Variolins and Related Alkaloids

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Contents

1. Introduction

The phosphorylation of proteins on serine, threonine, and tyrosine residues by the 518 protein kinases encoded in the human genome constitutes one of the major mechanisms used by cells to regulate their metabolism and functions.[1] The recent appreciation of the implication of abnormal protein phosphorylation in many human diseases has sparked considerable interest in the search for pharmacological inhibitors of kinases.[2,3]

Of the 518 kinases, cyclin-dependent kinases (CDKs) have attracted major interest because they regulate the cell-division cycle, apoptosis, transcription, and differentiation, as well as controlling functions in the nervous system.[4] Deregulation of CDKs in various diseases has stimulated an intensive search for selective pharmacological inhibitors of these kinases.[5] These inhibitors all target the ATP-binding pocket of the catalytic site of the kinase. Pharmacological inhibitors of CDKs are currently being evaluated in a wide range of therapeutic areas, and thus, the identification and development of new CDK inhibitors is an important area of research.

One such example of a new lead in this area is the discovery that variolin B (**1**), a novel marine alkaloid that was isolated from the rare, and difficult-to-access, Antarctic sponge *Kirkpatrickia varialosa* by the group of Blunt and Munro,[6] is a potent CDK inhibitor.[7−9] The unique heterocyclic framework has emerged as a new scaffold for the design of new inhibitors of CDKs. Indeed, the Spanish pharmaceutical company PharmaMar has been investigating the potential of variolin B, and its analogues, as antitumor agents. Furthermore, the related meridianins, and their

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azaindole analogues (referred to as the meriolins), have also been found to have important biological properties. Indeed, in the case of the meriolins, they have proved to be even more potent CDK inhibitors than variolin B.

In this review we summarize the isolation, structure determination, synthetic studies, and biological activities of the variolins, meridianins, and meriolins.

2. Isolation and Structure Determination of the Variolins and Related Alkaloids From Marine Organisms

2.1. Variolins

In 1994, Blunt, Munro, and co-workers reported the P388 murine leukemia bioassay-guided isolation of a new class of marine alkaloids from the Antarctic sponge *Kirkpatrickia varialosa*.[6] The variolins (**1**−**4**) all possess a fused tricyclic heteroaromatic core—formally a pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine—bearing a heterocyclic substituent at C5 in the cases of variolins B (**1**) and A (**2**) and *N*(3′)-methyl tetrahydrovariolin B (**3**) (Figure 1). A fourth compound,

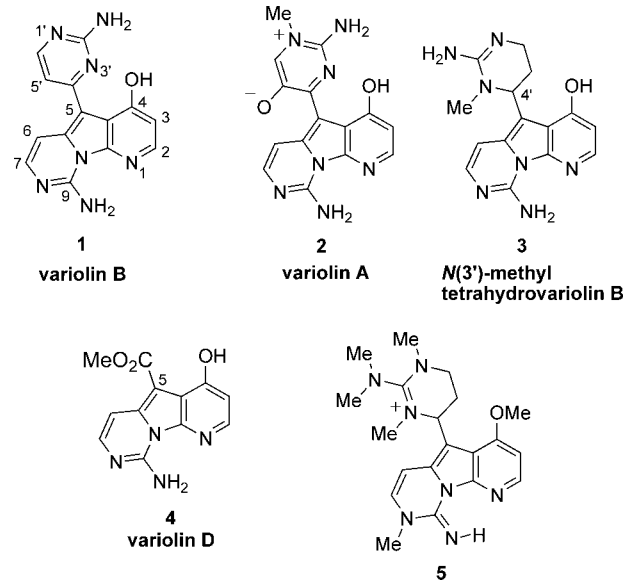


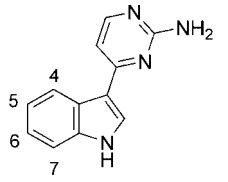
Figure 1. Variolins.

variolin D (**4**), bears an ester grouping at C5 and is thought to be a degradation product produced by aerial oxidation of the variolins during the extraction process. The pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine ring system has not been encountered in any other natural products.

Despite their modest size, the variolins presented quite a challenge in their structural elucidation. This was largely due to the presence of several ring-nitrogen atoms and relatively few hydrogen atoms in the structure, which made C−C connectivities difficult to establish using long-range NMR techniques. Extensive NMR analysis (HMQC, HMBC, NOE) did not result in definitive structures being assigned to the variolins. Very small crystals of variolin B were obtained from a trifluoroacetic acid (TFA)/water solution, and these crystals were studied by rotating-anode X-ray diffraction and allowed the assignment of variolin B to structure **1**.

Once the structure of variolin B (**1**) had been established with absolute certainty, the wealth of NMR data that had been collected could be interpreted. The appearance of two signals in the ^{1}H NMR spectrum for the amine protons on the C9 amine group indicated that rotation around the C9−NH_2 bond is somewhat restricted, presumably due to hydrogen bonding between one of the amine protons and N1 of the tricyclic core. The corresponding atoms appear within hydrogen bonding distance in the X-ray crystal structure, supporting the existence of this interaction. The downfield chemical shift (δ 16.1 ppm) of the ^{1}H NMR signal for the hydroxyl proton indicated the proton is in an extremely deshielded environment, and the broad nature of the signal indicated significant hydrogen bonding. Together this indicated the existence of a hydrogen bond between the hydroxyl group and the N3′ nitrogen of the pendant pyrimidine ring in solution, and the corresponding atoms are found within hydrogen bonding distance in the X-ray crystal structure. This interaction pulls the pendant pyrimidine ring out of the plane of the tricyclic core, which is observed in both the X-ray crystal structure and in solution by nuclear Overhauser effect (NOE) measurements.

As with variolin B, X-ray crystallography was required to establish the structure of variolin A (**2**). After significant efforts to obtain suitable crystals, a TFA/water solution yielded a few small needles of variolin A, whose structure was determined to be that of **2**. With the structure revealed,



	C4	C5	C6	C7
meridianin A (6)	OH	H	H	H
meridianin B (7)	OH	H	Br	H
meridianin C (8)	H	Br	H	H
meridianin D (9)	H	H	Br	H
meridianin E (10)	OH	H	H	Br
meridianin F (11)	H	Br	Br	H
meridianin G (12)	H	H	H	H

Figure 2. Meridianin family.

it was then possible to rationalize the NMR data, resulting in the complete assignment of all the ^{1}H and ^{13}C signals. The structure of variolin D (**4**) was determined by comparison to the spectroscopic data of variolin B and also by chemical transformation from variolin A.

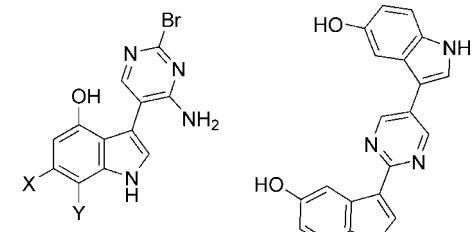
The NMR analysis of *N*(3′)-methyl tetrahydrovariolin B (**3**) was complicated by the existence of axial and equatorial conformers in the tetrahydropyrimidine. The natural product was, therefore, allowed to react with an excess of methyl iodide to give hexamethyl derivative **5** (Figure 1), whose structure could be solved by NMR methods. The results of the NMR analysis were then extrapolated to *N*(3′)-methyl tetrahydrovariolin B (**3**), enabling a satisfactory structure to be proposed.

During this isolation, preliminary studies on the biological activity of these compounds indicated that variolin B was the most cytotoxic, with an IC_{50} of 210 ng/mL in the P388 murine leukemia cell line assay. However, there was not enough material available from the sponge material collected to allow a full investigation. Aside from the cost and difficulties of accessing more of the sponge *K. varialosa* from Antarctica, access to the Antarctic was restricted when the 1991 Madrid Protocol to the Antarctic Treaty, which prohibits any person from removing flora or fauna from the region without the authorization of the government, came into force in 1998.[10] As a consequence, further work on the variolins was stymied until the first total synthesis of variolin B was completed (see section 3).

2.2. Meridianins and Other Natural Products With Related Structures

In addition to the more well-known variolins, several similar compounds sharing a common 3-(pyrimidyl)indole structure are known. In 1998, the groups of Hernandez Franco and Palermo reported the isolation of five 3-(2-aminopyrimidine)indoles, which were named meridianins A−E (**6**−**10**), from the tunicate *Apidium meridianum*, which had been collected at a depth of 100 m near the South Georgia Islands (South Atlantic) (Figure 2).[11] The structures were deduced by 2D NMR spectroscopic methods and comparison to literature compounds. Re-examination of the crude extract using tandem mass spectrometry lead to the discovery of two new meridianins—F **11** and G **12**.[12]

The psammopemmin family of natural products was isolated from an Antarctic marine sponge from the genus *Psammopemma* and comprises three structurally related compounds designated psammopemmins A−C (**13**−**15**)

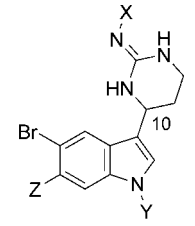


psammopemmins

A X = Y = H (**13**)
B X = H, Y = Br (**14**)
C X = Br, Y = H (**15**)

hyrtinadine A (**16**)

Figure 3. Psammopemmins and hyrtinadineA.



aplicyanins

A X = Y = Z = H (**17**)
B X = Ac, Y = Z = H (**18**)
C Y = OMe. X = Z = H (**19**)
D X = Ac, Y = OMe,Z = H (**20**)
E X = H, Y = OMe, Z = Br (**21**)
F X = Ac,Y = OMe, Z = Br (**22**)

Figure 4. Aplicyanins.

(Figure 3).[13] All of the psammopemmins contain a 4-hydroxyindole moiety substituted at the 3-position by an unusual 2-bromopyrimidine system, which was isolated from the natural source as an amine salt. Psammopemmins B and C contain further bromination on the indole ring. The assigned structure of the psammopemmin family remains to be confirmed by total synthesis. The possible biological activities of the psammopemmin family likewise remain unexplored.

Hyrtinadine A (**16**) was isolated from an Okinawan marine sponge from the genus *Hyrtios*.[14] Like the meridianin family, hyrtinadine A contains an indole-substituted pyrimidine ring, although in hyrtinadine A the pyrimidine ring forms a bridge between two 5-hydroxyindole systems. Hyrtinadine A was found to have a cytotoxic effect against murine leukemia L1210 and human epidermoid carcinoma KB cells, with IC_{50} values in the μg/mL range.

More recently, an additional series of compounds with a similar structure to the meridianins was discovered. The aplicyanin family was isolated from the Antarctic tunicate *Aplidium cyaneum* and consists of six variants on a core 3-(tetrahydropyrimidyl)indole structure (**17**−**22**) (Figure 4).[15] In the aplicyanin family, the indole ring is further modified by the addition of bromine substituents and, in several members, by the addition of an unusual *N*-methoxy group. Unlike the planar pyrimidine ring of the meridianins, the tetrahydropyrimidine system of the aplicyanins has a stereocenter at C10. Optical rotation measurements indicate that this stereocenter is nonracemic in the aplicyanins, and analysis of isolated aplicyanin E (**21**) by chiral high-performance liquid chromatography (HPLC) indicates the natural product was isolated as a single enantiomer. However, the absolute configuration of this center remains undetermined. Aplicyanins B (**18**), D (**20**), and F (**22**) have been

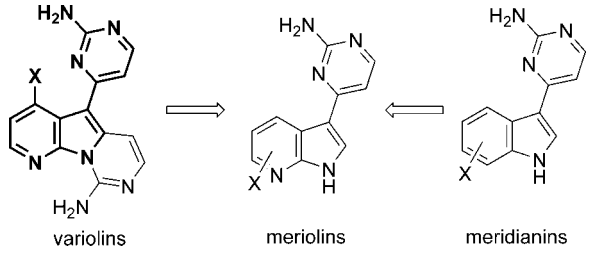


Figure 5. Meriolins are hybrid structures of variolins and meridianins.

found to have significant cytotoxic and antimitotic activities, with IC_{50} values in the low to sub-μM range. Aplicyanins A (**17**) and C (**19**) were found to possess no cytotoxic or antimitotic activity at the concentrations tested, while aplicyanin E (**21**) possessed weak cytotoxic activity. These results indicate a vital role for the acetyl portion of the acetylguanidine group.

2.3. Meriolins

Variolin B (**1**) and the meridianin family of natural products have some structural similarities, with the meridianins possessing a pyrimidyl-substituted indole skeleton and variolin B possessing a pyrimidyl substituted pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine skeleton. By combining the common features of these natural products, a new class of 7-azaindole-containing analogues was designed; Meijer and co-workers have coined the term "meriolin" to describe this hybrid structure (Figure 5).[8,9]

3. Synthesis

While promising biological activities had been reported for the variolins, there was not enough material available from the natural source to allow a full investigation. The combination of the cost and difficulties of accessing the sponge *K. varialosa* from Antarctica with the 1991 Madrid Protocol to the Antarctic Treaty limits the possibility of obtaining more variolin B from its natural source. As a consequence, there has been significant interest in the synthesis of variolin B, and the related meridianins, so that access to these interesting materials could be restored and full biological studies could be carried out.

3.1. Total Syntheses of Variolin B

The pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine ring system of variolin B (**1**) presents a significant synthetic challenge. Prior to the isolation of the variolins, the tricyclic system had been described only once (as a series of 9 synthetic derivatives), albeit in a different oxidation state to the variolin core.[16] Because variolin B is a polar molecule and insoluble in many organic solvents, it is important in the synthetic design to ensure that the core be assembled first, and then the appropriate functional groups appended.

There have been four total syntheses of variolin B reported and they can be divided into two different strategies—convergent and linear. The first total synthesis was reported by Anderson and Morris, utilizing a convergent strategy.[17] The other three syntheses all assembled the pyrido[3′,2′:4,5]pyrrolo[1,2-c]pyrimidine core in a stepwise fashion, with the pendant 2′-aminopyrimidine attached late in the synthesis.[18−20]

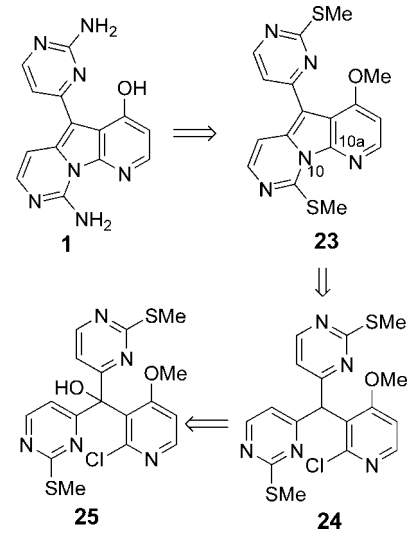
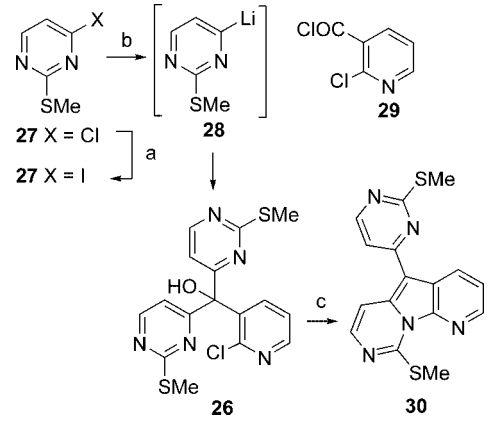


Figure 6. Morris retrosynthesis of variolin B (**1**).

Scheme 1[a]



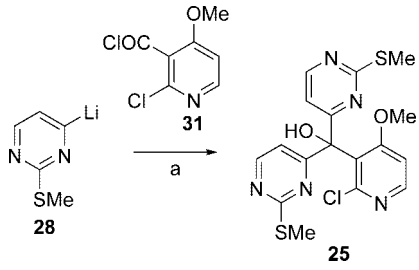
[a] Reagents and conditions: (a) HI, rt (79%); (b) (1) *n*-BuLi, tetrahydrofuran (THF), −95 °C, 20 min; (2) 2-chloronicotinyl chloride (**29**), THF, −95 °C (56%); (c) Et_3SiH (8 equiv), CF_3CO_2H (2 equiv), $ClCH_2CH_2Cl$, 80 °C (34%).

3.1.1. Convergent Synthesis of Variolin B—The Morris Approach

The retrosynthesis proposed by Anderson and Morris is outlined in Figure 6, with the key compound being the core structure **23**, which contains the appropriate functionality to introduce the highly polar amino groups.[17] Examination of **23** reveals a hidden symmetry element, which leads to the disconnection of the N10−C10a bond to give the symmetrical triarylmethane **24**. This triarylmethane could be prepared by deoxygenation of alcohol **25**, which itself could be prepared by double arylation of a suitable acyl compound. Exploiting the hidden symmetry in the structure of variolin B was anticipated to provide a more rapid approach to the natural product than traditional linear syntheses.

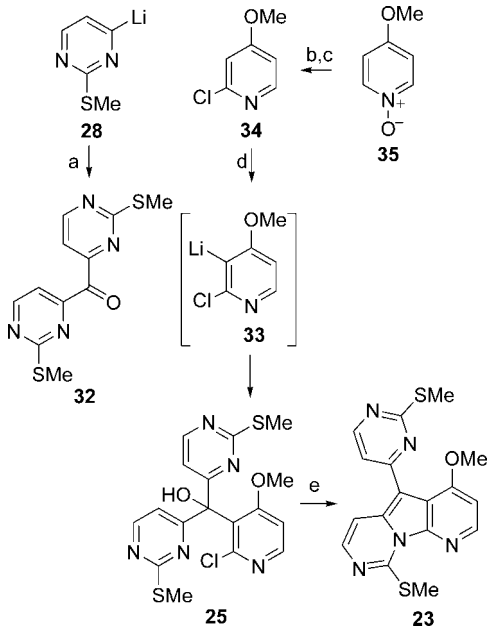
Because of the unprecendented nature of the variolin tricyclic system when this work was initiated, the key cyclization reaction was first investigated with the simplified deoxy system **26** (Scheme 1).[21] Accordingly, treatment of iodide **27** (R = I) (generated from the commercially available chloride **27** (R = Cl) by reaction with HI) with *n*-butyllithium at low temperature gave the highly reactive lithiopyrimidine **28**, which was treated with commercially available acid chloride **29** at −95 °C to give the triaryl alcohol **26**. Initial attempts to deoxygenate alcohol **26** with trifluoroacetic acid/

Scheme 2[a]



[a] Reagents and conditions: (a) **31**, THF, −95 °C (14%).

Scheme 3[a]



[a] Reagents and conditions: (a) $(EtO)_2CO$, THF, −95 °C (61%); (b) Ac_2O, reflux, then MeOH, H_2O (61%); (c) $POCl_3$, 95 °C (79%); (d) (1) **34**, *n*-BuLi, THF, −90 °C → −78 °C; (2) **32**, THF, −90 °C (76%); (e) Et_3SiH (8 equiv), CF_3CO_2H (2 equiv), $ClCH_2CH_2Cl$, 80 °C (47%).

triethylsilane were unsuccessful, giving rise to a variety of products that included a small quantity of the desired tricyclic system **30**. Optimization of the reaction conditions (2 equiv of TFA, 8 equiv of triethylsilane) led to a one-pot synthesis of the deoxyvariolin system **30** from alcohol **26**, in only three steps from commercially available materials.

With the reaction conditions for the key cyclization established, attention was turned to preparing the natural product using this methodology.[18] Attempts to prepare the triaryl alcohol **25** from acid chloride **31** resulted in only a low yield, and attempts to improve the yield were frustrated by the fact that the lithiopyrimidine **28** polymerized at temperatures above −95 °C (Scheme 2).

To overcome this problem, an alternative synthesis of the triaryl alcohol **25** was developed whereby the symmetrical ketone **32** was reacted with the lithiopyridine **33** (Scheme 3). The symmetrical ketone **32** was prepared in 61% yield by reaction of the lithiopyrimidine **28** with an excess of diethyl carbonate. The lithiopyridine **33** was obtained by lithiation of 4-methoxy-2-chloropyridine **34**, which could be readily prepared from the commercially available 4-methoxypyridine-*N*-oxide **35**. With these two reactants, the desired triaryl alcohol **25** was prepared in 76% yield. Application of the previously determined cyclization conditions (2 equiv of TFA, 8 equiv of triethylsilane) to the triaryl compound **25** led to the generation of the desired variolin core structure **23** in 47% yield.

Isolation and analysis of the reaction byproduct suggested that a competing reaction pathway involving nucleophilic attack of the alcohol group of **25** onto the pyrimidine ring was responsible for the reduced yield. To prevent this occurring, the acetate **36** was prepared by quenching the lithiation reaction with acetyl chloride (Scheme 4).[17b] Subjecting the triaryl ester **36** to the cyclization conditions returned a 75% yield of the variolin core structure **23**. The improved cyclization of the acetate ester was also found to be true for the deoxy analogue, resulting in a 69% yield of the corresponding deoxy core structure **30**. With the core structure in hand, the amino groups of variolin B could be introduced. Oxidation of the thiomethyl groups with *m*-chloroperbenzoic acid gave bis-sulfoxide **37**, which was heated in *p*-methoxybenzylamine to give protected variolin precursor **38**. Deprotection of the methyl ether with sodium ethanethiolate gave **39** in 95% yield; then cleavage of the amine protecting groups with triflic acid gave variolin B (**1**) in 80% yield.

Variolin B was produced in 8 steps and 17% overall yield, starting from the commercially available pyrimidine **27** (R = Cl).[17b] Application of the synthesis to the deoxy core **30** afforded deoxyvariolin B (**40**) in 6 steps, in 23% overall yield, and this analogue was found to be as biologically active as the natural product. The utility of this synthesis is highlighted by the fact that PharmaMar, a Spanish pharmaceutical company, filed two patents on the synthesis and have used it to generate multigram quantities of variolin B and deoxyvariolin B for preclinical investigation.[22]

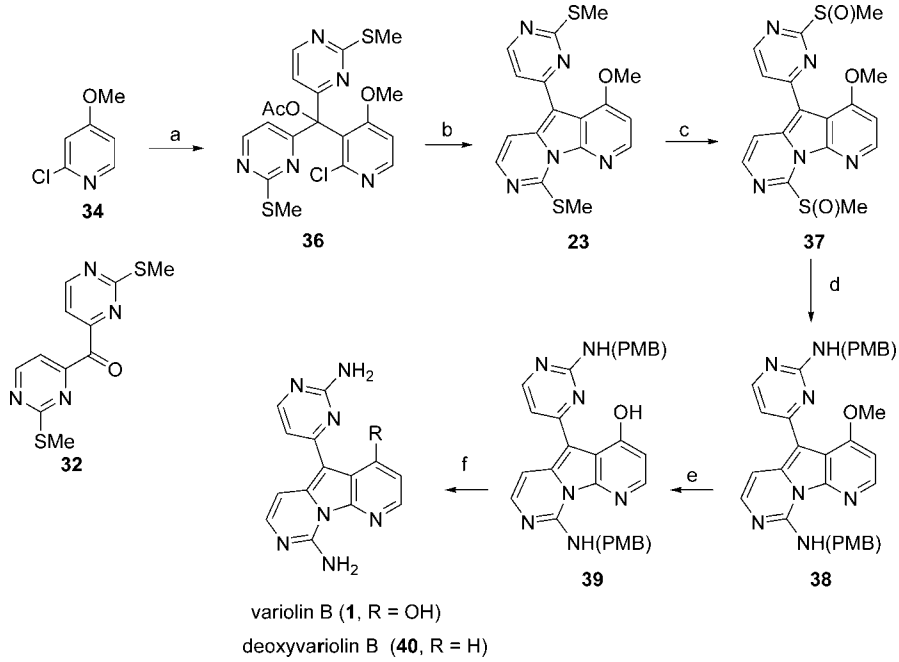
3.1.2. Synthesis of Variolin B Using a Linear Strategy

There have been three total syntheses of variolin B reported where the pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine core is assembled in a stepwise fashion, with the pendant 2′-aminopyrimidine attached late in the synthesis. The syntheses are presented in chronological order, and the relevant model studies have been included in the discussion of each synthesis.

3.1.2.1. Molina/Fresneda Synthesis of Variolin B. The group of Molina and Fresneda have pioneered the application of iminophosporanes in the synthesis of heterocyclic natural products.[23] Using this chemistry, they were the first to report a synthesis of the pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine core **41**, which is shown in Scheme 5.[24] Their approach began using 3-formyl-4-methoxypyridine (**42**), available in one step from 4-methoxypyridine (**43**).[25] Condensation with ethyl azidoacetate gave the vinyl azide **44**, which was cyclized via a nitrene insertion process to produce the 2-substituted azaindole **45**. The side-chain was extended through a four-step sequence (again, employing ethyl azidoacetate) to give the protected vinylazide **46**. This underwent the Staudinger reaction with triphenylphosphine to give (after deprotection of the SEM group) the iminophosphorane **47**, which was set up for ring-closure to the variolin core **41**. This could be effected using various dielectrophilic isocyanates in excellent yields. For the total synthesis of variolin B, α-methylbenzylisocyanate was used, providing the variolin core **41** in quantative yield.[20a]

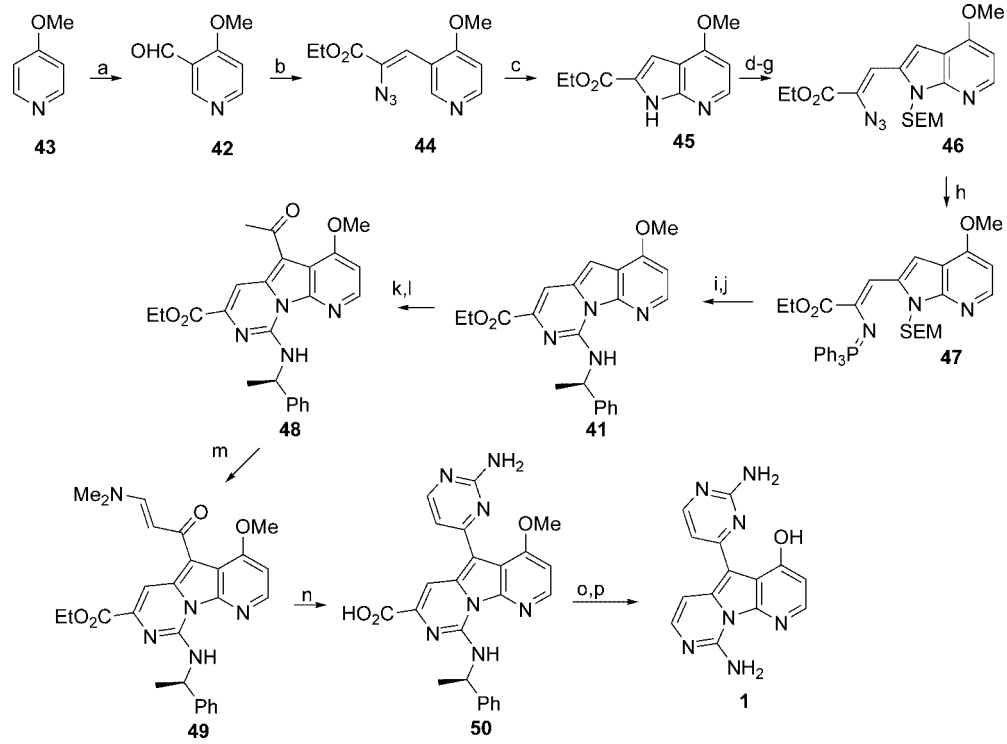
The construction of the pendant pyrimidine heterocycle was based on methodology Molina and Fresneda had used for the synthesis of meridianins C−E (see section 3.2).[24]

Scheme 4[a]



[a] Reagents and conditions: (a) (1) **34**, *n*-BuLi, THF, −90 °C → −78 °C; (2) **32**, −90 °C; (3) AcCl quench (68%); (b) Et_3SiH (8 equiv), CF_3CO_2H (2 equiv), $ClCH_2CH_2Cl$, 80 °C (75%); (c) MCPBA, $CHCl_3$, −40 °C; (d) *p*-$MeOC_6H_4CH_2NH_2$, 85 °C (78% over two steps); (e) NaSEt, DMF, 55 °C (95%); (f) TfOH, rt (80%).

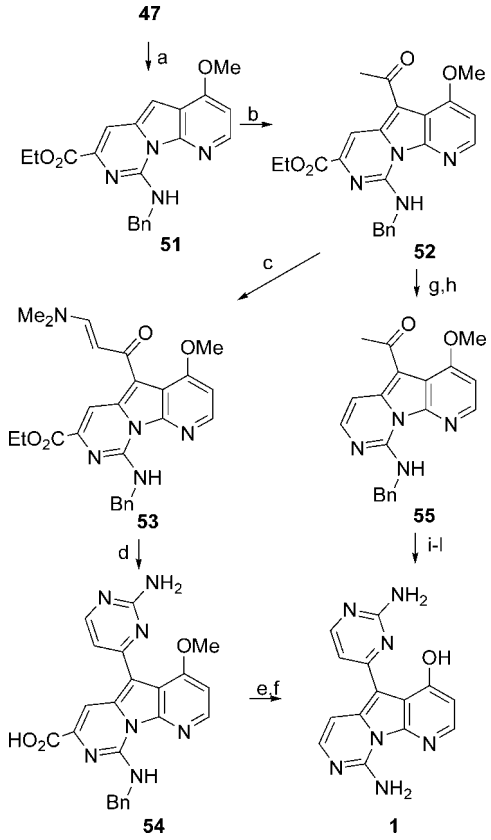
Scheme 5[a]



[a] Reagents and conditions: (a) mesityl lithium, −78 °C, then DMF (77%); (b) $N_3CH_2CO_2Et$, NaOEt, −15 °C (61%); (c) *o*-xylene, reflux (67%); (d) NaH, SEM−Cl, DMF (94%); (e) $LiAlH_4$, THF, reflux (93%); (f) MnO_2, CH_2Cl_2, rt (86%); (g) $N_3CH_2CO_2Et$, NaOEt, EtOH, −15 °C (85%); (h) Ph_3P, CH_2Cl_2, rt (82%); (i) TBAF−SiO_2, THF, MW (70%); (j) $PhCH(CH_3)NCO$, THF, rt (100%); (k) Br_2, pyridine, 0 °C (93%); (l) (α-ethoxyvinyl)trimethyltin, $PdCl_2(PPh_3)_2$ (4 mol %), DMF (65%); (m) *N*,*N*-dimethylformamide-di-*tert*-butyl acetal, DMF, 80 °C, (70%); (n) $H_2N(C{=}NH)NH_2.HCl$, K_2CO_3, 2-methoxyethanol, reflux (89%); (o) Ph_2O, 260 °C (35%); (p) triflic acid, rt (90%).

Functionalization at C5 of the variolin core was achieved by first reaction with bromine in pyridine and then Pd-catalyzed coupling with (α-ethoxyvinyl)trimethylstannane to give ketone **48** (Scheme 5). Treatment of **48** with dimethylformamide di-*t*-butyl acetal gave enaminone **49**, which, upon heating with guanidine hydrochloride and anhydrous K_2CO_3, formed the pendant 2-aminopyrimidine ring. Under these conditions, hydrolysis of the ester occurred concurrently, giving **50** in 89% yield. Decarboxylation of **50** proved to be rather difficult, but eventually, it was found that

Scheme 6[a]

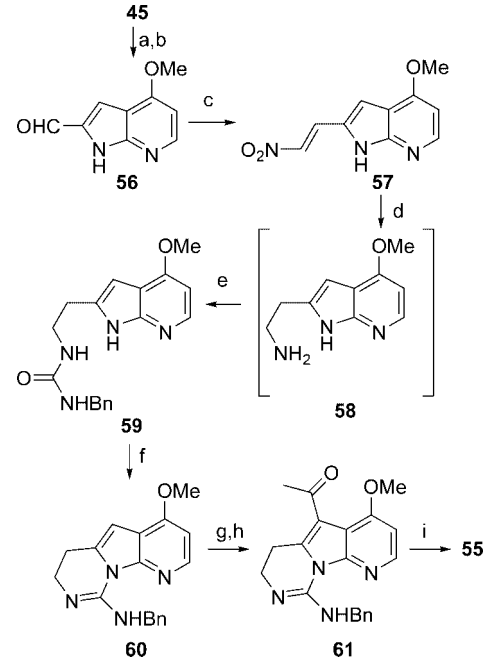


[a] Reagents and conditions: (a) BnNCO, THF, 50 °C (97%); (b) DMA, $POCl_3$ (90%); (c) DMF−DtBA, DMF, 80 °C (70%); (d) $H_2N(C{=}NH)NH_2 \cdot HCl$, K_2CO_3, 2-methoxyethanol, reflux (93%); (e) Ph_2O, 280 °C, 4 h (67%); (f) triflic acid, 50 °C (74%); (g) LiOH, THF−H_2O (100%); (h) Ph_2O, 250 °C (50%); (i) *N*,*N*-dimethylformamide-di-*tert*-butyl acetal, DMF, 80 °C (85%); (j) $H_2N(C{=}NH)NH_2 \cdot HCl$, K_2CO_3, 2-methoxyethanol, reflux (93%); (k) NaSMe, DMF, 80 °C (85%); (l) triflic acid, 50 °C (74%).

decarboxylation and simultaneous deprotection of the methoxy group could be accomplished in 35% yield by heating at 260 °C in diphenyl ether. Treatment of **50** with triflic acid at room temperature to remove the α-methylbenzyl protecting group gave the natural product variolin B (**1**) in 90% yield. The overall yield for the 16-step sequence was 1.2%.

Further work by the Molina and Fresneda group, reported in a full paper published in 2003, has led to several improvements in the total synthesis (Scheme 6).[20b] First, it was found that the acetyl functionality could be introduced directly by reacting **51** (prepared by reaction of benzyl isocyanate with **47**) with *N*,*N*-dimethylacetamide in the presence of $POCl_3$. The acetyl-substituted variolin core **52** was produced in 90% yield, which is a significant improvement on the prior two-step sequence (**41**−**48**, 60% overall yield). Conversion of **52** to variolin B was by the protocol reported in the first generation synthesis; however, the key decarboxylation/demethylation process has significantly improved—it was found that traces of water were detrimental to the process, and with appropriate precautions (preliminary drying and under nitrogen), the desired variolin core could be generated in 67% yield by heating **54** in dry diphenyl ether at 250 °C. It was also reported that **52** could be decarboxylated prior to installation of the pendant pyrimidine ring and the resulting acetyl compound **55** could be transformed to variolin B. Thus, Molina and Freneda have been able to complete a 14-step synthesis of variolin B,

Scheme 7[a]



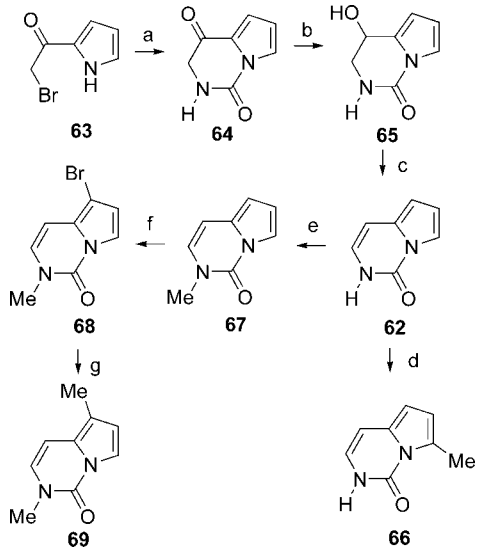
[a] Reagents and conditions: (a) $LiAlH_4$, THF, reflux (93%); (b) MnO_2, CH_2Cl_2, rt (60%); (c) CH_3NO_2, NH_4OAc, EtOH, 75 °C (80%); (d) $LiAlH_4$, THF, 0 °C → rt; (e) $PhCH_2NCO$, CH_2Cl_2, rt (60% over 2 steps); (f) CCl_4, Ph_3P, Et_3N, CH_2Cl_2, 50 °C (90%); (g) NBS, THF, CH_2Cl_2 (70%); (h) (α-ethoxyvinyl)trimethyltin, $PdCl_2(PPh_3)_2$ (4 mol %), DMF, 70 °C; then 1 M HCl, acetone, rt (65%); (i) DBU, $CBrCl_3$ (32%); or DDQ, CH_2Cl_2, (48% b.r.sm.).

starting from the commercially available 4-methoxypyridine, in 5% overall yield.[20b]

An alternate synthesis of **55** was also developed where a dihydro ring system was first prepared and then dehydrogenated (Scheme 7).[20b] To access this system, the ester **45** was converted to the aldehyde **56** via a two-step sequence. After conversion to the nitro compound **57** (CH_3NO_2, NH_4OAc, 80%), reduction with $LiAlH_4$ provided an unstable amine **58**, which was immediately reacted with benzyl isocyanate to afford the urea **59** in 60% yield. Treatment with carbon tetrachloride/triphenylphosphine provided the dihydro tricyclic core **60** in 90% yield. After introduction of the 5-acetyl substituent by the previously developed bromination/Stille protocol, the dihydroheteroaromatic **61** was converted to **55** by either reaction with bromotrichloromethane and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (32%) or with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (48% based on recovered starting material).

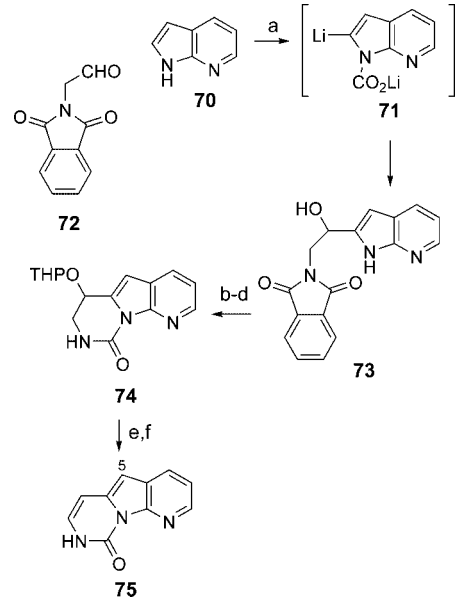
3.1.2.2. Álvarez-Joule Synthesis of Variolin B. Álvarez and Joule have been active in the area of variolin synthesis, with several reports on the synthesis of fragments of the variolin structure[26−31] and a total synthesis of variolin B completed in 2003.[21] Álvarez and Joule reported a synthesis of 1,2-dihydropyrrolo[1,2-*c*]pyrimidin-1-one (**62**), which was a model for the BC-fragment of the variolin core structure (Scheme 8).[26,29] The synthesis of **62** began with **63**, which itself was prepared by reacting pyrrole with bromoacetyl bromide in the presence of 2,6-lutidine. Reaction of **63** with potassium cyanate gave the bicyclic ketone **64** in 52% yield. Following borohydride reduction, exposure of the resulting alcohol **65** to methanesulfonyl chloride in the presence of triethylamine gave **62**. Lithiation of **62** with *t*-BuLi, followed by trapping with iodomethane, produced the 7-methyl

Scheme 8[a]



[a] Reagents and conditions: (a) KCNO, MeCN, reflux (52%); (b) $NaBH_4$, MeOH, 0 °C (83%); (c) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C then NaOH, MeOH, rt (84%); (d) *t*-BuLi, THF/−85 °C → 0 °C, then MeI (70%); (e) NaH, THF, 0 °C → rt, then MeI (93%); (f) NBS, CH_2Cl_2, rt (65%); (g) *n*-BuLi, THF, − 85 °C, then MeI (50%).

Scheme 9[a]



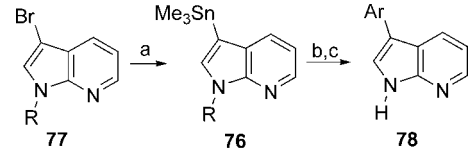
[a] Reagents and conditions: (a) (1) *n*-BuLi, THF, −78 °C → rt; (2) CO_2, −78 °C; (3) *t*-BuLi, THF, −78 °C; (4) **72**, THF, −78 °C → rt (44%); (b) DHP, HCl, benzene, $CHCl_3$, reflux (87%); (c) $NH_2NH_2 \cdot H_2O$, EtOH, reflux (100%); (d) $(Cl_3CO)_2CO$, EtN^iPr_2, CH_2Cl_2, rt (76%); (e) 4N HCl, CH_2Cl_2 (100%); (f) MsCl, NEt_3, CH_2Cl_2, 0 °C (95%).

derivative **66** in 70% yield. Methylation at C5 was accomplished by *N*-methylation to give **67** (NaH, MeI, 93%), followed by reaction with *N*-bromosuccinimide to yield the 5-monobromo derivative **68** in 65% yield. A bromine−lithium exchange followed by addition of iodomethane generated the 5-methylated derivative **69** in 50% yield. This was used to confirm the position of bromination.

Having established some protocols for the construction of the BC-fragment, Álvarez and Joule then investigated the synthesis of the non-natural analogue of variolin B—deoxyvariolin B (**40**)—because the starting material to prepare this was commercially available (Scheme 9).[28] Thus, 7-azaindole (**70**) was lithiated at C2, using Katritzky's method,[32] and the resulting lithio species **71** was quenched with *N*-pthalimidoaminoacetaldehyde (**72**) to give the 2-substituted azaindole **73** in 44% yield. Following protection/deprotection steps, cyclization to tetrahydropyrimidinone **74** was effected upon treatment with triphosgene and Hünig's base. Conversion to the desired pyrimidinone **75** was achieved in 95% yield by hydrolysis of the protecting group and dehydration via the mesylate derivative.

In an earlier study, they had established that the pendant pyrimidine ring could be added via a palladium-catalyzed cross-coupling (Scheme 10).[27] In this work, stannane **76**, which was obtained from the corresponding bromide **77**, was coupled with a range of aryl and heteroaryl halides under Pd-catalysis, to give aryl-substituted azaindoles **78** (Scheme 10). However, application of this methodology to the variolin system required some work for it to be a viable method.

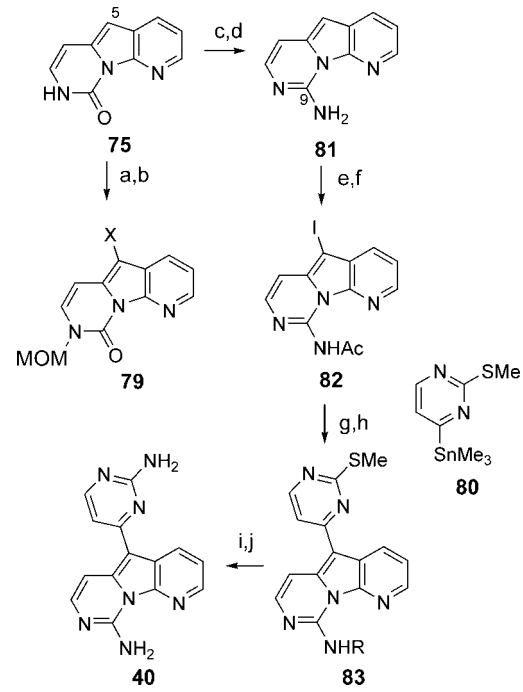
Scheme 10[a]



Ar	X	% Yield
4-methoxyphenyl	Br	38
4-nitrophenyl	Br	50
thien-3-yl	Br	57
2-(methylthio)pyrimidin-4-yl	Cl	60
2-(methylthio)pyrimidin-5-yl	Br	53

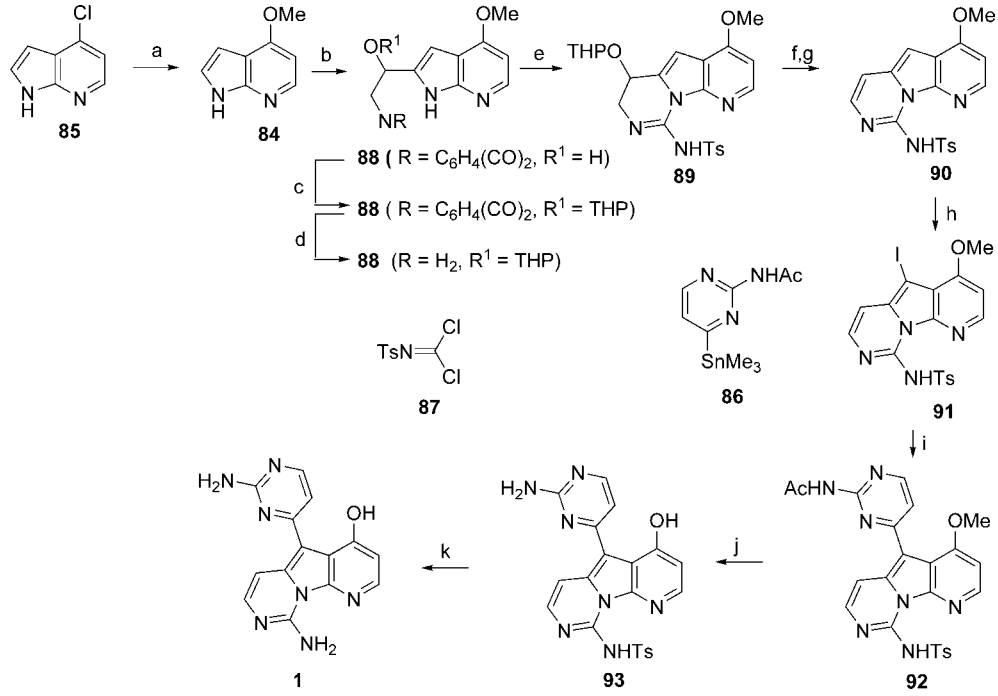
[a] Reagents and Conditions: (a) *t*-BuLi, THF, −90 °C, then Me_3SnCl (99%); (b) $Pd(PPh_3)_4$, ArX, LiCl, THF, reflux; (c) 10% aq HCl, rt (see yields in table above).

Scheme 11[a]



[a] Reagents and conditions: (a) MOMCl, NaH, DMF, 0 °C (87%); (b) I_2, DMF, NaOH, rt (62%); (c) TMSCl, HMDSA, 2,6-lutidine; (d) NH_3, 150 °C, 60 psi (30% over two steps); (e) Ac_2O, THF, rt (75%); (f) NIS, $CHCl_3$, rt (95%); (g) $Pd_2(dba)_3$, PPh_3, LiCl, CuI, dioxane, reflux, 4-trimethylstannyl-2-methylthiopyrimidine (**80**); (h) HCl, MeOH, reflux (45% over two steps); (i) MCPBA, CH_2Cl_2, 0 °C (90%); (j) aq. NH_4OH, dioxane, 80 °C (90%).

Scheme 12[a]



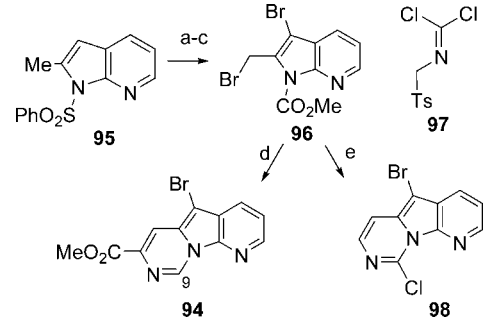
[a] Reagents and conditions: (a) NaOH, MeOH, 150 °C (70%); (b) (i) *n*-BuLi, THF, −78 °C to rt, (ii) CO_2, −78 °C, (iii) *t*-BuLi, THF, −78 °C, (iv) 2-phthalimidoacetaldehyde (**72**), THF, −78 °C to rt, (43%); (c) DHP, HCl, benzene, $CHCl_3$, reflux (65%); (d) $NH_2NH_2 \cdot H_2O$, EtOH, reflux (95%); (e) **87**, EtN^iPr_2, CH_2Cl_2, rt (65%); (f) 4N HCl, CH_2Cl_2, rt (80%); (g) NEt_3, MsCl, CH_2Cl_2, 0 °C (78%); (h) NIS, CH_2Cl_2, −30 °C (95%); (i) **86**, $Pd_2(dba)_3$ (22 mol %), PPh_3 (38 mol %), LiCl (3 equiv), CuI (20 mol %), dioxane, reflux (71%); (j) 48% aq HBr, reflux (60%); (k) 1,4-dimethoxybenzene, $NH_2NH_2.H_2O$, MeOH, *hν* (30%).

While **75** could be efficiently iodinated at the 5-position, the iodo-derivative **79** (X = I) could not be efficiently converted to the corresponding 5-stannyl compounds **79** (X = $SnMe_3$) (Scheme 11). Furthermore, it was found that the 1,2-dihydropyrrolo[1,2-*c*]pyrimidin-1-one **79** (X = I) could not be coupled with the stannylpyrimidine **80** in an effective manner.[19b,31] To circumvent these difficulties, **75** was converted to the amine **81**. However, this proved to be quite difficult, with the most successful method requiring a two-step procedure involving *O*-silylation followed by nucleophilic substitution with ammonia in a pressure vessel. This provided the desired amine **81** in just 30% yield for the two steps.

After *N*-acylation (Ac_2O, THF, 75%), iodination with *N*-iodosuccinimide gave **82** in 95% yield. A Pd-catalyzed coupling with **80** gave the acetamide **83** (R = Ac), but because some hydrolysis had occurred, the reaction mixture was reacted with methanolic hydrochloric acid to afford the amine **83** (R = H) in 45% yield for the two-step procedure. The synthesis was completed by *S*-oxidation of **83** (R = H), followed by substitution of the resulting sulfone with ammonia, producing deoxyvariolin B (**40**) in 81% yield for the two steps. The overall yield for the 14-step sequence was 2.2%.

In 2003 this strategy was applied to the total synthesis of variolin B,[20] resulting in a ten-step procedure starting from 4-methoxy-7-azaindole (**84**), which in turn can be prepared from commercially available 4-chloro-7-azaindole (**85**) (Scheme 12). This synthesis follows the same outline as the preceding synthesis of deoxyvariolin B, but with significant modifications to two of the key steps. First, the reagent used for the formation of the tricyclic system was altered, allowing the direct introduction of a protected amino group. Second, 2-acetylamino-4-trimethylstannylpyrimidine (**86**) was used for the coupling reaction so as to minimize the functional group interchanges at the end of the sequence.

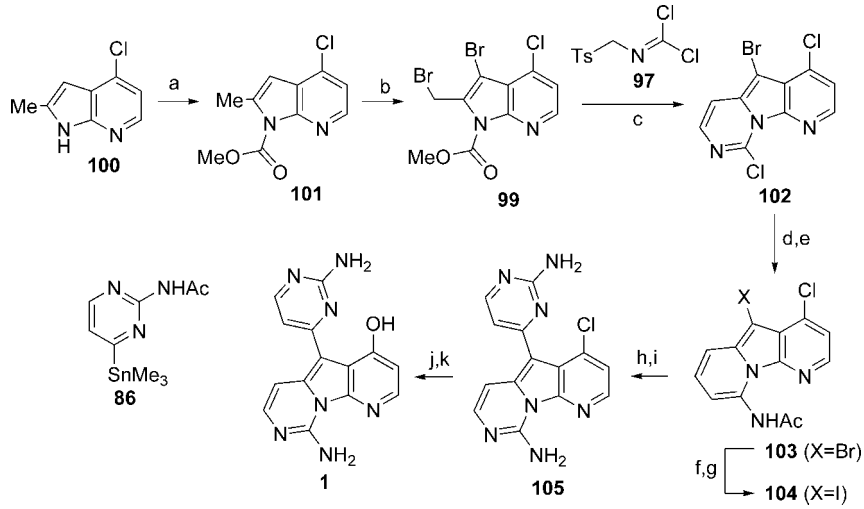
Scheme 13[a]



[a] Reagents and conditions: (a) K_2CO_3, MeOH, H_2O (80%); (b) LiHMDS, THF, −78 °C, then $ClCO_2Me$ (99%); (c) NBS, Bz_2O, CCl_4 (91%); (d) $TsCH_2NC$, NaOH, Bu_4NI, CH_2Cl_2, −10 °C (65%); (e) **97**, 30% aq. LiOH, Bu_4NCl, CH_2Cl_2, rt (58%).

In the deoxyvariolin synthesis, the introduction of the C9 amino group proved to be difficult, and as a consequence, in the total synthesis of variolin B, Álvarez and Joule employed *N*-tosyldichloromethanimine (**87**) as the cyclization reagent (Scheme 12).[20,30] Treatment of amine **88**, prepared from **84** in an analogous manner to the deoxy series, with *N*-tosyldichloromethanimine (**87**) and Hünig's base in dichloromethane provided the dihydropyrimidinone **89** in 65% yield. Removal of the protecting group (4N HCl) and dehydration (MsCl, NEt_3) gave the tricyclic heterocycle **90** in 66% yield for the two steps. As per the deoxy series, the pyrimidine ring was attached using cross-coupling chemistry, but the acetamide **86** was used. Thus, the iodide **91**, synthesized in 95% yield by reaction of **90** with *N*-iodosuccinimide, was heated with **86** in the presence of

Scheme 14[a]



[a] Reagents and conditions: (a) LiHMDS, THF, −78 °C; $ClCO_2Me$, (86%); (b) NBS (2.2 equiv), CH_2Cl_2, rt (75%); (c) **97**, 30% aq. LiOH, CH_2Cl_2, Bu_4NCl, rt (58%); (d) Ph_3SiNH_2, $Pd_2(dba)_3$ (5 mol %), (2-biphenyl)-di-*t*-butylphosphine (10 mol %), THF, then LiHMDS, rt; (e) Ac_2O, reflux (51% over 2 steps); (f) TTMSS (2 equiv), AIBN (2 equiv), THF, 80 °C; (g) NIS, CH_2Cl_2, 0 °C (75% over 2 steps); (h) **86** (2 equiv), $Pd_2(dba)_3$ (6 mol %), PPh_3 (12 mol %), LiCl (3 equiv), CuI (6 mol %), dioxane, reflux; (i) HCl−MeOH, reflux (48% over two steps); (j) $Pd_2(dba)_3$ (5 mol %), (2-biphenyl)-di-*t*-butylphosphine (10 mol %), NaO*t*-Bu (2 equiv), toluene−*t*-BuOH (5:1), MW 150 °C and 300 W; (k) H_2O−HCl, rt, (48% over two steps).

$Pd_2(dba)_3$ (22 mol %), PPh_3 (38 mol %), LiCl (3 equiv), and CuI (20 mol %) to generate the fully protected variolin B **92** in 71% yield. Removal of the *N*-acetate and the methoxy group was achieved by reaction with aqueous hydrobromic acid to afford the *N*-tosyl derivative **93** in 60% yield. Unfortunately, removal of the tosyl group proved to be very difficult, with the best conditions involving irradiation of **93** with a high-pressure Hg lamp in the presence of 1,4-dimethoxybenzene, hydrazine hydrate, and methanol. After purification of the reaction mixture by preparative HPLC, variolin B (**1**) was obtained in 30% yield.

3.1.2.3. Vaquero Total Synthesis of Variolin B. Vaquero and co-workers reported a four-step synthesis of the variolin core **94** in 2000 (Scheme 13).[33,34] In their approach, intact azaindoles were utilized as starting materials, with the variolin core being formed by annulation of a side-chain attached to C2 of the azaindole. This work was based on their prior work on the synthesis of pyrrolo[1,2-*c*]pyrimidines.[35] For example, azaindole **95** was protected at the indole nitrogen and dibrominated to give the bromomethyl compound **96** (Scheme 13). Extension of the bromomethyl group was achieved using tosylmethyl isocyanide under phase-transfer conditions to afford the variolin core structure **94**. While this elegant chemistry provides access to interesting structures, the use of tosylmethyl isocyanide limits the application to a total synthesis of variolin B as there is no amino substituent in the C9 position of the generated compounds. As a consequence, Vaquero investigated the use of tosylmethyldichloroformimide (**97**), which as a tosylmethyl isocyanide synthetic equivalent would allow the introduction of a chloro substituent at C9 (Scheme 13). For example, the model system **96** was reacted with **97** under phase-transfer conditions (30 mol % LiOH, CH_2Cl_2, Bu_4NCl, room temperature (rt)) to generate the 9-chloropyridopyrrolopyrimidine **98** in 58% yield.

Application of this chemistry to the total synthesis of variolin B requires access to dibromide **99** (Scheme 14).[21,36] The readily available azaindole **100** was acylated with methyl chloroformate to afford the carbamate **101** in 86% yield. Reaction of the carbamate **101** with 2.2 equiv of *N*-bromosuccinimide gave the dibromide **99** in 91% yield. Reaction of dibromide **99** with tosylmethyldichloroformimide (**97**) under the previously described conditions gave the desired heterocycle **102** in 58% yield. A palladium-catalyzed amination (Ph_3SiNH_2, $Pd_2(dba)_3$ (5 mol %), (2-biphenyl)di-*t*-butylphosphine (10 mol %), then LiHMDS), followed by acetylation of the resulting amine, afforded a 51% yield of the C9-acetylamino heterocycle **103**. As already reported by Álvarez and Joule on a related system,[20] attempts to couple the stannane **86** with the bromide **103** were unproductive, but this unreactivity was overcome by using the iodide group.

Vaquero and co-workers were able to convert **103** to the iodide **104** in 75% yield using a two-step procedure involving debromination (AIBN, TTMSS) and then iodination with *N*-iodosuccinimide. After a successful palladium coupling of the iodide **104** with stannane **86**, the reaction mixture was reacted with methanolic hydrochloric acid to afford the variolin derivative **105** in 48% yield. Completion of the synthesis required conversion of the 4-chloro substituent to the 4-hydroxy substituent, and this was achieved by reacting **105** with 2 equiv of sodium *t*-butoxide in the presence of $Pd_2(dba)_3$ (5 mol %) and (2-biphenyl)-di-*t*-butylphosphine (10 mol %) for 2 min at 300 W microwave power. Reaction of the material under acidic conditions (HCl, H_2O) removed the resulting *t*-butyl group and provided variolin B (**1**) in 48% yield for the two steps. The overall yield for the 11-step sequence was 3.3%.

3.2. Synthesis of the Meridianins and Related Compounds

Members of the meridianin family have been prepared by a number of groups. The current routes to meridianins and analogues involve disconnection of the indole as an intact unit and either construction of the pyrimidine ring onto an 5-acylated indole **106** (path a) or, in one example (path b), the connection of an intact pyrimidine ring **107** to an indole system **108** via a Suzuki coupling reaction (Figure 7).

The group of Molina and Fresneda were the first to report the synthesis of the meridianins A (**6**), C (**8**), D (**9**), and E

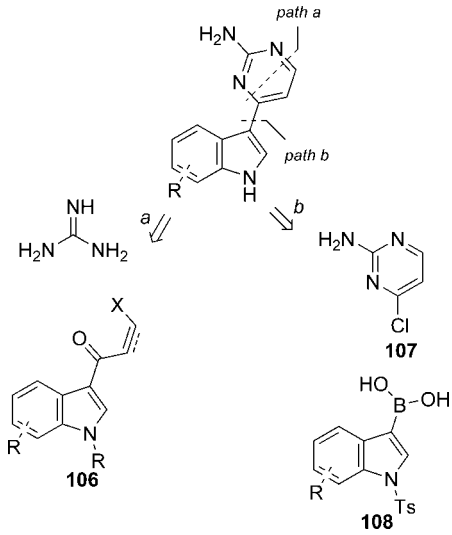


Figure 7. Retrosynthetic analyses of the meridianins.

(**10**) (Scheme 15).[24,37] These syntheses utilized the Bredereck protocol to construct the 2-aminopyrimidine ring onto an 5-acetylindole, namely, formation of an enaminone with dimethylformamide dimethylacetal (DMF−DMA) followed by heterocyclization with guanidine hydrochloride under basic conditions. In order to synthesize meridianins A (**6**) and E (**10**), the previously unknown indole **109** was required. This was prepared in 75% overall yield by protection of the commercially available phenol **110** with benzyl bromide, followed by conversion to vinyl azide **111** by treatment with ethyl azidoacetate under basic conditions. Under thermal conditions, this vinyl azide **111** cyclized to form carboxyindole **112** in 92% yield and then converted to the desired indole **109** by saponification and decarboxylation. Friedel−Crafts acylation of **109**, followed by reaction with tosyl chloride, gave the *N*-tosyl methyl ketone **113** in 49% overall yield. Treatment of the *N*-tosyl methyl ketone **113** with DMF−DMA gave enaminone **114** in 82% yield. Treatment of the enaminone **114** with guanidine hydrochloride and potassium carbonate formed the 2-aminopyrimidine ring, in addition to deprotecting the *N*-tosyl group to give the meridianin precursor **115** in 82% yield. This common precursor was then used to prepare both meridianin A (**6**) and meridianin E (**10**). Hydrogenolysis of both the benzyl ether and bromide groups in **115** provided meridianin A (**6**) in 83% yield, while acidic cleavage of the benzyl ether group of **115** gave meridianin E (**10**) in 65% yield.

Meridianins C (**8**) and D (**9**) are isomeric (aminopyrimidyl)bromoindoles and were prepared by a similar application of the Bredereck protocol to that used for the synthesis of meridianins A (**6**) and E (**10**) (Scheme 16). Reaction of the bromoindoles **116a**/**116b** with tosyl chloride, then acylation under Friedel−Crafts conditions, gave methyl ketones **117a**/**117b**. Treatment of **117a**/**117b** with *N*,*N*-dimethylformamide−dimethyl acetal (DMF−DMA) gave rise to the desired enaminones **118a**/**118b**, which upon treatment with guanidine hydrochloride and potassium carbonate gave meridianins C (**8**) and D (**9**), respectively.

The approach of Müeller and co-workers to the synthesis of meridianins involved a carbonylative Sonagashira coupling to introduce the carbon framework of the meridianins, followed by a heterocyclization to complete the pyrimidine ring (Scheme 17).[38] Suitable Boc-protected 3-iodoindoles **119** were prepared in two steps from the corresponding indoles **120** (88−98% yield).[39] These iodoindoles **119** underwent carbonylative Sonagashira coupling with trimethylsilylacetylene under an atmosphere of carbon monoxide to give good yields (64−68%) of the corresponding silyl alkynones **121**. Interestingly, a mixed catalyst system of $Pd(PPh_3)_2Cl_2$ and $Pd(dppf)Cl_2$ was required for this coupling to proceed; use of either catalyst alone resulted in significantly reduced yields. Treatment of **121** with aqueous guanidine resulted in both formation of the 2-aminopyrimidine ring and concomitant removal of the trimethylsilyl and Boc groups, to give meridianins C (**8**), D (**9**), and G (**12**) in good yields. This methodology provides access to the meridianins in six steps from the corresponding indoles **120**, in addition to providing access to other arylated pyrimidines.

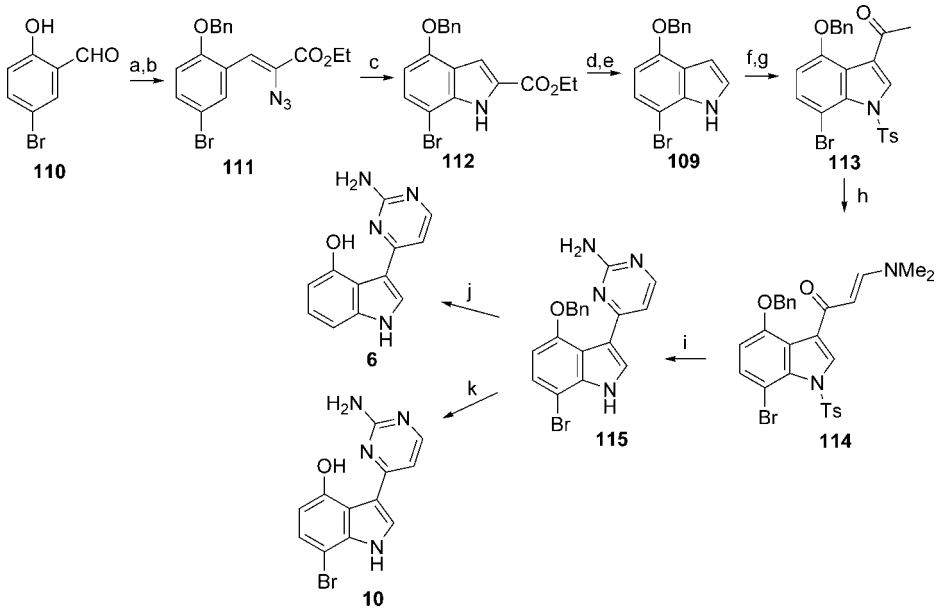
A different approach to producing meridianins D (**9**) and G (**12**) was taken by Jiang and co-workers (Scheme 18).[40] Instead of introducing a pyrimidine ring onto an indole system by sequential acylation and heterocyclization, the entire pyrimidine ring system was introduced by coupling an intact chloropyrimidine with a indoylboronic acid. For example, meridianin D (**9**) was prepared by Suzuki coupling of chloropyrimidine **122** with the indoylboronic acid **123** (X = $B(OH)_2$). This afforded the protected meridianin **124** in 53% yield. Base hydrolysis removed the *N*-tosyl group to afford meridianin D (**9**) in 76% yield. The same methodology was used to access analogues of the meridianin family with pyrimidines bearing indole substituents at both the 2- and 4-positions.

Palermo and co-workers investigated the preparation of pyrimidylindoles **125** that are isomeric to the meridianans, with the aminopyrimidine ring connected to the 2-position of the indole (Scheme 19).[41] Treatment of pyrimidine **126** with di-(*tert*-butyl)pyrocarbonate in hot pyridine, followed by chlorination with phosphorus oxychloride, gave Boc-protected chloropyrimidine **127**. Stille coupling of chloropyrimidine **127** with (α-ethoxyvinyl)tributylstannane gave a good yield of the enol ether **128**, which was subsequently cleaved under acidic conditions to give the methyl ketone **129**. Treatment of the methyl ketone **129** with phenylhydrazine **130** (X = H), followed by heating with zinc chloride, gave the desired 2-pyrimidylindole **125** (R = H) via a Fischer indole synthesis and concomitant Boc deprotection. Treatment of the methyl ketone **130** with 4-bromophenylhydrazine **130** (X = Br) and then reaction with zinc chloride gave the corresponding bromoindole **125** (X = Br). Both 2-subsituted indoles **125** were found to have poor biological activity, highlighting the importance of the 3-pyrimidyl substituent in the biological activity of the meridianins.[42]

Radwan and co-workers prepared a small series of meridianins substituted on the pyrimidine ring at the 5-position (Scheme 20).[43] The analogues were prepared by elaboration of indole (**131**) in a similar manner to that reported by Fresneda and co-workers. Treatment of indole (**131**) with cyanoacetic acid and acetic anhydride gave the α-cyanoketone **132**, which was converted to the (*Z*)-enaminone **133** by heating with DMF−DMA. Treatment of enaminone **133** with guanidine directly gave 5′-cyanomeridianin G (**134**).

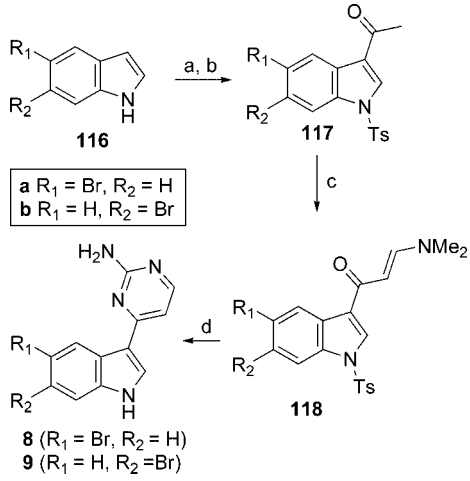
5′-Cyanomeridianin G (**134**) was then converted to other 5′-substituted compounds (Scheme 21). Treatment of nitrile **134** with hydrazine hydrate gave the amidrazone **135**, while hydrolysis of **134** gave the carboxylic acid **136**. Acid **136** could also be decarboxylated to meridianin G (**12**) by treatment with hot sulfuric acid, providing a different route to the natural product. The 5′-substituted meridianin G

Scheme 15[a]



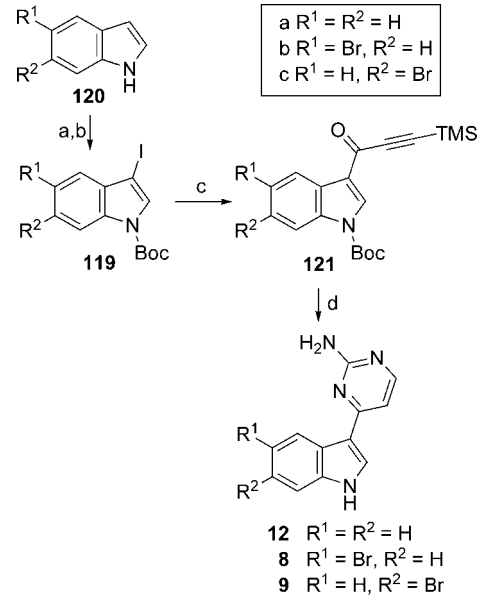
[a] Reagents and conditions: (a) NaH, DMF, 0 °C; BnBr (100%); (b) $N_3CH_2CO_2Et$, EtONa, EtOH, −15 °C (75%); (c) PhMe, reflux (92%); (d) LiOH, aq. THF (99%); (e) Cu, quinoline, 235 °C (72%); (f) AcCl, $SnCl_4$, PhH (89%); (g) TsCl, NaH, DMF (55%), (h) DMF−DMA, DMF, 110 °C (45%); (i) $H_2N(=NH)NH_2 \cdot HCl$, K_2CO_3, 2-methoxyethanol, reflux (82%); (j) H_2, Pd/C, EtOAc (83%); (k) TFA, thioanisole (65%)

Scheme 16[a]



[a] Reagents and conditions: (a) TsCl, NaH, DMF (90−93%); (b) Ac_2O, $AlCl_3$, CH_2Cl_2 (80−84%); (c) DMF−DMA, DMF, 110 °C (82−83%); (d) $H_2N(=NH)NH_2 \cdot HCl$, K_2CO_3, 2-methoxyethanol, reflux (72−78%).

Scheme 17[a]



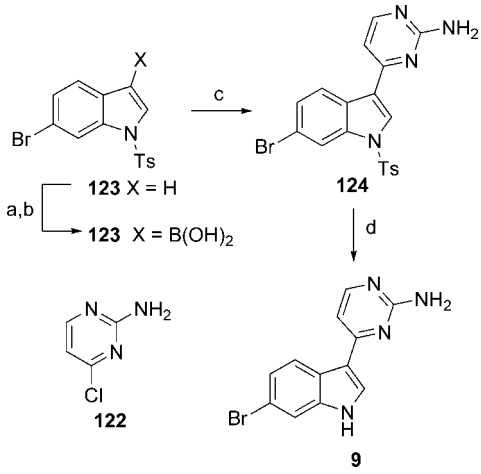
[a] Reagents and conditions: (a) I_2, KOH, DMF, 20 °C (100%); (b) Boc_2O, Et_3N, DMAP (10 mol %), CH_2Cl_2, 20 °C (88−98%); (c) TMS−acetylene, NEt_3, cat. $Pd(PPh_3)_2Cl_2$, cat. $Pd(dppf)Cl_2$, cat. CuI, THF, 1 atm CO (**121a** 68%, **121b** 68%, **121c** 64%); (d) 2.5 equiv of $H_2N(=NH)NH_2$, 1 equiv of Na_2CO_3, *t*-BuOH, MeCN, 80 °C (**12** 66%, **8** 73%, **9** 78%).

analogues all possessed cytotoxic activity against breast carcinoma (MCF7) cell lines, with the amidrazone **135** proving the most active with an IC_{50} of 0.25 μg/mL. This is a considerable improvement over the parent natural product meridianin G (**12**), which is reportedly inactive against MCF7 cultures.

In a further investigation of 5′-substituted meridianins, Anizon and co-workers synthesized a series of 5′-arylated meridianin G analogues (Scheme 22).[44] Treatment of meridianin G (**12**) or its *N*-methylated analogue (**137**) (both obtained in two steps from 3-acetylindole) with *N*-bromosuccinimide in cold THF cleanly brominated the 5′-position, affording bromopyrimidines **138**. Bromopyrimidines **138** were then coupled with a variety of arylboronic acids under Suzuki conditions to give 5′-arylated meridianin analogues **139**. The 5′-brominated meridianin G analogue **138** (R = H) was shown to have improved activity compared to the natural product when tested against a variety of kinases.

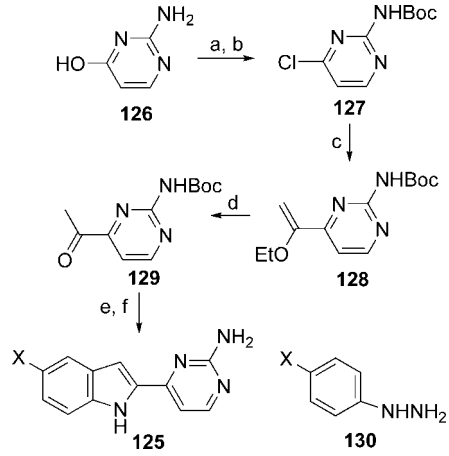
Stanovnik investigated analogues of the meridianins where the pendant pyrimidine ring is replaced with other heterocyclic systems, either as a monocyclic pyrimidinone or as a fused bicyclic system containing a pyrimidinone motif (Scheme 23).[45] The monocyclic pyrimidinone derivatives **140** were prepared in a three-step sequence from the indole ester **141**. Treatment of ester **141** with DMF−DMA gave the (*E*)-enaminoate **142** in good yield. Treatment of the enaminoate **142** with a variety of (thio)urea compounds in the presence

Scheme 18[a]



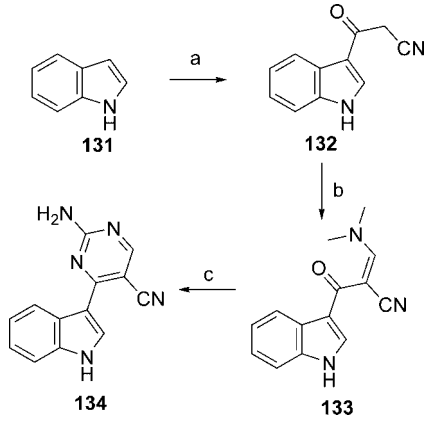
[a] Reagents and conditions: (a) Br_2, CCl_4, (96%); (b) *t*-BuLi, THF, −78 °C, then $B(OMe)_3$, −78 °C, (60%), (c) **122**, $Pd(PPh_3)_4$ (10 mol %), aq Na_2CO_3, PhH/MeOH, reflux (53%); (d) NaOH, MeOH, reflux (76%).

Scheme 19[a]



[a] Reagents and conditions: (a) Boc_2O, pyridine, 85 °C, (63%); (b) $POCl_3$, $PhNMe_2$, CH_2Cl_2, (76%); (c) (α-ethoxyvinyl)tributyltin, $Pd(PPh_3)_4$, LiCl, THF, reflux, (91%); (d) TsOH, acetone, reflux, (88%); (e) **130**, NaOAc, MeOH, (X = H, 90%; X = Br, 90%); (f) $ZnCl_2$, DMF, microwave, (X = H, 75%; X = Br, 55%).

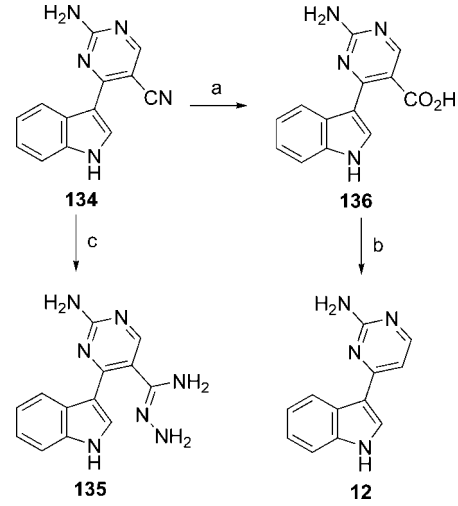
Scheme 20[a]



[a] Reagents and conditions: (a) cyanoacetic acid, Ac_2O, reflux (91%), (b) DMF−DMA, PhMe, 120 °C (85%); (c) $H_2N(C{=}NH)NH_2{\cdot}HCl$, K_2CO_3, EtOH, reflux (71%).

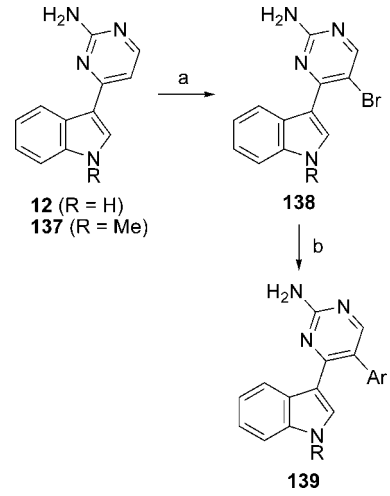
of acid caused exchange of the enamine amine, giving the corresponding enamino(thio)ureas **143**, generally as a separable mixture of (*E*)- and (*Z*)-isomers. Treatment of a mixture of (*E*)- and (*Z*)-enamino(thio)ureas **143** with an alkoxide base induced cyclization, returning the desired monocyclic pyrimidinone derivatives **140** in low-to-good yields. These compounds exist primarily in their oxo(thione) tautomers in d_6-DMSO, as determined by ^{13}C NMR spectroscopy. A similar method was used to prepare additional meridianin analogues with varied fused bi- and tricyclic heterocycles attached to the indole 3-position. The wide variety of 3-substituted indole structures generated by this methodology bear some analogy to the meridianins. The variety of structures might be able to shed some insight into the role of the pendant pyrimidine ring in the biological activity of the meridianins, but an investigation of the biological properties of these compounds is yet to be reported in the literature.

Scheme 21[a]



[a] Reagents and conditions: (a) aq. NaOH, EtOH, reflux, (83%); (b), conc. H_2SO_4, 90−120 °C (87%); (c) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux (79%).

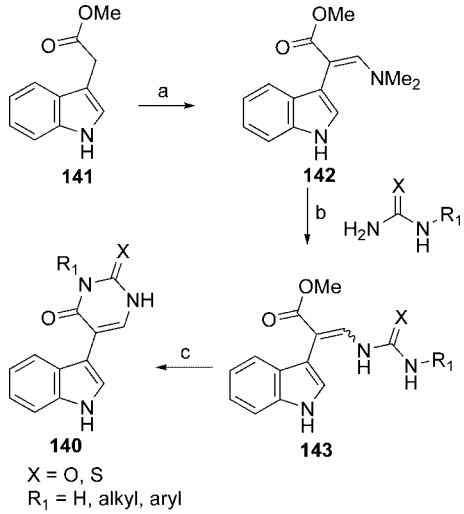
Scheme 22[a]



[a] Reagents and conditions: (a) NBS, THF, 0 °C (94−95%); (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, PhMe, EtOH, H_2O (21−60%).

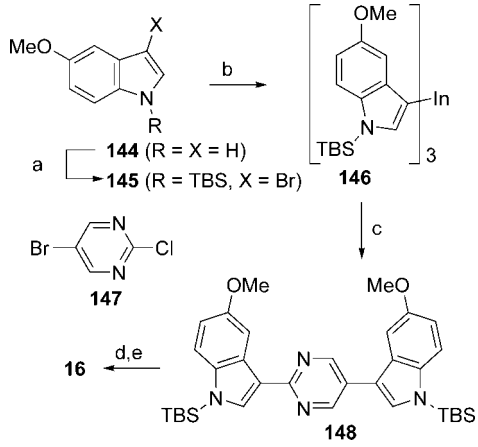
The structure of the related alkaloid hyrtinadine A (**16**) was recently confirmed by total synthesis (Scheme 24).[46] Protection and bromination of commercially available 5-methoxyindole (**144**, R = X = H) gave bromoindole **145** (R = TBS, X = Br), which was treated sequentially with *n*-butyllithium and indium(III) chloride to give the triarylindium

Scheme 23[a]



[a] Reagents and conditions: (a) DMF−DMA, DMF, reflux (85%); (b) conc. HCl or TFA, DMAA, 50 °C (43−89%); (c) MeONa or *t*-BuOK, DMAA (46−83%).

Scheme 24[a]



[a] Reagents and conditions: (a) (1) *n*-BuLi, THF, −78 °C, (2) *t*-BuMe$_2$SiCl, THF, 0 °C, (3) NBS, −78 °C (85%); (b) *n*-BuLi, THF, −78 °C; then InCl$_3$, THF; (c) **147**, Pd(PPh$_3$)$_4$ (5 mol %), THF, 80 °C, (87% over two steps); (d) BBr$_3$, CH$_2$Cl$_2$ (72%); (e) TBAF, THF, (81%).

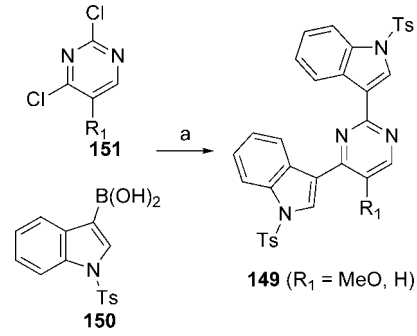
species **146**. This species was not isolated, but was coupled in situ with 2-chloro-5-bromopyrimidine (**147**) under palladium catalysis to give the core structure of hyrtinadine A **148** in 87% yield. Deprotection of the methoxy groups with boron tribromide, and removal of the *N*-silyl groups with tetrabutylammonium fluoride (TBAF), gave the natural product (**16**) in an overall 48% yield from 5-methoxyindole.

Jiang and co-workers prepared a series of indolyl pyrimidines **149** related to hyrtinadine A via a Suzuki coupling of *N*-tosylindole-3-boronic acid **150** with 2,4-dichloropyrimidines **151** (Scheme 25).[47] These compounds exhibited cytotoxic activities against several cell lines in the mid-to-low μM range.

3.3. Synthesis of the Meriolins

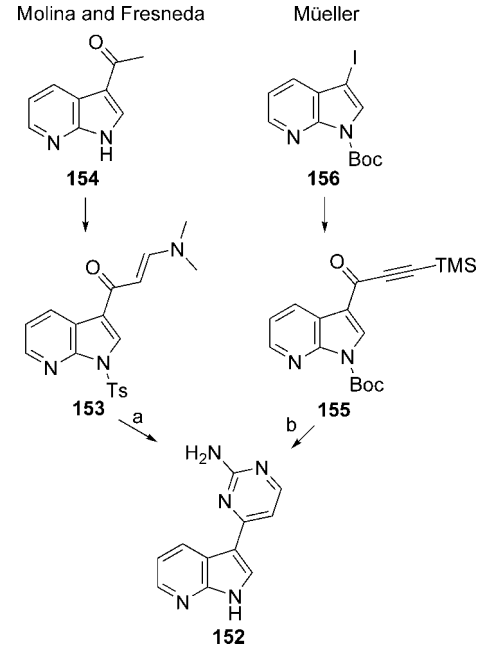
In addition to modifications to the pyrimidine system of the meridianins, several groups have produced meridianin derivatives with modifications to the indole system, which are structurally related to the variolin system—Meijer and co-workers have coined the term "meriolin" to describe this

Scheme 25[a]



[a] Reagents and conditions: (a) Pd(PPh$_3$)$_4$ (10 mol %), 2 M Na$_2$CO$_3$, MeOH, PhH, reflux (R$_1$ = H, 82%; R$_1$ = OMe, 82%).

Scheme 26[a]

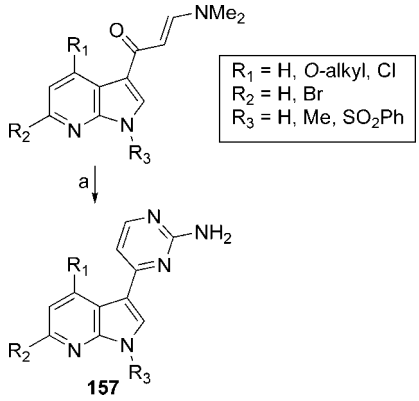


[a] Reagents and conditions: (a) H$_2$N(*C*=NH)NH$_2$·HCl, K$_2$CO$_3$, 2-methoxyethanol, 110 °C (70%); (b) H$_2$N(*C*=NH)NH$_2$·HCl, Na$_2$CO$_3$, *t*-BuOH, MeCN, 80 °C (59%).

hybrid structure. The parent 3-(pyrimidyl)-7-azaindole compound **152** has been prepared by the groups of Molina and Fresneda[24,37] and Müeller,[38] utilizing the same strategy as used in each of their respective syntheses of the meridianins (Scheme 26). In both syntheses, the pyrimidine ring is appended onto the preformed indole system by reaction of an unsaturated indole 3-ketone with guanidine. In the Molina−Fresneda synthesis, the required enaminone **153** was formed from the corresponding methyl ketone **154**, while for the Müeller synthesis, the desired alkynone **155** was formed via a carbonylative Sonagashira reaction on iodide **156**. Investigation of the biological activity of azaindole **152** by Müeller and co-workers found it had improved inhibitory activity against the protein kinase hSGK1, with an IC$_{50}$ of 2.4 μM compared to an IC$_{50}$ of >10 μM for the deaza compound meridianan G (**12**).

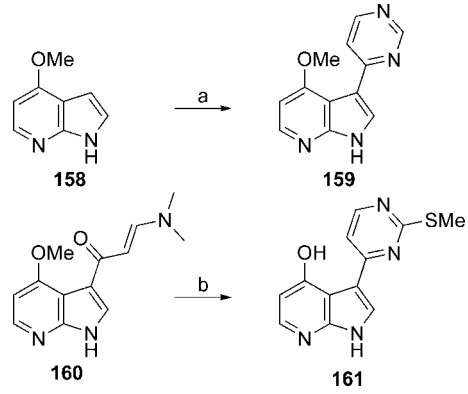
The improved biological activity of the azaindole **152** was further investigated by Meijer and co-workers, who prepared a series of related pyrimidyl 7-azaindoles **157** (Scheme 27).[8,9,48] A series of these compounds, containing a 2-aminopyrimidyl substituent, was prepared by the enaminone cyclization method of the groups of Molina and Fresneda.

Scheme 27[a]



[a] Reagents and conditions: (a) $H_2N(C{=}NH)NH_2 \cdot HCl$, K_2CO_3, 2-methoxyethanol, 110 °C (31−92%).

Scheme 28[a]



[a] Reagents and conditions: (a) CuCl, formamide, 170 °C, (26%); (b) methyl-2-thiopseudourea sulfate, K_2CO_3, 2-methoxyethanol, 110 °C, (30%).

In addition, a small series of analogues with different substituents on the 2′-position on the pyrimidine ring was also prepared (Scheme 28).[8,9] Direct treatment of the azaindole **158** with copper(I) chloride and formamide at elevated temperature gave the 2′-unsubstituted pyrimidine **159**, while cyclization of the enaminone **160** with 2-methyl-2-thiopseudourea sulfate under similar conditions to those used with guanidine hydrochloride gave both cyclization and *O*-dealkylation to generate the 2′-thiomethyl compound **161**. In both cases, low yields of the target compound were observed.

4. Biological Activity

4.1. Variolins

From the initial isolation studies of the variolin family, it was established that variolin B (**1**) had an IC_{50} of 210 ng/mL against the P388 murine leukemia cell line and possessed some antiviral activity but was inactive against a range of bacteria and fungi.[6] Of the other members of the family, variolin A (**2**) and *N*(3′)-methyl tetrahydrovariolin B (**3**) had only modest activities against the P388 murine leukemia cell line, while variolin D (**4**) was completely inactive. On the basis of the DNA intercalating behavior of other nitrogen-rich cytotoxic natural products such as ellipticine, Munro and Blunt speculated that variolin B may exert its cytotoxic activity via DNA intercalation.

In 1996, D'Incalci and co-workers reported that variolin B was an efficient activator of apoptosis, showing potent cytotoxic activity against a variety of human cancer cell lines, including those overexpressing *p*-glycoprotein (*pgp*), a cell efflux pump responsible for the resistance of cancerous cells to multiple chemotherapy agents.[49] However, further investigation of the molecular basis of variolin B activity was hampered by the limited amount of compound available from natural sources. With the encouragement of PharmaMar, work on the synthesis of variolin B was initiated.

The completion of the first total synthesis of variolin B in 2001[17] provided significant quantities of material, so that detailed studies into the mechanism of action of variolin B could begin. In 2005 PharmaMar, in collaboration with the group of D'Incalci, were able to provide further details about the biological properties of variolin B.[7] Cytotoxicity studies indicated that both variolin B (**1**) and its analogue deoxyvariolin B (**40**) possessed similar levels of cytotoxic activity, with both compounds exhibiting IC_{50} values of 50−100 nM against a variety of cell lines.

Interestingly, both compounds exhibited similar levels of activity in sublines of cells producing either normal or an inactive mutant of the tumor suppressor protein *p*53. Likewise no difference in activity was observed in sublines of cells expressing normal or increased levels of the efflux pump *pgp*. These mutations have been found to be responsible for clinically observed resistance of tumors to other chemotherapy agents, suggesting that variolin B and deoxyvariolin B may be useful in the treatment of cancers resistant to other methods of chemotherapy. Further analysis of the effects of variolin B and deoxyvariolin B showed that both compounds cause perturbations in the progression of cells through the cell cycle, and both compounds were demonstrated to induce apoptosis in Jurkat cells.

With the similar cytotoxicity of variolin B (**1**) and deoxyvariolin B (**40**) established, studies were undertaken in order to identify the molecular target of these compounds. On the basis of the previous speculation that variolin B may intercalate DNA, a study of the DNA-binding ability of the variolins was undertaken. These studies utilized deoxyvariolin B (**40**), which has improved stability and water solubility compared to the natural product.

Deoxyvariolin B (**40**) showed marked changes in its UV−visible spectrum on titration with calf-thymus DNA, indicating a change in the environment of the tricyclic chromophore of deoxyvariolin B, consistent with DNA intercalation. Further analysis of the interaction between deoxyvariolin B and DNA by surface plasmon resonance (SPR) confirmed the ability of deoxyvariolin B to bind to DNA. However, in both cases, the binding to DNA was observed to be modest, with no increase in the melting temperature of DNA determined by UV−visible spectrophotometry and a modest equilibrium constant ($7 \times 10^5\ M^{-1}$ for $[CG]_4$ DNA) with rapid association and dissociation kinetics observed by SPR.

Given that some cytotoxic agents act by stabilizing the interaction of topoisomerase with DNA, it was decided to examine how deoxyvariolin B interacted with topoisomerase. However, no such stabilization was observed with either topoisomerase I or topoisomerase II, but deoxyvariolin B was able to affect the topoisomerase I mediated unwinding of supercoiled DNA, with a dose-dependent shift in topoisomer distribution observed. This is consistent with the proposed ability of deoxyvariolin B (and presumably variolin

B) to intercalate with DNA. However, no significant increase in DNA strand breaks were observed in a Comet assay when whole cells were treated with either deoxyvariolin B or variolin B, which would suggest that directly induced DNA damage is not the cause of the observed cytotoxicity of this family of compounds

Both variolin B (**1**) and deoxyvariolin B (**40**) had been observed to affect cell cycle progression. In order to further investigate this phenomenon, the ability of variolin B and deoxyvariolin B to inhibit cyclin-dependent kinases was investigated, with a mind to the pivotal role of cyclin-dependent kinases in the regulation of the cell cycle. Both variolin B and deoxyvariolin B were observed to inhibit the phosphorylation of histone H1 mediated by cyclin E-CDK2, cyclin A-CDK2, cyclin B-CDK1, cyclin H-CDK7, and cyclin D-CDK4, with IC_{50} values in the micromolar range. A preferential inhibition of CDK1 and CDK2 over CDK4 and CDK7 was observed. The inhibition of CDK1 and CDK2 is consistent with the observed cell cycle effects of the variolins. On the basis of these results, it was proposed that the primary mechanism of action of variolin B and deoxyvariolin B is to inhibit cyclin-dependent kinases and interrupt the normal progression of the cell cycle.

On the basis of these exciting results, PharmaMar have been carrying out preclinical investigations on the variolins. They have filed patents with the Morris group[22] and the groups of Álvarez and Joule.[50] They have also, in collaboration with Molina and Fresneda, reported on the cytotoxic potential of variolin B and eight synthetic derivatives with different substituents at positions C-5 and C-7.[51]

It appears that PharmaMar have a particular focus on deoxyvariolin B (**40**) because it has better water solubility and higher stability than variolin B. Accordingly, they have developed a rapid and sensitive high-performance liquid chromatography/tandem mass spectrometry method so as to be able to quantify deoxyvariolin B, which they have termed PM01218, in mouse and rat plasma.[52] This work has provided information on the pharmacokinetics of deoxyvariolin B.

Further understanding of the interaction between variolin B and cyclin-dependent kinases was provided by Meijer and co-workers, who analyzed the interaction of variolin B with a variety of kinases.[8,9] Inhibition of the cyclin-dependent kinases was again observed, with the same preference of CDK1 and CDK2 inhibition over CDK4 and CDK7 inhibition. However, the inhibition of CDK9 (IC_{50} = 26 nM) was determined to be even more potent than that of either CDK1 (IC_{50} = 60 nM) or CDK2 (IC_{50} = 80 nM). Strong inhibition by variolin B of several other kinases was observed, including casein kinase-1 (CK1), FMS-like tyrosine kinase 3, and glycogen synthase kinase-3 (GSK3).

In order to gain insight into the nature of kinase inhibition by variolin B, the structure of a variolin B/ pCDK2-cyclin A complex was determined by X-ray crystallography (Figure 8). The bulk of the protein portion of the structure is similar to that observed in complexes with other kinase inhibitors, with the exception of the glycine-rich loop region. Variolin B binds in the kinase ATP site, between the smaller N-terminal and larger C-terminal lobes of CDK2. Variolin B makes two hydrogen bonding contacts with the hinge region of CDK2 via its tricyclic system, with the main-chain amide carbonyl of Glu81 and the amide NH of Leu83 interacting with the pyrimidine portion of the tricyclic system. In addition, the hydroxyl group of variolin B forms a hydrogen bond with Lys33 on the N-terminal lobe of CDK2.

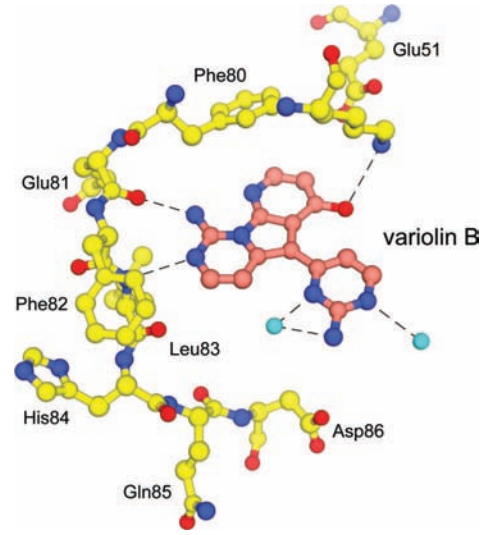
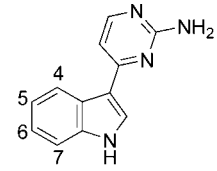


Figure 8. Detail of the interaction between the ATP binding site of pCDK2/cyclin A and variolin B (**1**) (salmon) as determined by X-ray crystallography. Key residues of CDK2 are shown in yellow. Hydrogen bonds between variolin B and residues of CDK2 (lysine 33, glutamate 81, leucine 83) and ordered water molecules (cyan) are indicated. Figure generated from the published structures of pCDK2/cyclin A/variolin B (PDB code 3BHV),[8] with UCSF Chimera.[53]



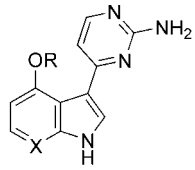
	4	5	6	7	IC_{50} (CDK1)
meridianin A (**6**)	OH	H	H	H	2.50
meridianin B (**7**)	OH	H	Br	H	1.50
meridianin C (**8**)	H	Br	H	H	3.00
meridianin D (**9**)	H	H	Br	H	13.00
meridianin E (**10**)	OH	H	H	Br	0.18
meridianin F (**11**)	H	Br	Br	H	20.00
meridianin G (**12**)	H	H	H	H	150.00

Figure 9. IC_{50} values for the meridianins.

The pendant aminopyrimidine ring of variolin B interacts with two water molecules that are bound to the glycine-rich loop region of CDK2; however, this region of the structure displays some signs of flexibility. The glycine-rich loop region is known to adopt varying conformations in the structures of CDK2 bound to other inhibitors, and these differences may reflect an inherently flexible region of CDK2.

4.2. Biological Properties of the Meridianins and Meriolins

From initial isolation studies of the meridianins, it was known that members of the meridianin family had cytotoxic properties.[11] The origin of these properties was investigated by Meijer and co-workers, who discovered that the meridianin family were inhibitors of several protein kinases, including CDK1, GSK3, and protein kinase A.[42] The variety of naturally occurring meridianins allowed a small investigation of structure−activity relationships (Figure 9). The unsubstituted meridianin skeleton found in meridianin G (**12**) was only weakly inhibitory, while a single bromine for hydrogen substitution at the 5- or 6- position of the indole



		X	R	IC_{50} (CDK1)	IC_{50} (SH-SY5Y)
meridianin A	**6**	CH	H	2.50	>30.00
meriolin 1	**164**	N	H	0.057	0.41
meriolin 3	**162**	N	Me	0.17	0.073
meriolin 5	**163**	N	n-Pr	0.007	0.026

Figure 10. Comparison of the CDK inhibition and cytotoxicity of the meridianins and meriolins.

ring (meridianin C (**8**) and D (**9**), respectively) resulted in considerable improvements in potency, with up to 1000-fold decreases in IC_{50} observed in favorable cases. Interestingly, in meridianin F (**11**), the presence of two bromine atoms, at both the 5- and 6-positions, resulted in improved potency relative to meridianin G (**12**) but decreased potency compared to either monobrominated meridianin C (**8**) or D (**9**). Substitution of a hydroxyl group for hydrogen at the indole 4-position, as seen in meridianin A (**6**), resulted in smaller improvements in potency, while a bromine at position 7 and a hydroxyl group at the 4-position (as seen in meridianin E (**10**)) resulted in the most potent compounds, with IC_{50} values in the low micromolar to high nanomolar range, depending on the target kinase.

The kinase inhibitory activity of the meridianin family is yet to be investigated by structural biology; however, complexes of CDK2/cyclin A with the corresponding 7-azaindole analogues **162**−**163** have been investigated by Meijer and co-workers.[8,9] These 7-azaindoles can be considered hybrid structures between the variolin and meridianin classes of natural products and are referred to as "meriolins". These compounds **162**−**164** display considerably enhanced cyclin-dependent kinase inhibition and cytotoxicity compared to their meridianin counterparts (Figure 10).

The structures of meriolin 3 (**162**) and meriolin 5 (**163**) bound to CDK2/cyclin A have been determined by X-ray crystallography (Figure 11).[8,9] The compounds differ only by the nature of the alkoxy group attached at position 4 of the 7-azaindole ring, and both compounds exhibit similar biological activity, with cytotoxicity and cyclin-dependent kinase IC_{50} values in the nanomolar range. Both compounds, broadly speaking, share a similar binding mode, occupying the ATP binding cleft of CDK2. The azaindole portion of the molecule forms two hydrogen bonds to CDK2, from the azaindole N1 to the main chain carbonyl of Glu81, and from the azaindole N7 to the peptide bond N−H of Leu83. The pendant pyrimidine ring also contributes two hydrogen bonds to binding: from N1′ to the side chain of Lys33 and from the 2′-amino group to the side chain of Glu51. The phenyl ring of Phe80 lies a short distance from both the indole and the pyrimidine portion of the inhibitors. Small differences in the conformation of the glycine-rich loop region of CDK2/cyclin A are observed between the two structures; however, this area is known to possess considerable flexibility and adopts varying conformations in complexes of CDK2 with different ATP-competitive inhibitors. In the case of **162** and **163**, the propyl group of **163** may pack more tightly against this glycine-rich loop region than the smaller methyl group in **162**, which may explain the higher potency of **162** against CDK2.

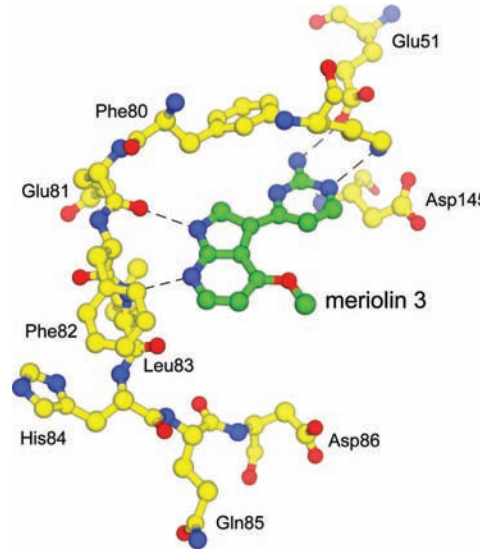


Figure 11. Detail of the interaction between the ATP binding site of pCDK2/cyclin A and meriolin 3 (**162**) (green), as determined by X-ray crystallography. Key residues of CDK2 are shown in yellow. Hydrogen bonds between meriolin 3 and residues of CDK2 (glutamate 51, glutamate 81, leucine 83, aspartate 145) are indicated. Figure generated from the published structures of pCDK2/cyclin A/meriolin 3 (PDB code 3BHT),[8] with UCSF Chimera.[53]

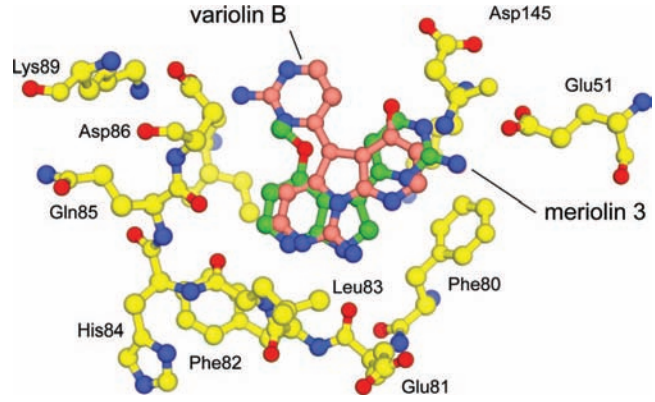


Figure 12. Detail of the interaction between the ATP binding site of pCDK2/cyclin A and variolin B (**1**) (salmon) and meriolin 3 (**162**) (green), as determined by X-ray crystallography. Key residues of CDK2 from the meriolin 3 structure are shown in yellow. Figure generated from the published structures of pCDK2/cyclin A/variolin B and pCDK2/cyclin A/meriolin 3 (PDB codes 3BHV and 3BHT, respectively),[8] with UCSF Chimera.[53]

Interestingly, despite sharing a similar azaindole moiety, variolin B (**1**) and the meriolins (**162** and **163**) bind to CDK2 in reversed orientations (Figure 12). The pendant pyrimidine ring of variolin B does not bind in the same orientation as that of meriolin 3 (**162**) but instead occupies another portion of the active site not utilized by **162**. Likewise, variolin B does not make the same azaindole-mediated contacts as **162**, instead utilizing its fused aminopyrimidine ring to make the equivalent hydrogen bonds. It has been suggested that these large differences in binding modes are due to steric interactions of the variolin chromophore with the side chain of Phe80.

This difference in orientation in the CDK2 binding site is significant because a selectivity study of 32 kinases showed that, compared with variolin B, the meriolins display enhanced specificity toward CDKs, with marked potency on CDK2 and CDK9 (see Figure 13 for selected data).[8]

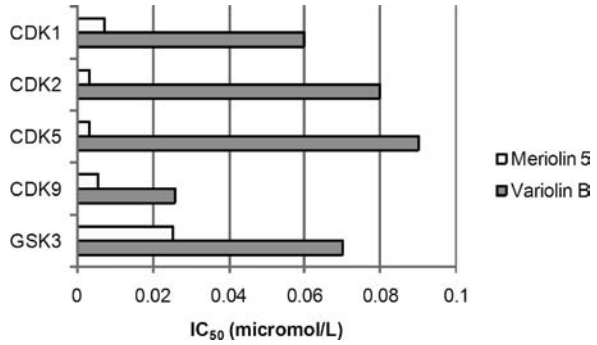
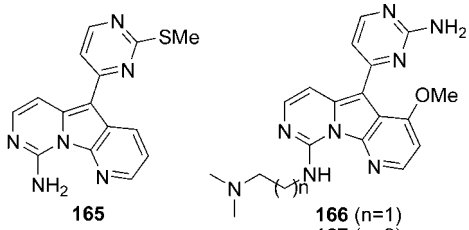


Figure 13. Comparison of the kinase inhibition by meriolin 5 (**163**) and variolin B (**1**) against CDK1, CDK2, CDK5, CDK9, and GSK3.



	B. malayi AsnRS	*H. sapiens AsnRS*
165	51±8 mM	101±10 mM
166 (n=1)	173±90 mM	>500 mM
167 (n=2)	123±54 mM	>1000 mM

Figure 14. Inhibition of *B. malayi* and *H. sapiens* AsnRS by variolin analogues **165**−**167**.

Furthermore, the meriolins display better antiproliferative and proapoptotic properties in human tumor cell cultures than their parent natural products. Two mouse xenograft cancer models, Ewing sarcoma and LS174T colorectal carcinoma, have also been investigated, and it has been found that meriolin 3 (**162**) potently inhibits tumor growth in vivo.[8]

4.3. Antifiliarial Activity of Variolin B and Analogues

From the crystal structure of variolin B in complex with CDK2, it can be seen that variolin B binds to the enzyme in a similar position to the phosphoryl transfer substrate ATP. This mimicry of ATP may indicate the potential utility of variolin B as a lead structure toward the inhibition of other ATP-utilizing enzymes. One such example has already been reported, with a computational screening study identifying variolin B as a potential inhibitor of asparaginyl *t*-RNA synthetase (AsnRS) from *Brugia malayi*, a parasite responsible for the debilitating tropical disease lymphatic filariasis.[54] The asparaginyl-*t*-RNA synthetase from *Brugia malayi* is an excellent target for filarial drug development because it is expressed in both sexes, adults, and in several lifecycle stages.[55]

Kuhn and co-workers used their modeling software SLIDE to identify variolin B as the top candidate for the inhibition of the AsnRS found in *Brugia malayi*.[54] Preliminary work in vitro confirmed that variolin B was capable of inhibiting *B. malayi* AsnRS, with ∼50% reduction of enzyme activity observed in the presence of 50 μM variolin B. A small series of variolin B analogues (**165**−**167**) were also found to be capable of inhibiting *B. malayi* AsnRS, with small changes in structure providing improved selectivity for *B. malayi* AsnRS over human AsnRS (Figure 14). On the basis of computational docking results, compounds **166** and **167** cannot bind to the adenosyl pocket of the closed conformation of the AsnRS due to steric clashes. It is hypothesized that the *Brugia* AsnRS has more main-chain conformational flexibility due to slight differences in the protein sequence from the human enzyme, which allows **166** and **167** to bind in the adenosine pocket of the *Brugia* AsnRS more readily.

5. Conclusion

The identification and development of new CDK inhibitors is an important area of research, particularly in the area of cancer chemotherapeutics. The isolation of variolin B and the meridianins has resulted in a new scaffold being identified. However, access to these compounds from their natural source, particularly in the case of variolin B, is very difficult, and as such, total synthesis was required to restore access. This has allowed for more thorough biological testing and allowed insights into the mode of action of these compounds.

It is difficult to say whether variolin B or its deoxy analogue will make it to clinical trials because there are issues with their solubility in water and their pharmokinetic behavior. However, work on these natural products has led to the discovery that the meriolins, synthetic hybrids that contain common structural features from each family, possess bioactivities that surpass those of either class of natural products. Work by Meijer and co-workers[8,9] suggests that the meriolins constitute a potent kinase inhibitory scaffold, and as such, these compounds may in turn inform the development of the next generation of cancer therapeutics.

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