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CNS and antimalarial activity of synthetic meridianin and psammopemmin analogs

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ABSTRACT

The marine invertebrate-derived meridianin A, the originally proposed structure for psammopemmin A, and several related 3-pyrimidylindole analogs were synthesized and subsequently investigated for central nervous system, antimalarial, and cytotoxic activity. A Suzuki coupling of an indoleborate ester to the pyrimidine electrophile was utilized to form the natural product and derivatives thereof. The 3-pyrimidineindoles were found to prevent radioligand binding to several CNS receptors and transporters, most notably, serotonin receptors (<0.2 μM K_i for $5HT_{2B}$). Two compounds also inhibited the human malaria parasite *Plasmodium falciparum* (IC_{50} <50 μM). Only the natural product was cytotoxic toward A549 cells (IC_{50} = 15 μM).

1. Introduction

We recently reported[1] the 6′N-HX salt of psammopemmin A (**1**),[2] isolated from the Antarctic sponge *Psammopemma* sp., actually possesses the structure of meridianin A (**2**), a similar 3-pyrimidylindole alkaloid. The meridianins, isolated from the Antarctic tunicates *Aplidium meridianum*,[3–5] *A. falklandicum*,[5] as well as *Synoicum* sp.,[1] are low micromolar inhibitors of various cyclin-dependent kinases, glycogen synthase kinase-3, protein kinase A, and other protein kinases.[6,7] Meridianin A, which has been previously synthesized using a Bredereck protocol[8] and through a one pot Masuda borylation–Suzuki coupling,[9] was found to inhibit CDK1 (IC_{50} = 2.5 μM), CDK5 (3.5 μM), PKA (11.0 μM), PKG (200 μM), and GSK3-β (1.3 μM) while showing no toxic effects toward Hep2, HT29, and LMM3 cell lines (IC_{50} >100 μM).[6] Additionally, meridianin A inhibited CDK2 (IC_{50} = 3.10 μM), CDK9 (2.40 μM), CK1 (1.10 μM) but was nontoxic toward SH-SY5Y cells (IC_{50} >30 μM).[10] Naturally occurring meridianins were found to deter predation against the common Antarctic omnivorous predator *Odontaster validus*.[5] A variety of synthetic routes to form meridianins and related bioactive analogs have been devised, and subsequently reviewed.[11] Several more syntheses of meridianin derivatives have been recently reported.[7,12–14]

Our previous study utilized a Suzuki–Miyaura reaction to synthesize the reported structure for psammopemmin A by coupling 4-amino-2-bromo-5-iodopyrimidine to a 4-hydroxyindole moiety. Using this strategy, we now report the synthesis of meridianin A (**2**), 4-methoxymeridian A (**3**), meridoquin (**4**), and 2′-debromo-2′-chloro-**1** (**5**). The remarkable biological activity previously reported for natural occurring meridianins and synthetic analogs thereof impelled us to examine the potential pharmacological effects of our analogs.

We were prompted to investigate the binding affinity of several of our 3-pyrimidylindoles to various serotonin receptors due to the structural features (amine-containing indolol) common to our compounds and serotonin (5-hydroxytryptamine, 5-HT). Serotonin transmission is thought to play a role in central nervous system (CNS) disorders. Compounds that bind to specific serotonin receptor subtypes could lead to treatment of CNS diseases.[15] Selective antagonists of $5\text{-}HT_{2C}$ helped to establish the receptor's role in behaviors such as feeding[16] and anxiety.[17] Neuropsychiatric disorders such as major depression as well as anxiety,[18] and migraine[19] are currently being treated with 5-HT selective receptor ligands while drugs that target the $5\text{-}HT_{2A}$ receptor[20] are under clinical investigation for the treatment of schizophrenia.

Pyrimidine containing compounds (e.g., pyrimethamine) have been used to combat malaria, a devastating disease affecting disadvantaged populations worldwide.[21,22] Because many current treatments for malaria are losing efficacy due to drug-resistant

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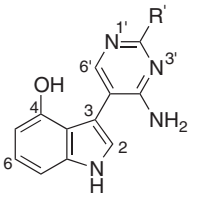
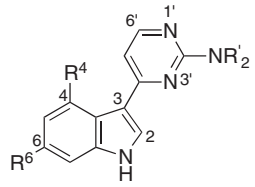
parasites,[23–25] new drugs are required to overcome resistance. Meridianin analogs substituted with both 2′-piperidinyl and 6′-aryl moieties have been reported to display a minimum inhibitory concentration of 1–10 μg/mL (MIC = ~3–30 μM) versus the malaria parasite *Plasmodium falciparum* NF-54.[26] We were curious if our 3-pyrimidylindole compounds could also inhibit the malaria parasite.

3-Pyrimidylindoles **1**–**5** were screened against a variety of CNS receptors and transporters,[27] the malaria parasite *P. falciparum*, and A549 lung cancer cells affording valuable SAR insight as small structural changes have a considerable effect on biological activity.



1: R′=Br, proposed psammopemmin A
5: R′=Cl, 2′-debromo-2′-chloro-**1**



2: R^4=OH, R^6=H, R′=H, meridianin A
3: R^4=OMe, R^6=H, R′=H, 4-methoxymeridianin A
4: R^4=H, R^6=Cl, R′=Et, meridoquin

2. Results and discussion

2.1. Chemistry

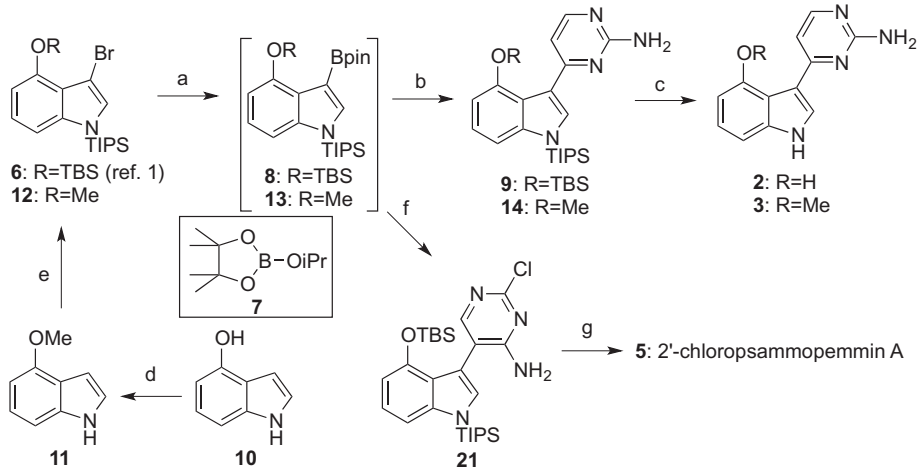
We formed meridianin A (**2**) using a synthetic route developed earlier for the synthesis of proposed psammopemmin A (Scheme 1). Halogen–lithium exchange of previously reported disilyl-protected-3-bromo-indole **6**[1] then addition of borate **7** generated heteroaryl borate **8** which was immediately coupled to 2-amino-4-chloropyrimidine using a Suzuki coupling reaction (46%, two steps).[28] The resulting intermediate (**9**) bearing the 4-hydroxymeridianin skeleton was deprotected with TBAF to afford meridianin A in 87% yield. Physical data of synthetic meridianin A were identical those of meridianin A reported from *A. meridianum*[3] and isolated from *Synoicum* sp.[1]

The 4-methoxy analog of meridianin A (**3**) was synthesized using a route analogous to that producing meridianin A (Scheme 1). 4-Indolol (**10**) was refluxed with methyl iodide under basic conditions to form 4-methoxyindole (**11**, 77%). After silylation and bromination resulting in the formation of **12** (85%),[29] halogen–lithium exchange followed by addition of borate **7** gave vinyl borate **13** (not isolated). Coupling **13** to 2-amino-4-chloropyrimidine with Pd^0 (10 mol %) resulted in 3-pyrimidylindole **14** (two steps, 57%). Upon treatment with TBAF, 4-methoxymeridianin A was obtained (90%).

Inspired by meriolin,[10,30] a synthetic hybrid of naturally occurring meridianin and variolin, we envisioned a meridianin-chloroquine hybrid we have dubbed meridoquin (**4**, Fig. 1). By combining the bioactive meridianin skeleton with characteristics from the widely used 7-chloro-4-aminoquinoline antimalarial chloroquine, we hoped to build a new bioactive scaffold as a starting point for future studies as well as demonstrate the utility of our pyrimidine–indole coupling protocol. It has been shown that the chloro- substituent in 4-aminoquinoline antimalarials is essential for potent antiplasmodial activity while alkylation of the terminal amine improves inhibition moderately.[31]

The synthesis of meridoquin (**4**) was achieved thorough coupling of a suitably substituted indole moiety to the desired pyrimidine (Scheme 2). A low yielding but rapid reaction of neat diethylamine and 2,4-dichloropyrimidine (**15**) resulted in the formation of 2-chloro-4-*N*,*N*-diethylaminopyrimidine (**16**, 59%) as well as the desired 4-chloro-2-*N*,*N*-diethylaminopyrimidine (**17**, 13%). Construction of the indole-coupling partner began with the one pot N1 silylation, 3-bromination of commercially available 6-chloroindole (**18**) resulting in dihaloindole **19** (89%). Halogen–lithium exchange of **19** with *t*-butyllithium then addition of **7** afforded intermediate **20** which was then coupled to pyrimidine **17** using the previously developed Suzuki reaction conditions. Due to the electron withdrawing effects of the ring nitrogens, 4-halopyrimidines are effectual electrophiles for use in Suzuki coupling reactions,[32] explaining the absence of any bisindoles in the reaction mixture. The indole–pyrimidine coupling product (**20**) was observed exclusively (two steps, 69%). Desilylation of **20** with TBAF afforded meridoquin (**4**, 86%).

The 2′-debromo-2′-chloro analog of psammopemmin A (**5**) was formed through a Suzuki coupling of 4-amino-2-chloro-5-iodopyrimidine[33] and borate ester **8** (Scheme 1, two steps, 56%). As was reported previously in the synthesis of proposed psammopemmin A (2′-bromo),[1] deprotection of **21** with TBAF resulted in mainly degradation with isolation of **5** in trace amounts while treatment with HF·pyr gave higher yields to afford the desired 2′-debromo-2′-chloro analog in 32% yield.



Scheme 1. Reagents and conditions: (a) *t*-BuLi then **7**, −78 °C; (b) 2-amino-4-chloropyrimidine, 10 mol % $Pd(PPh_3)_4$, 2 M Na_2CO_3, MeOH, benzene, reflux, **9**: 46% from **6**, **14**: 57% from **12**; (c) TBAF, THF, rt, **2**: 87% from **9**, **3**: 90% from **14**; (d). MeI, K_2CO_3, acetone, reflux, 77%; (e) *n*-BuLi, TIPSCl, THF, −79–−10 °C then NBS, −78 °C, **12**: 85%; (f) 4-amino-2-chloro-5-iodopyrimidine, $Pd(PPh_3)_4$, 2 M Na_2CO_3, MeOH, benzene, reflux, 56% from **6**; (g) HF·pyr, pyridine, acetonitrile, 32%.

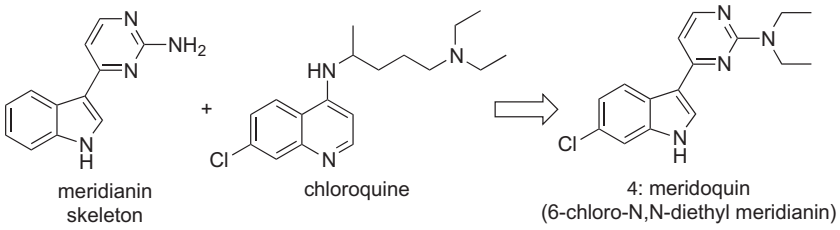
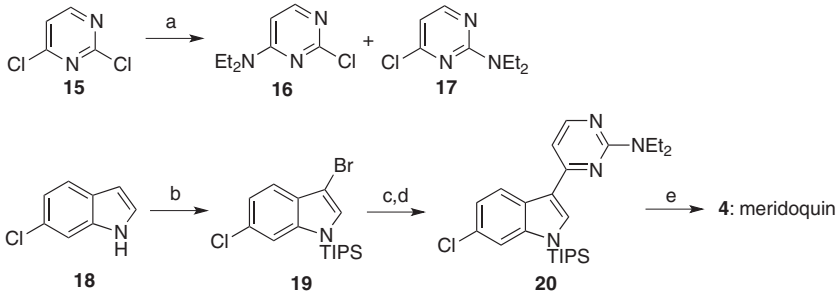


Figure 1.



Scheme 2. Reagents and conditions: (a) Et_2NH, neat, 5 min, 59% **16**; 13% **17**; (b) *n*-BuLi, TIPSCl, THF, −79 to −10 °C then NBS, −78 °C, 89%; (c) *t*-BuLi then **7**, −78 °C; (d) **17**, 10 mol % $Pd(PPh_3)_4$, 2 M Na_2CO_3, MeOH, benzene, reflux, 62% from **19**; (e) TBAF, THF, 86%.

2.2. Activity at CNS receptors and transporters

The binding affinity of proposed psammopemmin A (**1**), meridianin A (**2**), 4-methoxymeridianin A (**3**), and analog **5** toward eleven 5-HT receptor subtypes was evaluated. Primary screening was conducted in vitro by measuring the percent inhibition of radioligand bound to the receptor in question (% inhibition = 100% − % radioactively bound). Secondary screening was performed in vitro on compounds showing >50% inhibition (Table 1).

Meridianin A inhibited binding of the radioligand to 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2B} and 5-HT_{2C} in primary screening. Secondary screening revealed meridianin A did not inhibit binding of 5-HT_{1D} and 5-HT_{2C} with [^{3}H]5-carboximidotryptamine and [^{3}H]mesulergine, respectively. Meridainin A did, however, significantly inhibit the binding of radioligand [^{3}H]lysergic acid diethylamide ([^{3}H]LSD) with 5-HT_{2B} (K_i = 150 nM) and also displayed weaker binding inhibition of [^{3}H]8-OH-DPAT (an aminotetralin analog) to 5-HT_{1A}.

Primary screening of 4-methoxymeridida nin A (**3**) showed the compound could inhibit radioligand binding to 5-HT_{1A}, 5-HT_{2B}, 5-HT_{2C} and 5-HT_{5A}, and 5-HT_7. Secondary screening toward 5-HT_{1D} and 5-HT_{2C} revealed 4-methoxymeridianin A did not inhibit binding of the radiolabled compounds. Like meridianin A, the 4-methoxy analog significantly inhibited binding of radioligand [^{3}H]LSD with 5-HT_{2B} (K_i = 88 nM). 4-Methoxymeridianin A also inhibited, to a lesser extent, binding of [^{3}H]8-OH-DPATto 5-HT_{1A} as well as [^{3}H]LSD to 5-HT_{5A} and 5-HT_7. Secondary screening showed proposed psammopemmin A (**1**) did not inhibit binding of [^{3}H]LSD to 5-HT_{1D}. These initial findings indicate meridianin A is a slightly more selective inhibitor of serotonin receptor subtypes than other compounds tested while methylation of the 4-OH group leads to more potent inhibition of radioligand binding to 5-HT_{2B} as well as broader but less potent activity versus other receptor subtypes.

In addition to 5-HT receptors, the binding effects of 3-pyrimidylindoles on other CNS receptors and transporters were investigated. The dopamine active transporter (DAT) was screened for radioligand [^{3}H]WIN35428 (a synthetic tropane, an analogue to cocaine) inhibition. Meridianin A (**2**) was the only compound to display activity in primary and secondary DAT screens (K_i = 2.35 μM). Analog **5** was also found to inhibit (K_i = 7.04 μM) radioligand binding ([^{3}H]3-quinuclidinyl benzilate, [^{3}H]QNB) of the muscarinic acetylcholine receptor (M_5).

Table 1
Bioactivity of 3-pyrimidylindoles **1**–**5**: CNS binding, antimalarial and cytotoxic activity[a]

	5-HT_{1A}[b]	5-HT_{1D}[b]	5-HT_{2B}[b]	5-HT_{2C}[b]	5-HT_{5A}[b]	5-HT_7[b]	D5[b]	DAT[b]	M5[b]	*P. falciparum*[c]	A549[c]
1	×	>10	×	×	×	×	>10	×	×	>33	>219
2	0.684	>10	0.150	>10	×	×	>10	2.350	>10	12	15
3	1.354	>10	0.088	>10	1.525	1.985	>10	×	×	40	>420
4	—	—	—	—	—	—	—	—	—	200	>333
5	×	×	×	×	×	×	>10	×	7.036	>38	>256
control	0.049[d]	0.006[e]	0.003[d]	0.027[f]	0.014[e]	0.027[f]	0.124[g]	0.022[h]	0.0004[i]	0.006[j]	<9.7[k]

[a] Compound **4** was not assayed against CNS receptors or transporters.
[b] K_i (μM); ×, indicates primary screen IC_{50}>10 μM; —, indicates not tested.
[c] IC_{50} (μM).
[d] Methsergide.
[e] Ergotamine.
[f] Chlorpromazine.
[g] SKF38393.
[h] Vanoxerine.
[i] Atropine.
[j] Chloroquine.
[k] Roridin E.

CNS therapeutic agents that target receptors in the brain (i.e., 5-HT, M_5) are required to pass the blood brain barrier (BBB). Meridianin derivatives have the appropriate mass (M_r <400 Da) to permeate the BBB.[34] Psilocybin and 5-methoxy-*N*,*N*-dimethyltryptamine,[35] oxygenated indole-containing natural products similar to meridianins but possessing a tertiary amine moiety, are able to transverse the BBB and interact with CNS receptors. *N*-Alkylation of meridianin derivatives should, presumptively, enhance BBB permeability.[36]

2.3. Antimalarial and cytotoxic activity

The potential antimalarial activity and cytotoxicity of 3-pyrimidylindoles **1**-**5** were investigated. Meridianin A (**2**), 4-methoxymeridianin A (**3**), and meridoquin (**4**) were active against the malaria parasite *P. falciparum* in initial screening. Secondary screening was conducted to determine IC_{50} values toward *P. falciparum*. Meridianin A was the most potent (IC_{50} = 12 μM) but 4-methoxymeridianin A (IC_{50} = 40 μM) and meridoquin (IC_{50} = 200 μM) also displayed modest antimalarial activity. Meridianin A was cytotoxic toward A549 lung cancer cells at nearly equimolar concentrations to its antimalarial activity (IC_{50} = 15 μM). This was surprising because cytotoxic effects of meridianin A toward Hep2, HT29, LMM3 and SH-SY5Y cell lines (IC_{50} >100 μM) were not observed, previously.[6,10] Interestingly, Meijer et al. found all natural meridianin analogs were cytotoxic with the exception of meridianin A.[6] Both 4-methoxymeridianin A and meridoquin displayed no toxic effects at the highest concentration examined. Proposed psammopemmin A (**1**) and its 2′-debromo-2′-chloro analog (**5**) showed no activity against *P. falciparum* or A549 at the highest concentration tested. 4-Hydroxy methylation of meridianin A appears to significantly decrease the cytotoxicity of the compound, while retaining antiparasitic activity. It is important to note that BBB permeability of meridianin derivatives, while desirable for CNS drugs, would be detrimental to their use as antimalarials. Although their size is appropriate for BBB permeability, meridianin derivatives may not be lipophilic enough to cross phospholipid bilayers. For instance, serotonin itself is unable to traverse from blood to the brain.[37]

3. Conclusion

In summary, several meridianin and psammopemmin analogs were synthesized and examined for biological activity. Meridianin A (**2**) inhibited binding of [^{3}H]LSD to 5-HT_{2B}, inhibited radioligand binding to DAT, and inhibited growth of *P. falciparum* but was, unfortunately, found to be cytotoxic versus A549. 4-Methoxymeridianin A (**3**) inhibited binding of [^{3}H]LSD with 5-HT_{2B}, 5-HT_{5A}, and 5-HT_7, inhibited growth of *P. falciparum*, and displayed no cytotoxic effects at the highest concentrations tested (>300 μM). Meridoquin (**4**) had moderate antimalarial activity and was found to be nontoxic. Proposed psammopemmin A (**1**) was devoid of activity but its 2′debromo-2′-chloro analog (**5**) inhibited radioligand binding of M_5. Further study on 3-pyrimidylindole analogs is ongoing and will reveal if the preliminary promising bioactivity and selectivity findings reported herein can be improved.

4. Experimental

4.1. General experimental procedures

Unless otherwise stated, all experiments were performed under inert atmosphere (N_2 or Ar) in oven- or flame-dried glassware equipped with a magnetic stir bar and a rubber septum. All solvents used were reagent grade. Anhydrous THF was obtained by distillation from sodium/benzophenone. All other chemicals were purchased from Sigma–Aldrich and used as received. Low-temperature baths of −78 °C were obtained with an immersion cooler bath using acetone with dry ice. TLC was carried out using Whatman normal phase Silica gel 60 Å Partisil®. TLC plates were visualized with UV (254 nm) or 5% phosphomolybdic acid (PMA) in EtOH and heating. Products were chromatographed on a Teledyne Isco Combiflash Companion MPLC instrument using normal phase silica Gel cartridges purchased from Teledyne Isco. Melting points were recorded on an Electrothermal Mel-Temp 3.0 instrument. HRMS data were obtained on an Agilent LC/MSD TOF electrospray ionization mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 MHz or 500 MHz instrument using residual protonated solvent as ^{1}H internal standard or ^{13}C absorption lines of solvents for ^{13}C internal standard in $CDCl_3$ or DMSO-d_6 (Cambridge Isotope Labs).

4.2. General procedure for the synthesis of 2–4

To a stirring solution of mono- or disilyl- protected 3-pyrimidylindole (0.15 mmol) in dry THF (2 mL) at rt was added dropwise tetra-*n*-butylammonium fluoride (TBAF: 1.0 M; monosilyl: 0.15 mmol; disilyl: 0.3 mmol). After 5 min aqueous sodium carbonate (Na_2CO_3, 2 M, 2 mL) was added. The mixture was then partitioned between sat NH_4Cl/10% MeOH in EtOAc. The aqueous layer was extracted with another aliquot of 10% MeOH in EtOAc. The combined organic layers were dried over anhyd $MgSO_4$, concentrated, and purified via MPLC (silica gel, **2** and **3**: 10% MeOH/DCM; **4**: 35% EtOAc:hexanes).

4.2.1. Meridianin A (2)

Yellow needles from MeOH/H_2O; 87% yield; mp = 168 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (multiplicity, *J* (Hz), integration): 6.36 (d, 7.6, 1H), 6.73 (br s, 2H), 6.79 (d, 7.9, 1H), 6.96 (dd, 7.6, 7.9, 1H), 7.10 (d, 5.4, 1H), 8.10 (d, 5.4, 1H), 8.21 (d, 2.6, 1H), 11.73 (br s, 1H), 13.58 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 102.4, 104.4, 105.5, 113.8, 114.4, 124.4, 128.5, 139.3, 152.0, 158.5, 160.5, 161.8; ESI HRMS [M+H]$^+$ calcd for $C_{12}H_{11}N_4O$: 227.0927, found: 227.0929.

4.2.2. 4-Methoxymeridianin A (3)

Yellow solid; 90% yield; mp = 213–215 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (multiplicity, *J* (Hz), integration): 3.87 (s, 3H), 6.27 (br s, 2H), 6.64 (d, 7.2, 1H), 7.08 (m, 1H), 7.09 (m, 1H), 7.25 (d, 5.3, 1H), 7.84 (d, 2.6, 1H), 8.15 (d, 5.3, 1H), 11.64 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.0, 101.2, 105.6, 109.6, 114.5, 115.4, 122.7, 127.5, 138.8, 153.3, 157.0, 161.8, 163.1; ESI HRMS [M+H]$^+$ calcd for $C_{13}H_{13}N_4O$: 241.1084, found: 241.1084.

4.2.3. Meridoquin (4)

White solid; 86% yield; mp = 166 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (multiplicity, *J* (Hz), integration): 1.29 (t, 6.9, 6H), 3.75 (q, 6.9, 4H), 6.78 (dd, 5.3, 2.1, 1H), 7.24 (d, 8.6, 1H), 7.43 (dd, 2.1, 2.1, 1H), 7.83 (s, 1H), 8.27 (dd, 5.3, 2.1, 1H), 8.45 (d, 8.6, 1H), 8.60 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.2 (2C), 42.1 (2C), 104.3, 111.2, 116.5, 121.9, 123.1, 124.2, 126.5, 128.7, 137.2, 156.9, 161.1, 161.7; ESI HRMS [M+H]$^+$ calcd for $C_{16}H_{18}ClN_4$: 301.1215, found: 310.1220.

4.3. Synthesis of 2′-debromo-2′-chloro analog (5)

The synthesis of compound **5** was carried out according to the reported procedure.[1]

Tan powder; 32% yield; ^{1}H NMR (400 MHz, DMSO-d_6) δ (multiplicity, *J* (Hz), integration): 6.39 (d, 7.3, 1H), 6.89 (*d*, 8.3, 1H), 6.90 (vbr s, 2H), 6.92, (dd, 7.3, 7.8, 1H), 7.27 (s, 1H), 7.87 (s, 1H), 9.30 (s, 1H), 11.28 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 103.1,

103.8, 105.6, 112.6, 115.5, 122.6, 123.7, 138.6, 151.2, 156.4, 157.0, 164.0; ESI HRMS $[M+H]^+$ calcd for $C_{12}H_{10}ClN_4O$: 261.0538, found:261.0536.

4.4. General procedure for the synthesis of 9, 14, 20, and 21

To a stirring solution of **6**[1] or **19** (1.0 mmol) in dry THF (5 mL) at −78 °C under N_2 was added dropwise *tert*-butyllithium (1.6 M in pentane) until the solution remained yellow then and additional aliquot (1.1 mmol) was added dropwise. The solution stirred for 15 min. Neat 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**7**) was added dropwise until the solution became colorless (~1.5 mmol). The mixture stirred for 1 h at −78 °C and was quenched with sat. NH_4Cl. The mixture warmed to rt and was partitioned between Et_2O/satd NH_4Cl. The aqueous layer was extracted 2 times with EtO_2. The combined organic layers were dried (anhyd $MgSO_4$), concentrated to afford crude borate ester which was used immediately in the next reaction without further purification.

A stirring mixture of crude borate ester, tetrakis(triphenylphosphine)palladium (0.1 mmol), the suitably substituted pyrimidine (2-amino-4-chloropyrimidine, **17**, or 4-amino-2-chloro-5-iodopyrimidine, 0.9 mmol), benzene (25 mL, degassed by sparging with N_2), methanol (5 mL, degassed), and aqueous sodium carbonate (1.25 mL, 2 M, degassed) was refluxed under N_2 for 24 h. The mixture was allowed to cool to rt, diluted with EtOAc and dried with anhyd $MgSO_4$. The filtrate was concentrated onto silica and purified via MPLC (silica gel, **9** and **14**: 40% EtOAc/hexanes; **20** and **21**: 18% EtOAc/hexanes).

4.4.1. 4-(4-(*tert*-Butyldimethylsilyloxy)-1-(triisopropylsilyl)-1*H*-indol-3-yl)pyrimidin-2-amine (9)

White solid; 46% yield; mp = 83–85 °C; ^1H NMR (500 MHz, $CDCl_3$) δ (multiplicity, *J* (Hz), integration): 0.10 (s, 6H), 0.90 (s, 9H), 1.17 (d, 7.6, 18H), 1.73 (sept., 7.6, 3H), 5.01 (br s, 1H), 5.04 (br s, 1H), 6.63 (d, 7.8, 1H), 7.05 (dd, 7.8, 8.1, 1H), 7.15 (d, 8.1, 1H), 7.22 (d, 5.3, 1H), 7.69 (s, 1H), 8.19 (d, 5.3, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: −0.4 (2C), 12.8 (3C), 18.2 (6C), 18.6, 26.0 (3C), 107.9, 110.9, 113.4, 118.4, 120.7, 122.5, 133.7, 144.2, 149.5, 156.6, 162.5, 163.1; ESI HRMS $[M+H]^+$ calcd for $C_{27}H_{45}N_4OSi_2$: 497.3126, found: 497.3116.

4.4.2. 4-(4-Methoxy-1-(triisopropylsilyl)-1*H*-indol-3-yl)pyrimidin-2-amine (14)

White solid; 57% yield; mp = 106 °C; ^1H (400 MHz, DMSO-d_6) δ (multiplicity, *J* (Hz), integration): 1.11 (d, 7.5, 18H), 1.74 (sept., 7.5, 3H), 3.86 (s, 3H), 6.33 (br s, 2H), 6.71 (d, 7.7, 1H), 7.16 (m, 3H), 7.77 (s, 1H), 8.16 (d, 5.2, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.0 (3C), 17.9 (6C), 55.0, 102.0, 107.5, 110.2, 117.4, 118.5, 123.1, 133.0, 143.1, 153.3, 157.0, 161.14, 163.2; ESI HRMS $[M+H]^+$ calcd for $C_{22}H_{33}N_4OSi$: 397.2418, found: 397.2418.

4.4.3. 4-(6-Chloro-1-(triisopropylsilyl)-1*H*-indol-3-yl)-*N*,*N*-diethylpyrimidin-2-amine (20)

White solid; 62%; mp = 126 °C; ^1H NMR (400 MHz, $CDCl_3$) δ (multiplicity, *J* (Hz), integration): 1.18 (d, 7.4, 18H), 1.29 (t, 7, 6H), 1.72 (sept., 7.3, 3H), 3.76 (q, 7.3, 4H), 6.80 (d, 5.5, 1H), 7.22 (dd, 8.7, 1.8, 1H), 7.49 (d, 1.8, 1H), 7.88 (s, 1H), 8.26 (dd, 5.4, 1H), 8.46 (d, 8.7, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.7 (3C), 13.3 (2C), 18.06 (6C), 42.04 (2C), 104.3, 113.8, 118.0, 121.7, 122.8, 127.4, 128.1, 133.8, 142.4, 157.1, 161.2, 161.6; ESI HRMS $[M+H]^+$ calcd for $C_{25}H_{38}ClN_4Si$: 457.2549, found: 457.2569.

4.4.4. 5-(5-(4-(tert-Butyldimethylsilyloxy)-1-(triisopropylsilyl)-1*H*-indol-3-yl)-2-chloropyrimidin-4-amine (21)

Compound **21** (123 mg, 0.23 mmol, 56% yield); mp = 83–85 °C; ^1H NMR (400 MHz, $CDCl_3$) δ (multiplicity, *J* (Hz), integration, position): 0.10 (s, 6H), 0.83 (s, 9H), 1.16 (d, 7.3, 18H), 1.71 (sept, 7.3, 3H), 5.40 (br s, 2H), 6.55 (d, 7.8, 1H), 7.05 (dd, 7.8, 8.2, 1H), 7.09 (s, 1H), 7.16, (d, 8.2, 1H), 8.02 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −4.3 (2C), 12.8 (3C), 18.1 (6C), 18.2, 25.6 (3C), 107.7, 108.9, 109.1, 113.5, 121.6, 123.0, 130.2, 143.7, 149.4, 156.1, 158.6, 164.1; ESI HRMS $[M+H]^+$ calcd for $C_{27}H_{43}ClN_4OSi_2Na$: 553.2556, found: 553.2540.

4.5. The synthesis of 4-methoxy-1*H*-indole (11)

A stirring mixture of K_2CO_3 (3.9 g, 28.5 mmol), **10**[1] (380 mg, 2.85 mmol), and iodomethane (4.05 g, 28.5 mmol, 1.8 mL) in dry acetone (10 mL, Sigma–Aldrich) under N_2 was refluxed for 6 h. The mixture was then cooled to rt, and partitioned between EtOAc/H_2O. The aqueous layer was extracted 2× with aliquots of EtOAc. The combined organic layers were dried over anhyd. $MgSO_4$, concentrated, and purified via MPLC (silica, eluting at 22% EtOAc:Hex) to afford **11** as white crystals (326 mg, 2.22 mmol, 78% yield). mp = 66 °C; ^1H NMR (400 MHz, $CDCl_3$) δ (multiplicity, *J* (Hz), integration): 3.98 (s, 3H), 6.55 (d, 7.7, 1H), 6.68 (m, 1H), 7.05 (d, 8.2, 1H), 7.12 (m, 2H), 8.16 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.3, 99.5, 99.8, 104.4, 118.5, 122.61, 122.72, 137.18, 153.33; ESI HRMS $[M+H]^+$ calcd for $C_9H_{10}NO$: 148.0757, found: 148.0757.

4.6. General procedure for the synthesis of 12 and 19

The syntheses of compounds **12** and **19** were carried out according to the reported procedures.[1,29]

4.6.1. 3-Bromo-4-methoxy-1-(triisopropylsilyl)-1*H*-indole (12)

White crystalline solid; 85% yield; mp = 65 °C; ^1H NMR (400 MHz, DMSO-d_6) δ (multiplicity, *J* (Hz), integration): 1.06 (d, 7.4, 18H), 1.71 (sept., 7.6, 3H), 3.84 (s, 3H), 6.61 (d, 7.6, 1H), 7.10 (d, 8.2, 1H), 7.13 (dd, 8.24, 7.6, 1H), 7.26 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.9 (3C), 17.76 (6C), 55.24, 89.5, 101.1, 107.3, 118.0, 123.4, 129.4, 141.4, 153.0; ESI HRMS $[M+H]^+$ calcd for $C_{18}H_{28}BrNOSiNa$: 404.1016, found: 404.1025.

4.6.2. 3-Bromo-6-chloro-1-(triisopropylsilyl)-1*H*-indole (19)

White crystalline solid; 89% yield; mp = 52 °C; ^1H NMR (400 MHz, $CDCl_3$) δ (multiplicity, *J* (Hz), integration): 1.15 (d, 7.6, 18H), 1.67 (q, 7.6, 3H), 7.18 (dd, 8.3, 1.4, 1H), 7.22 (s, 1H), 7.46 (d, 1.4, 1H), 7.48 (d, 8.3, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.7 (3C), 18.0 (6C), 93.6, 113.8, 119.9, 121.3, 128.6, 128.7 130.4, 140.4; ESI HRMS $[M+H]^+$ calcd for $C_{17}H_{26}BrClNSi$:386.0701, found: 386.0698.

4.7. The synthesis of aminochloropyrimidines 16 and 17

At rt, neat diethylamine (2 mL) was slowly added to 2,4-dichloropyrimidine (**15**, 500 mg, 3.38 mmol). The mixture stirred for 1 min. The resulting white precipitate was dissolved in EtOAc and concentrated onto silica. Purification via MPLC (silica, gradient from 0 to 35% EtOAc:hexane) afforded 4-chloro-2-*N*,*N*-diethylaminopyrimidine (**17**, eluting at 12% EtOAc:Hex, 54 mg, 0.44 mmol, 13% yield) and 2-chloro-4-*N*,*N*-diethylaminopyrimidine (**16**, eluting at 26% EtOAc:hexane, 372 mg, 2.0 mmol, 59% yield).

4.7.1. 4-(*N*,*N*-Diethyl)-2-chloroaminopyrimidine (16)

Viscous colorless liquid, solidifies in freezer; ^1H NMR (400 MHz, $CDCl_3$) δ (multiplicity, *J* (Hz), integration): 1.18 (t, 7.0, 6H), 3.48 (br s, 4H), 6.26 (d, 5.9, 1H), 7.96 (d, 5.9, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 12.5, 42.4, 100.9, 156.5, 160.7, 161.7; ESI HRMS $[M+H]^+$ calcd for $C_8H_{13}ClN_3$: 186.0793, found: 186.0794.

4.7.2. 2-(*N*,*N*-Diethyl)amino-4-chloropyrimidine (17)

Viscous colorless liquid; ^1H NMR (400 MHz, $CDCl_3$) δ (multiplicity, *J* (Hz), integration): 1.19 (t, 7.1, 6H), 3.61 (q, 7.1, 4H), 6.44 (d,

4.7, 1H), 8.14 (d, 4.7, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.9, 42.0, 108.0, 158.7, 160.9, 161.0;ESI HRMS $[M+H]^+$ calcd for $C_8H_{13}ClN_3$: 186.0793, found: 186.0791.

4.8. In vitro *P. falciparum* assay

The transgenic *P. falciparum* clone 3D7 expressing luciferase was grown in continuous culture using the method outlined by Trager and Jenson.[38] Briefly, parasites were grown in RPMI 1640 media without phenol-red and contained 10% heat-inactivated type A+ human plasma, sodium bicarbonate (2.4 g/L), HEPES (5.94 g/L) and 4% washed human type A+ erythrocytes. Cultures were gassed with a 90% N_2, 5% O_2 and 5% CO_2 mixture followed by incubation at 37 °C. *P. falciparum* 3D7 cultures at ⩾4% parasitemia and having >70% of parasites in the ring-stage were used in the assay. Parasites were diluted to 0.5% parasitemia and 1.5% hematocrit using the RPMI 1640 media described above. Test compounds were diluted to a final concentration of 100 μg/mL and then serially diluted in duplicate over 11 concentrations using a Beckman Coulter Biomek 3000. The final test compound concentrations ranged from 100 to 0.098 μg/mL. In 96-well microtiter plates a volume of 90 μl/well of 0.5% parasitized erythrocytes at 1.5% hematocrit was added on top of 10 μL/well of the test compound. A separate 96 well plate containing chloroquine, dihydroartemisinin and atovaquone was added to each set of assay plates as control drugs. The Beckman Coulter Biomek 3000 was used to dispense test compounds, control drugs and parasitized erythrocytes into the microtiter plates. Positive and negative controls were included in each microtiter plate. Positive controls consisted of drug-free parasitized erythrocytes and negative controls consisted of parasitized erythrocytes dosed with a high concentration of chloroquine or dihydroartemisinin that ensured 100% parasite death. Assay plates were placed into a plastic gassing chamber and equilibrated with a 90% N_2, 5% O_2 and 5% CO_2 mixture then incubated at 37 °C for 72 h. After 72 h of incubation excess culture media was aspirated off of each well using the Biomek 3000. Plates were then frozen at −20 °C until later processed for parasite growth determinations. For processing, assay plates were removed from −20 °C and allowed to thaw at room temperature. Assay plates were processed using Luciferase Assay System reagents purchased from Promega. Briefly, 50 μL of Promega's Cell Culture Lysis Reagent was added to each well of the 96 well microtiter plate. Plates were then shaken for a minimum of 5 min to lyse the red blood cells. Subsequently, 50 μL of Promega's Luciferase Assay Substrate was then added to each well. Relative Luminescence Units (RLU) were read using a Perkin Elmer Topcount NXT Microplate Scintillation and Luminescence Counter. RLU's were captured for each well using an integration time of 5 s. Data analysis was performed using a custom database manager (Dataspects, Inc). Nonlinear regression analysis was used to calculate IC_{50}.

4.9. In vitro A549 cytotoxicity assay

Cell line A549 (adenocarcinomic human alveolar epithelial cells) was cultured in F-12 K Nutrient Mixture (Kaighn's Modification) media containing L-GLUTAMINE, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. For the assay, the A549 cells were diluted to 1.33×10^5 cells/mL in DMEM F12 media with L-GLUTAMINE, without HEPES or phenol red, and supplemented with 2% fetal bovine serum and 1% penicillin–streptomycin. The A549 cells were dispensed into 96 well plates at a volume of 90 μL/well giving a final concentration of 12,000 cells per well. Plates were incubated for 24 h at 37 °C and 5% CO_2. After 24 h, the test compounds were added to the 96 well plates containing A549 cells followed by 72 h of incubation at 37 °C and 5% CO_2. A Beckman–Coulter Biomek NX 3000 was used to dispense cells and prepare and dispense test compounds to the 96 well plates. Positive and negative controls were included on each assay plate. After the incubation period, cell proliferation was assessed using Promega's CellTiter 96 Aqueous One Solution Cell Proliferation Assay reagent. Into each well 20 μL reagent was added followed by incubation for 1 h at 37 °C and 5% CO_2. A Biotek plate reader was used to read absorbance at 490 nM and IC_{50} values were determined for each test compound.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.08.033.

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