of which then gave the corresponding methyl ether. Successive treatment of this ether with aqueous sulfuric acid and TFA in the presence of anisole and then with sodium hydroxide in ethanol afforded compound **40**, the same key intermediate reported by Rapoport. Once again, this ester was converted into MBC **28** using the M-S reduction protocol.



Scheme 2. Wasserman's synthesis of MBC **28**.

A distinct but still somewhat lengthy route to MBC was described by Boger and Patel.[52] So, as shown in Scheme 3, when tetrazine **47** was reacted with 1,1-dimethoxyethylene **48** the initial Diels-Alder adduct underwent a cycloreversion reaction (involving loss of nitrogen) and so forming the 1,2-diazine **49** (94%). Treatment of this last compound with metallic zinc in acetic acid resulted in a reductive ring-contraction to deliver pyrrole diester **50** (68%) that could be selectively saponified, under carefully controlled conditions, using LiOH, and so affording, after acidic work-up, mono-acid **51** (91%). Iodinative decarboxylation of compound **51** using a mixture of sodium iodide and molecular iodine then gave pyrrole **52** (89%), two-fold reductive deiodination of which could be achieved under conventional hydrogenolytic conditions. The resulting pyrrole (96%) could then be deprotonated at nitrogen using sodium hydride and the product salt **53** was reacted, in the presence of 18-C-6, with chloride **54** to give the carbonyl-bridged dipyrrole **55** (96%). Intramolecular oxidative coupling of the pyrrole residues within compound **55** could be effected using polymer-bound palladium(II) acetate in acetic acid at 80 °C and so affording the dehydro-analogue **56**

(96%). Upon treating this compound with lithium methoxide in methanol, the carbonyl-bridge could be excised and so delivering the methyl ester equivalent, **57** (88%), of ethyl ester **40** associated with the Rapoport and Wasserman syntheses. Once again, a three-step M-S reduction protocol was then applied and so effecting the conversion of compound **57** into MBC **28** which was obtained 38% overall yield.

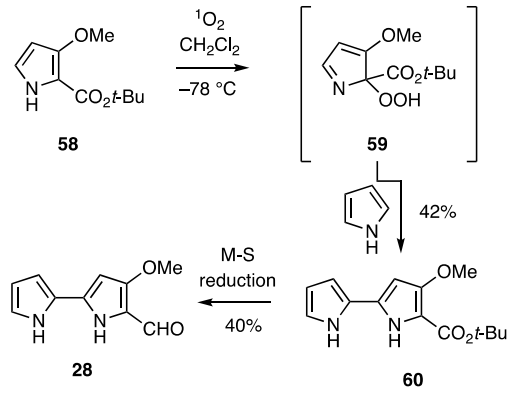


Scheme 3. Boger's synthesis of MBC **28**.

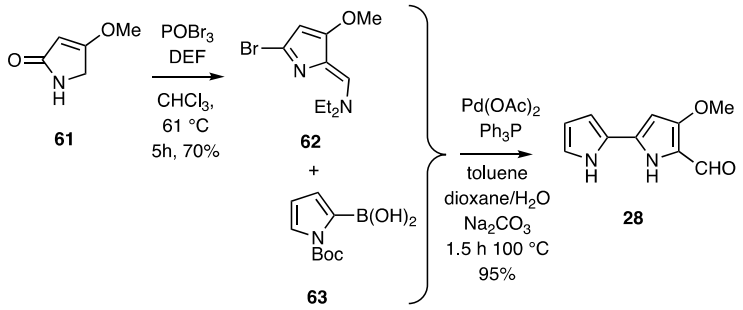
In 1999, Wasserman and co-workers described[53] a second-generation synthesis of MBC (Scheme 4) and wherein pyrrole **58** was treated with photochemically-generated singlet oxygen at –78 °C and the ensuing hydroperoxide **59** intercepted by added pyrrole and so thus affording dipyrrole **60** in 42% yield. On applying the M-S reduction protocol to this last compound, MBC **28** was obtained in 40% yield. Detracting somewhat from this reaction sequence is that the "starting" pyrrole **58** had to be prepared in five steps from β-chloropropionyl chloride.

A "game-changing", two-step route to MBC was reported by Tripathy, Lavallée and co-workers in 2006.[54] This involved, as shown in Scheme 5 and in a simple modification of protocols reported by D'Alessio and Rossi,[55] conversion of the commercially available lactam **61** into the bromopyrrole enamine **62** (70%) using the Vilsmeier-Haack reagent obtained from $POBr_3$ and diethylformamide (DEF).

Suzuki-Miyaura-type cross-coupling of the last compound with the commercially pyrrole-2-boronic acid **63** then afforded MBC in 95% yield.



Scheme 4. Wasserman's second-generation synthesis of MBC **28**.

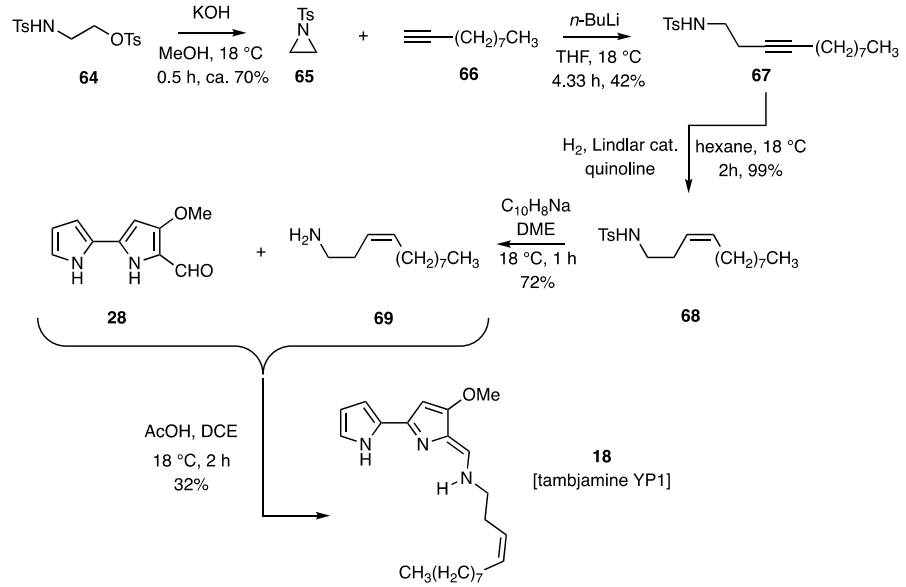


Scheme 5. The Tripathy/Lavallée two-step synthesis of MBC **28**.

The ready access to MBC provided by the route shown in Scheme 5 allowed us to prepare tambjamine YP1 **18** by the simple route shown in Scheme 6.[56] So, bis-tosylation of 2-aminoethanol afforded compound **64** that upon treatment with aqueous potassium hydroxide gave aziridine **65** and this could be ring-opened with the anion derived from the terminal alkyne **66** and so delivering the homopropargylic amine derivative **67** (42%). Controlled hydrogenation of this last compound using the Lindlar catalyst then afforded the corresponding *Z*-configured alkene **68** (99%), the associated sulfonamide group of which was reductively cleaved using sodium naphthalenide to give the primary amine **69** (72%). Finally, a Schiff-base type condensation of this last compound with MBC **28** promoted by acetic acid gave the target tambjamine **18** (32%), the spectral data for which proved identical with those derived from the natural product.[56]

More recently, the macrocyclic analogue of tambjamine YP1 **18**, namely tambjamine MYP1 **19**, has been prepared. So, in a sequence (Scheme 7) bearing some resemblance to the closing stages of work reported Fürstner *et al*,[57] Kelly and-co-workers[58] first added the Grignard reagent **70** to the readily prepared pyrrole 2-carboxaldehyde **71** and then treated product **72** (59%) with lithium aluminium hydride ($LiAlH_4$) so as to effect reductive cleavage of both the phenylsulfonyl and hydroxy groups and thus affording compound **73** (71%). *N*-Boc protection then followed and the ensuing carbamate **74** (93%) was selectively deprotonated at C5 and the ensuing anion captured using trimethyl borate to afford boronic acid diester **75**. The instability of this last compound meant that it was immediately engaged in Suzuki-Miyaura cross-coupling (S-M X-c) with the brominated azafulvene **62** and upon base-promoted alkaline hydrolysis of the primary coupling product then aldehyde **76** (81%) was obtained. Schiff-base condensation of this last compound with but-3-en-1-amine **77** then gave the enamine hydrochloride **78** (96%), but since this could not be directly engaged in a ring-closing metathesis (RCM) reaction it was converted into the Boc-derivative **79** (94%). The

last compound then served as a substrate in a RCM processes catalysed by the Grubbs' II system and after deprotection of the primary reaction products, using trifluoroacetic acid (TFA), a mixture (ratio not defined) of target **19** and the corresponding *E*-isomer **80** was obtained albeit in just 13% combined yield.



Scheme 6. A total synthesis of tambjamine YP1 **18**.

Relatively simple modifications to the end-game associated with this synthesis allowed for a more effective outcome. So, as shown in Scheme 8, an olefin cross-metathesis (OCM) reaction between terminal alkenes **76** and **81** using the Grubbs' II catalyst afforded product **82** in 67% yield and seemingly as a mixture of geometric isomers (ratio not specified). Finally, treatment of compound **82** with oxalyl chloride in methanol at ambient temperatures resulted in cleavage of the associated Boc group and an intramolecular Schiff-base condensation reaction to afford a 1:14 mixture of products **19** and **80** in 54% combined yield.

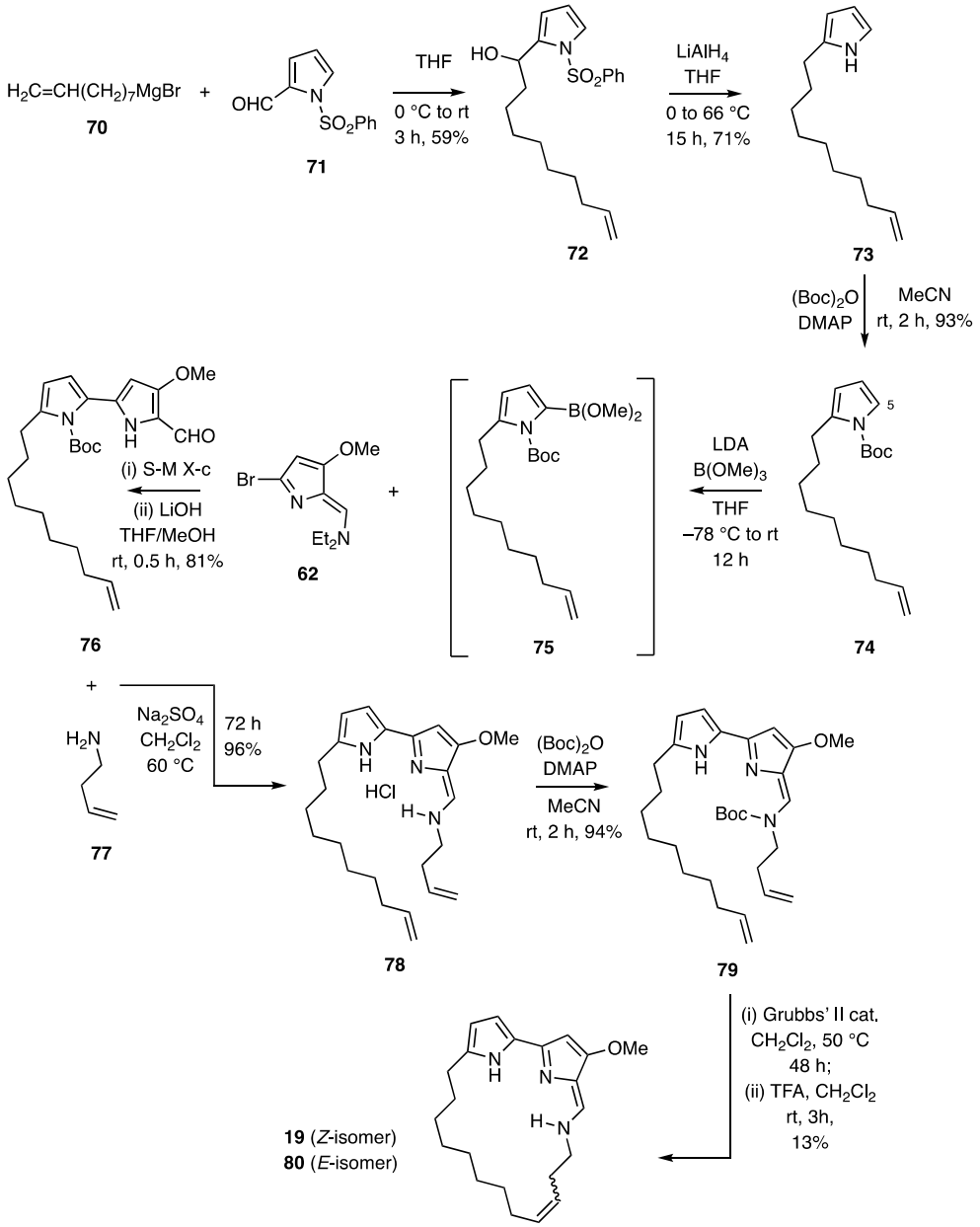
Syntheses of tambjamines N-O **21**-**23**, respectively, have also been reported recently[19] and each was obtained directly (albeit in just 13% yield in each instance) by condensing MBC **28** with the relevant α-amino acid, *viz.* L-valine, L-leucine and L-isoleucine, respectively.

6. Synthesis of analogues and their biological profiles

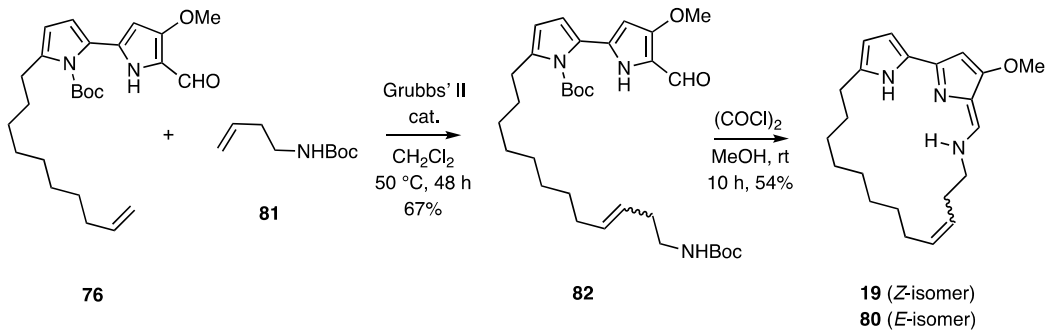
The Tripathy/Lavallée two-step synthesis of MBC **28** shown in Scheme 5 and the ease with which this and related compounds (*e.g.* **76**) can be engaged in Schiff-base type condensations with amines has inspired the preparation of a wide range of analogues by related means. For example, Reynolds and co-workers[59] have prepared various B-ring functionalised tambjamines as exemplified by the reaction sequence shown in Scheme 9. So, Suzuki-Miyaura cross-coupling (S-M X-c) of commercially available boronic acid **63** with the readily prepared C5-brominated pyrrole 2-carboxaldehyde **83** followed by LiOH-promoted cleavage of the Boc group gave bipyrrole **84** (64%) and Schiff-base condensation of this with, for example, *i*-butylamine than afforded the tambjamine C-analogue **85** in 92% yield. Analogous condensations involving pyrroles has provided access to prodigiosin-type analogues.[59,60]

Analogous chemistry has enabled the preparation of tambjamine analogues that allow for the identification of those structural features that contribute to the capacities they display for transporting anions across membranes.[61-64] Quesada and co-workers, for example, have prepared a suite of such analogues through Schiff-base condensation of a range of aromatic and other amines with MBC **28** and its *O*-benzyl congener. Studies of such systems reveal that many are potent anion transporters in liposome models and that they can, presumably as a consequence, trigger apoptosis, in the low nanomolar range, in a small panel

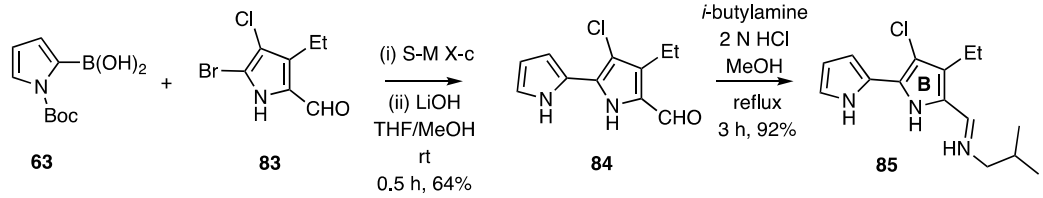
of cancer cell lines.[61,62] Unsurprisingly, perhaps, lipophilicity plays an important role in the transport properties of such systems.[62] Interestingly, it has been suggested that the anion transporting capacities of some such compounds could be harnessed in the development of a new means for treating cystic fibrosis.[63] The ability of tambjamine-type systems to selectively transport larger hydrophilic anions such as gluconate in preference to chloride ions is notable and may have implications for their deployment in therapeutic settings.[64]



Scheme 7. A total synthesis of tambjamine MYP1 **19** and *E*-isomer **80**.



Scheme 8. An alternative, higher-yielding end-game leading to tambjamine MYP1 **19**.



Scheme 9. Synthesis of an analogue **85** of tambjamine C using Suzuki-Miyaura cross-coupling and Schiff-base condensation reactions.

The cytotoxic/anti-proliferative effects of various tamjamine analogues have been investigated and revealing that, for example, the indole-based compounds **86**, **87**, and **88** (Figure 5) are highly active against lung cancer cells and exert their effects by triggering a ROS-activated stress kinase pathway.[65] These same types of analogues have been shown to inhibit the enzyme EZH2, a component of PRC2 that acts as a transcriptional repressor.[66]

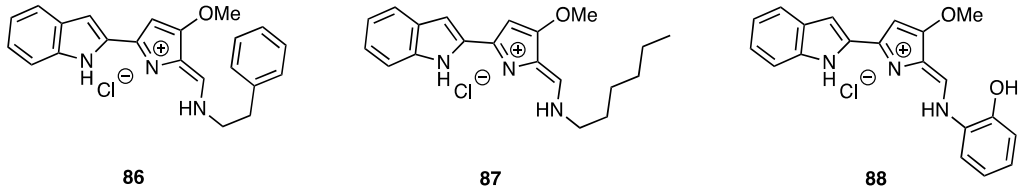
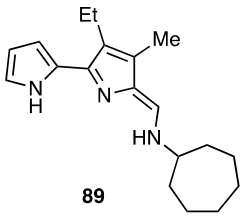


Figure 5. Structures of the cytotoxic, indole-based tambjamine analogues **86**, **87** and **88** (shown as the corresponding hydrochloride salts).

The above-mentioned suite of tambjamine analogues derived from condensation of MBC **28** and its *O*-benzyl congener with aromatic amines, have also been shown to effect autophagic blockading and necrotic cell death in lung cancer cell lines and they appear to do so by inducing mitochondrial swelling and lysosomal disfunction.[67]

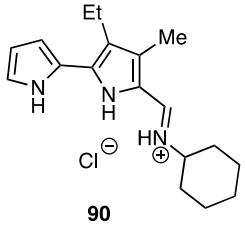
As is the case with certain prodigines and some analogues,[68] potent antimalarial effects have attributed to a corresponding range of tambjamine-type systems. Indeed, in a number of respects one such analogue, namely KAR425 **89** (Figure 6), has been identified as a potentially significant lead for the development of clinically relevant antimalarial agents. The compound has oral efficacy in female mice with no obvious signs of toxicity or induced behavioural changes. Furthermore, it is of low molecular weight (298 amu) and has a good lipophilic profile (cLogP<2.7). Perhaps, most notably, the compound was superior, in terms of efficacy, to all of the tripyrrole-containing prodigine analogues that were also studied.[69]



89

Figure 6. The structure of KAR425 **89**, a synthetically-derived tambjamine analogue displaying significant antimalarial properties.

Intriguingly, the lower homolgue of KAR425 **89**, namely MM3 **90** (Figure 7), has been investigated as a treatment for Chagas disease that is caused by *Trypanosoma cruzi*, a protozoan endemic in all of Latin America and one that is now spreading to countries such as Australia, Japan, North America and Spain.[70]

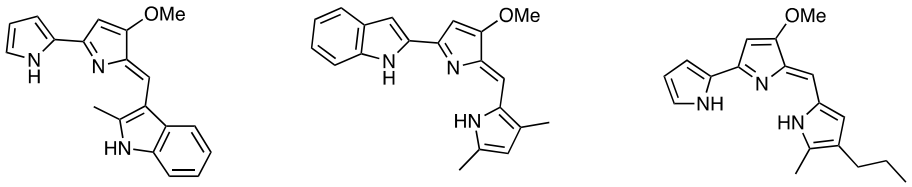


90

Figure 7. The structure of MM3 **90**, the lower homogue of KAR425 **89** showing biocidal activity against *Trypanosoma cruzi*.

7. Prospects for the development of the tambjamines as therapeutic agents

The intriguing range of biological activities displayed by the tambjamines and their analogues would seem to augur well for their development as therapeutic agents. Certainly, some such encouragement derives from the manner in which systems inspired by the prodigiosins are moving forward in this regard. Indole-containing analogues of prodigiosin such as compounds **91** and **92** (Figure 8) are perhaps the most conspicuous examples,[71,72] with the latter (a.k.a obatoclax) being a potent antagonist of the BCL-2 family of apoptotic proteins and thus having been deployed in Phase I and II clinical trials against a number of cancers.[73] Interestingly, genetic engineering of the prodigiosin pathway together with its "salting" by synthetic intermediates has provided a range of analogues including the lower homologue **93** that is exhibits more potent autophagy inhibitory activity than the parent compound **2**.[74]



91 **92** [obatoclax] **93**

Figure 8. Biologically-active analogues **91**, **92** (obatoclax) and **93** of prodigiosin.

While, based on *in vitro* studies, the therapeutic indices of the tambjamines would seem rather poor (see Table 1 above), targeted delivery strategies using, for example, nanocapsules,[75,76] would appear to offer a means for addressing matters. Additionally, the beneficial effects of tambjamine J on mice implanted with sacrcoma 180 tumor cells provide some encouragement that *in vivo* deployment of the title compounds

offers distinct clinical possibilities.[77] Their use as components of combination therapies would also seem to be a distinct possibility given the outcomes of studies involving obatoclax **92**.[72b,73b]

8. Conclusions

The tambjamines represent a fascinating group of alkaloids that display distinctive structural features and a diverse range of biological properties. With the development of concise methods for their synthesis from the now readily available precursor MBC **28**, the capacities to more fully explore "tambjamine chemical and biological space" have improved significantly in recent times. A complementary and equally promising approach follows from the rapidly emerging understanding of the details of the biogenesis of the tambjamines and the attendant capacity to manipulate this for the purposes of creating libraries of analogues. It seems reasonable to expect that such studies will deliver "tambjamine-inspired" compounds of therapeutic interest and ultimately, perhaps, of clinical value.

Acknowledgements

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