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Flinderoles A−C: Antimalarial Bis-indole Alkaloids from *Flindersia* Species

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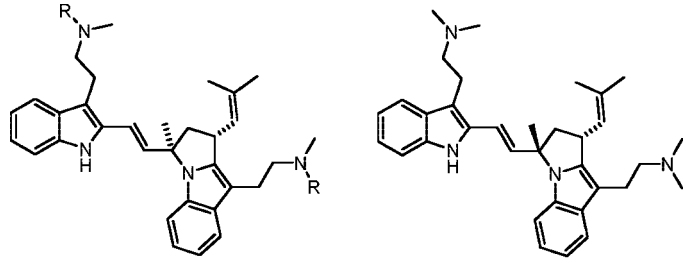
ABSTRACT



1 R = H
2 R = CH_3

3

With the aim of finding new natural product antimalarials, the novel indole alkaloids flinderole A−C were found to have selective antimalarial activities with IC_{50} values between 0.15−1.42 μM. Flinderole A was isolated from the Australian plant *Flindersia acuminata* and flinderoles B and C from the Papua New Guinean plant *F. amboinensis*. Flinderoles A−C contain an unprecedented rearranged skeleton compared to their related isomers of the borreverine class of compounds.

Malaria is caused by an infection by the protozoan parasite belonging to the genus *Plasmodium*. Effective treatment of *P. falciparum* infection, which causes the most clinically severe disease,[1] is increasingly being hampered by the presence of multidrug-resistant parasites. New antimalarial compounds that display the required chemical diversity to help combat drug resistance are urgently required.

Considering that antimalarial chemotherapy has been dominated by natural products (quinine and artemisinin) or compounds based on a natural product pharmacaphore (chloroquine, atovaquone), a screening program was undertaken of natural product extracts and compounds from Australian and Papua New Guinean plants using a whole parasite radiometric growth inhibition assay.[2]

The new antimalarial, flinderole A (**1**) was discovered from the natural product compound library at the Eskitis Institute and was isolated from the plant *Flindersia acuminata* (Rutaceae). Flinderole A (**1**) however, was only assayed after the isolation of flinderoles B (**2**) and C (**3**) from bioassay-guided fractionation of the extract from *F. amboinensis* (Figure 1), identified through an initial antimalarial natural product extract screening program.[3]

Compounds **1**−**3** have a new rearranged skeleton compared to the known borreverine class of tryptamine-isoprene derived compounds previously isolated from *F. fournieri*,[4] which include borreverine (**4**), isoborreverine (**5**), and dimethylisoborreverine (**6**).

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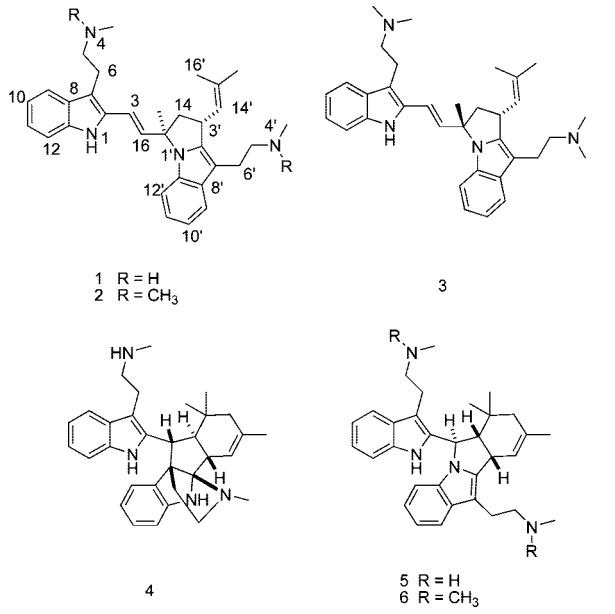

Figure 1. Structural formula of flinderoles A−C (**1**−**3**), borreverine (**4**), isoborreverine (**5**), and dimethylisoborreverine (**6**).

The bark of *F. acuminata* was collected in Lake Barrine National Park, Queensland, Australia, and a MeOH extract was prepared, which was then passed through a SCX resin. The $MeOH/H_2O/NH_3$ eluent gave an alkaloid-enriched extract that was further purified by three C_{18} HPLC purification steps yielding flinderole A (**1**) and isoborreverine (**5**) as their trifluoroacetate salts. Flinderole A (**1**) was isolated in low yield, 0.001% of the dried plant material, and had a molecular formula of $C_{32}H_{40}N_4$ by (+)-HRESIMS (*m/z* 481.33493 [$C_{32}H_{40}N_4$ + H]$^+$, calcd 481.33257). Compound **1** was isomeric with the indole alkaloids borreverine (**4**) and isoborreverine (**5**). The ^{1}H and ^{13}C NMR data (Table 1) showed general characteristics of the borreverine class of alkaloid but with distinct differences, suggesting a modified skeleton.[5]

Compound **1** consisted of two *N*-methyltryptamines with a substitution pattern similar to that of isoborreverine (**5**). Therefore, the ^{1}H NMR spectrum contained signals for two aromatic ring ABCD systems {δ_H 7.53 (brd, 7.5 Hz); 6.98 (td, 7.5, 1.1 Hz); 7.08 (td, 7.5, 1.1 Hz); 7.24 (brd, 7.5 Hz) and 7.56 (dd, 7.0, 1.9 Hz); 7.02 (dd, 7.0, 1.9 Hz); 7.04 (dd, 7.0, 1.9 Hz); 7.41 (dd, 7.0, 1.9 Hz)}, an indole NH (δ_H 11.08), four aminium H's {δ_H 8.60, 8.54, 8.48 (2H)}, eight methylene protons {δ_H 3.01 (2H), 2.99 (4H), 2.90 (2H)} and two *N*-methyls (δ_H 2.56, 2.62). Similarly to isoborreverine (**5**), compound **1** had a five-membered ring fused to one of the *N*-methyltryptamines. However, the substitution pattern of the five-membered ring in **1** compared to that in **5** was different. This was clearly indicated from ^{1}H and ^{13}C NMR chemical shifts and 2D NMR data (Table 1). The methylene protons H-14α {δ_H 2.31 (dd, 13.2, 7.5)} and H-14β {δ_H 2.90 (dd, 13.2, 8.0)} had gCOSY correlations to H-3′ {δ_H 4.28 (ddd, 9.6, 8.0, 7.5)}.

There were also gHMBC correlations between H-14α and the C-15 quaternary carbon (δ_C 63.2) and C-17 methyl group (δ_C 24.7; δ_H 1.94) suggesting the C-14 methylene was attached at one side to a quaternary carbon, with attached methyl group, and the other side to a methine. An isobutene group was identified from the ^{1}H NMR spectrum {δ_H 5.32 (dsep., 9.6, 1.3 Hz); 1.80 (d, 1.3 Hz, 3H); 1.84 (d, 1.3 Hz, 3H)}. Evidence supporting the presence of an isobutene group were gHMBC correlations between the olefinic methyls (3H-16′ and 3H-17′) and both C-14′ (δ_C 125.0) and C-15′ (δ_C 133.0), and mutual 3-bond gHMBC correlations between the olefinic methyl carbons and protons. A gCOSY correlation between H-14′ and H-3′ revealed the attachment of the isobutene group to the five-membered ring. All that remained to fit into the structure was a *trans*-disubstituted double bond {δ_C 116.4, δ_H 6.56 (d, J = 16.2 Hz); δ_C 132.1, δ_H 6.50 (d, J = 16.2 Hz)}. Clearly this was attached at C-2 and C-15, linking one tryptamine unit to one dihydropyrrole-tryptamine unit. The gHMBC correlations between H-16 and C-2, and between H-3 and C-15 supported this attachment.

The UV data λ_{max} (log ϵ) 223 (4.51), 301 (4.09), 311 (4.08) nm was in accord with a chromophore consisting of an indole with increased conjugation from an attached double bond. This completed the planar structure for compound **1**.

The relative stereochemistry of compound **1** was determined from a ROESY experiment (Figure 2). Weak ROESY correlations between H-3′ and H-16 and between 3H-17 and H-14′ indicated that the C-17 methyl and the isobutene group must be on the same side of the five-membered ring. Furthermore, there was no ROESY correlation observed between 3H-17 and H-3′. From the above evidence flinderole A was assigned structure **1**, *N*-methyl-2-{(1*R**,3*R**)-3-methyl-3-[(*E*)-2-{3-[2(methylamino)ethyl]-1*H*-indol-2-yl}vinyl]-1-(2methylprop-1-en-1-yl)2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl}ethanamine.[6]

The bark of *F. ambionensis* was collected from Papua New Guinea, and a MeOH extract was prepared, which was purified by four C_{18} HPLC bioassay-guided purification steps yielding flinderole B (**2**), flinderole C (**3**), and dimethylisoborreverine (**6**) as their trifluoroacetate salts. Flinderole B (**2**) had a pseudomolecular ion at *m/z* 509.363275 in the (+)-HRESIMS allowing a molecular formula $C_{34}H_{44}N_4$ to be assigned to **2**. Thus, **2** was larger than **1** by 28 Da. Analysis of the ^{1}H and ^{13}C NMR data for **2** (Table 1) clearly revealed that the two ethylamine groups were *N*,*N*-dimethylated in **2**, compared to *N*-methylated in **1**. Therefore, in **2** gHMBC correlations were observed between the methyl singlets at δ_H 2.85 and carbons at δ_C 57.6 and 41.8, while the six proton methyl singlet at δ_H 2.25 correlated to carbons at δ_C 56.4 and 41.8. Flinderole B was therefore assigned structure **2**, 2-[(1*R**,3*R**)-3-[(*E*)-2-{3-[2-(dimethylamino)ethyl]-1*H*-indol-2-yl}vinyl]-3-methyl-1-(2-methylprop-1-en-1-yl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl}-*N*,*N*-dimethylethanamine.

The third bis-indole alkaloid, flinderole C (**3**), had a molecular formula of $C_{34}H_{44}N_4$ (*m/z* 509.363369 [M + H]$^+$). Thus, **3** is an isomer of **2**. The ^{1}H and ^{13}C NMR data for **2**

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(6) Asterisk (*) = relative stereochemistry.

Table 1. NMR Data for Flinderoles A−C (**1**−**3**) in DMSO-d_6

	1			**2**		**3**	
position	δ_C[a]	δ_H[g] (mult, *J* Hz)	$^{2,3}J_{CH}$ HMBC (C no.)	δ_C[h]	δ_H[g] (mult, *J* Hz)	δ_C[a]	δ_H[h] (mult, *J* Hz)
1 (N)		11.08 (s)	2, 7, 8, 13		11.12 (s)		11.19 (s)
2	132.4			132.1		132.3	
3	116.4	6.56 (d, 16.2)	7, 15	115.9	6.35 (d, 16.2)	117.4	6.85 (d, 16.2)
4 (N)		8.60 (m)a			8.75 (m)/8.47 (m)		n.o.
		8.54 (m)b					
N4-Me	32.5	2.62 (t, 5.4)	5	41.8	2.75 (s, 6H)	41.9	2.86 (s, 6H)
5	48.83	2.99 (m, 2H)[d]		56.4	3.12 (m)a	56.8	3.17 (m, 2H)
					2.98 (m)b		
6	20.2[b]	3.01 (m)a[e]		18.5	2.98 (m, 2H)	18.9	3.17 (m, 2H)
		2.90 (brt, 7.5)b[f]					
7	109.1			108.8		109.1	
8	127.6			127.4		127.4	
9	118.2[c]	7.53 (brd, 7.5)	7, 8, 11, 13	118.0	7.54 (d, 7.8)	118.1	7.60 (d, 7.8)
10	118.8	6.98 (dd, 7.5, 7.5)	8, 12	118.5	6.98 (dd, 7.8, 7.8)	118.5	7.01 (dd, 7.8, 7.8)
11	122.4	7.08 (dd, 7.5, 7.5)	13	122.0	7.09 (dd, 7.8, 7.8)	122.2	7.10 (dd, 7.8, 7.8)
12	110.8	7.24 (brd, 7.5)	8, 10	110.6	7.25 (d, 7.8)	110.6	7.26 (d, 7.8)
13	136.4			136.2		136.3	
14	50.6	2.90 (dd, 13.2, 8.0)β[f]		50.5	2.88 (dd, 12.6, 8.0)β	50.5	2.73 (dd, 12.6, 7.8)β
		2.31 (dd, 13.2, 7.5)α	2′, 15, 16, 17, 3′, 14′		2.32 (dd, 12.6, 8.4)α		2.33 (dd, 12.6, 9.0)α
15	63.2			63.7		62.9	
16	132.1	6.50 (d, 16.2)	2, 3, 14, 15, 17	132.2	6.52 (d, 16.2)	132.9	6.66 (d, 16.2)
17	24.7	1.94 (s)	14, 15, 16	24.7	1.95 (s)	22.2	1.74 (s)
2′	143.6			143.5		143.1	
3′	34.5	4.28 (ddd, 9.6, 8.0, 7.5)	2′, 14′	34.1	4.27 (ddd, 9.0, 8.4, 8.0)	34.5	4.40 (ddd, 9.6, 9.0, 7.8)
4′ (N)		8.48 (m, 2H)			8.75 (m)/8.47 (m)		n.o.
N4′-Me	32.4	2.56 (brt, 5.4)	5′	41.8	2.85 (s, 6H)	41.9	2.85 (s, 6H)
5′	48.79	2.99 (m, 2H)[d]		56.7	3.07 (m)a	57.0	3.22 (m)a
					3.24 (m)b		3.08 (m)b
6′	20.3[b]	3.01 (m)a[e]		18.5	2.98 (m, 2H)	18.4	2.95 (m, 2H)
		2.90 (brt, 7.5)b[f]					
7′	100.2			99.9		99.7	
8′	132.2			131.8		131.5	
9′	118.2[c]	7.56 (d, 7.5)	7′, 11′, 13′	118.0	7.58 (d, 7.8)	118.0	7.56 (m)
10′	118.6	7.02 (dd, 7.5, 7.5)	8′, 12′	118.4	7.03 (dd, 7.8, 7.8)	118.5	7.00 (m)
11′	120.3	7.04 (dd, 7.5, 7.5)	13′	120.1	7.05 (dd, 7.8, 7.8)	119.5	7.00 (m)
12′	110.1	7.41 (d, 7.5)	8′, 10’	109.9	7.43 (d, 7.8)	109.6	7.29 (m)
13′	130.9			130.9		131.0	
14′	125.0	5.32 (dsept., 9.6, 1.3)		124.4	5.32 (brd, 9.0)	124.2	5.28 (brd, 9.6)
15′	133.0			133.3		133.4	
16′	18.0	1.84 (d, 1.3)	14′, 15′, 17′	17.8	1.83 (s)	17.8	1.86 (s)
17′	25.4	1.80 (d, 1.3)	14′, 15′, 16′	25.1	1.80 (s)	25.2	1.80 (s)

[a] ^{13}C, 125 MHz. [b] Chemical shifts are interchangable. [c] Chemical shifts are interchangable. [d] Chemical shifts are interchangable. [e] Chemical shifts are interchangable. [f] Chemical shifts are interchangable. [g] ^{1}H, 600 MHz. [h] Chemical shifts determined from 2D experiments, ^{13}C, 150 MHz, n.o. = not observed.

and **3** were very similar (Table 1). Analysis of the ROESY experiment showed that the only structural difference was at the C15 stereocenter where there was the opposite stereochemistry for compounds **2** and **3**. Thus, for compound **3** there were ROESY correlations between 3H-17 (δ_H 1.74) and both H-3′ (δ_H 4.40) and H-14β (δ_H 2.73). This concluded that flinderole C (**3**) had the structure 2-[(1*R**,3*S**)-3-[(*E*)-2-{3-[2-(dimethylamino)ethyl]-1*H*-indol-2-yl}vinyl]-3-methyl-1-(2-methylprop-1-en-1-yl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl}-*N*,*N*-dimethylethanamine.[7]

Flinderoles A−C (**1**−**3**), isoborreverine (**5**), and dimethylisoborreverine (**6**) showed inhibition of parasite growth with IC_{50} values between 0.08 and 1.42 μM against the Dd2 (chloroquine-resistant) *P. falciparum* strain with selectivity assessed using the HEK-293 mammalian cell line (Table 2). IC_{50} values are the mean of three dose−response curves performed on different days using duplicate data points. Dimethylisoborreverine (**6**) was the most active and selective of the five compounds screened. Further characterization of the antimalarial activity exhibited by this novel class of compounds is underway.

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(7) The triplet at δ_H 7.03, 7.12, and 7.20 can be assigned to $^{14}NH_4^+$ (atmospheric ammonia absorbed by the HPLC solvent containing TFA leaving a NH_4^+ (CF_3COO^-) residue upon evaporation).

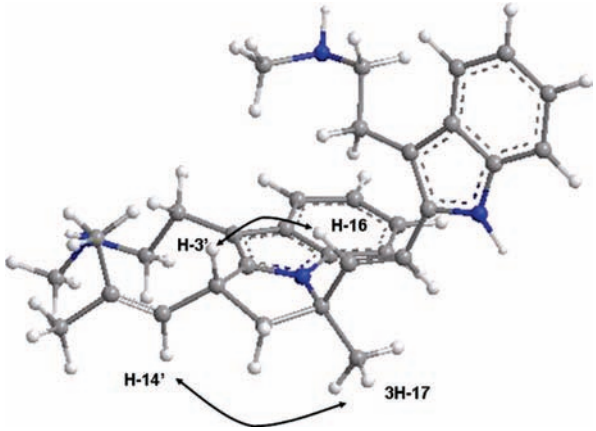

Figure 2. Selected ROESY correlations and relative stereochemistry for flinderole A (**1**).

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Table 2. Antimalarial Activity and Cytotoxicity of Indole Alkaloids of *Flindersia* Species

	$IC_{50} \pm SE$ (μM)		
compound	Dd2	HEK-293	selectivity index
1	1.42 ± 0.07	19.97 ± 1.26	14
2	0.15 ± 0.02	2.13 ± 0.08	14
3	0.34 ± 0.03	9.75 ± 0.46	29
5	0.32 ± 0.02	8.99 ± 0.73	28
6	0.08 ± 0.01	4.09 ± 0.69	51
chloroquine	0.22 ± 0.04	23.91 ± 2.21	108
artemisinin	0.02 ± 0.01	>100	>6250

Supporting Information Available: Detailed description of general experimental procedures, collection details, extraction and isolation, chemical characterization and 1D and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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