Antitrypanosomal Quinoline Alkaloids from the Roots of Waltheria indica

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Antitrypanosomal Quinoline Alkaloids from the Roots of *Waltheria indica*

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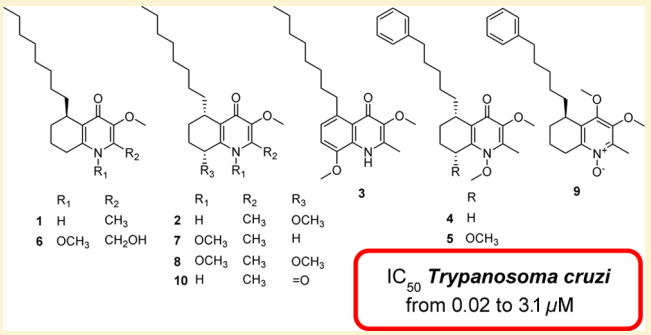
Supporting Information

ABSTRACT: Chemical investigation of the dichloromethane root extract of *Waltheria indica* led to the isolation and characterization of 10 quinoline alkaloids, namely, 8-deoxoantidesmone (**1**), waltheriones E−L (**2**−**9**), and antidesmone (**10**). Among these, compounds **2**−**9** have not yet been described in the literature. Their chemical structures were established by means of spectroscopic data interpretation including ^{1}H and ^{13}C NMR, HSQC, HMBC, COSY, and NOESY experiments and UV, IR, and HRESIMS. The absolute configurations of the compounds were established by comparison of experimental and TDDFT-calculated ECD spectra. In addition, the isolated constituents were evaluated for their in vitro antitrypanosomal activity. Compounds **4**, **5**, and **8** showed potent and selective growth inhibition toward *Trypanosoma cruzi* with IC_{50} values between 0.02 and 0.04 μM. Cytotoxicity for mouse skeletal L-6 cells was also determined for these compounds.



American trypanosomiasis (AT), also known as Chagas disease, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). About 7 to 8 million people are estimated to be infected worldwide, mostly in Latin America, where Chagas disease is endemic.[1] AT is characterized essentially by two clinical forms, an acute stage and a chronic stage. To date, benznidazole and nifurtimox are the only two available chemotherapeutic agents. These compounds are effective for the acute phase of the disease. In contrast, during the chronic phase their utility is limited due to low efficacy.[2,3] In addition, both drugs produce severe side effects occurring in up to 40% of treated patients.[1]

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a fatal disease caused by *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) and *Trypanosoma brucei gambiense* (*T. b. gambiense*). It threatens millions of people in 36 countries in sub-Saharan Africa[4] and progresses in two stages. In the first stage, the trypanosomes are located mainly in the hemolymphatic system. In the second stage, the parasites overcome the blood−brain barrier and invade the brain. Few drugs are available to treat the disease and include pentamidine and suramin for the first stage and melarsoprol, eflornithine, and the nifurtimox−eflornithine combination for the second stage of the disease.[5]

Another parasite of the *Trypanosoma* genus, *Trypanosoma brucei brucei* (*T. b. brucei*), is pathogenic to wild and domestic animals. In cattle, the disease is called "Nagana" and is a major obstacle to the economic development of affected rural areas.[4]

Waltheria indica L. (Malvaceae) is a short-lived shrub that can reach up to 2 m in height and is widespread in subtropical and tropical regions of the world. In traditional medicine, the roots, aerial parts, and whole plant either are taken as a decoction, infusion, or macerate or are chewed or directly applied on wounds. Many ailments such as skin ulcers, rheumatism, diarrhea, hemorrhoids, asthma, or tooth infections are treated with this plant.[6] In Burkina Faso, the roots and aerials parts are used against malaria,[7] and in Niger and Nigeria,

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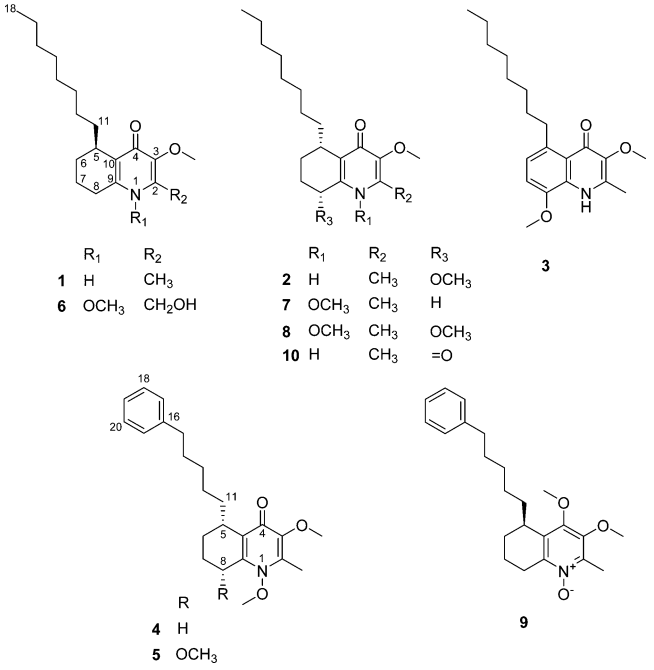
traditional healers give the whole plant to cattle as a tonic, suggesting a possible activity against "Nagana".[8]

Although *W. indica* is commonly used in traditional medicine, very few phytochemical studies have been performed on this plant. Flavonoids and four cyclopeptide alkaloids named adouetins X, Y, Y1, and Z were isolated and identified from the whole plant or aerial parts.[9−13]

Dichloromethane extracts of the aerial parts and roots of *W. indica* were prepared and screened with the aim of discovering new natural products with antitrypanosomal activity. This contribution describes the isolation and characterization of 10 quinoline alkaloids from the roots of *W. indica*. Among these, eight compounds (**2**−**9**) are new natural products. The antitrypanosomal activity of the isolated compounds was then determined.



RESULTS AND DISCUSSION

The powdered, air-dried roots of *W. indica* were extracted with dichloromethane at room temperature. After filtration and evaporation under reduced pressure, the residue was separated by medium-pressure liquid chromatography (MPLC) into 320 fractions by RP C_{18} silica gel column chromatography. Compounds **1**−**3** were readily isolated. Further purifications of the fractions were carried out by repeated column chromatography and afforded seven additional compounds, **4**−**10**.

High-resolution electrospray ionization mass spectrometry (HRESIMS) measurement of all compounds suggested the presence of a nitrogen atom in the molecular formula, indicating the putative presence of alkaloids. NMR data (Tables 1 and 2) and 2D NMR experiments suggested a quinoline skeleton for this series of compounds.

HRESIMS of compound **1** showed a pseudomolecular ion peak at *m/z* 306.2428 $[M + H]^+$ (calcd for $C_{19}H_{32}NO_2$, 306.2433), indicating a molecular formula of $C_{19}H_{31}NO_2$. The ^{13}C NMR and HSQC spectra revealed the presence of a carbonyl group at δ_C 174.0 (C-4), four quaternary aromatic carbons at δ_C 145.6, 144.8, 140.4, and 129.4 (C-3, C-9, C-2, and C-10, respectively), a methine at δ_C 32.3 (C-5), 10 methylenes at δ_C 33.7, 33.1, 31.0, 30.8, 30.5, 29.0, 27.6, 25.8, 23.7, and 18.0 (C-11, C-16, C-12 to C-15, C-8, C-6, C-17, and C-7, respectively), a methoxy at δ_C 60.3, and two methyls at δ_C 14.4 and 13.5 (C-18 and CH_3-2, respectively). The HMBC correlations from the methoxy to C-3, from the methyl CH_3-2 to C-2 and C-3, from H-5 to C-6, C-7, C-9, C-10, and C-11, and from CH_3-18 to C-17 and C-16 as well as the COSY correlations from H-5 (δ_H 2.91) to H_2-6 (δ_H 1.91, 1.54) and H_2-11 (δ_H 1.75, 1.26) and from H_2-7 (δ_H 1.82, 1.77) to H_2-6 and H_2-8 (δ_H 2.62) led to the conclusion that **1** is 3-methoxy-2-methyl-5-octyl-5,6,7,8-tetrahydroquinolin-4(1*H*)-one (8-deoxoantidesmone, Figure 1).

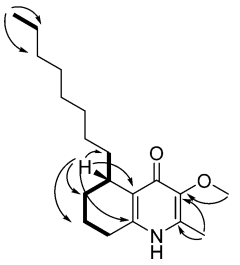


Figure 1. Key COSY (bold lines) and HMBC (arrows) correlations of **1**.

This molecule (**1**) has already been described from plants classified in the tribe Antidesmeae (Euphorbiaceae) by Buske et al.[14] However, this is the first time that 8-deoxoantidesmone has been isolated and characterized using NMR spectroscopy. The absolute configuration at C-5 was established by comparison of experimental and calculated ECD spectra. The experimental ECD spectrum exhibited positive and negative Cotton effects (CEs) at 285 and 261 nm, respectively. The corresponding absorption bands could be attributed to $\pi \rightarrow \pi^*$ electronic transition from the bonding to antibonding molecular configuration. The calculated ECD spectrum for the (5*R*)-stereoisomer showed an excellent fit with the experimental data, with positive and negative CEs around 295 and 260 nm, respectively (Figure 2). Thus, the absolute configuration of compound **1** was established as (5*R*)-8-deoxoantidesmone.

The HRESIMS of compound **2** exhibited an $[M + H]^+$ ion at *m/z* 336.2534 (calcd for $C_{20}H_{34}NO_3$, 336.2539), corresponding to the molecular formula $C_{20}H_{33}NO_3$. In comparison with **1**, an extra methoxy group (+30 amu) was confirmed by NMR spectroscopy, where an additional methine at δ_H 4.23 (1H, dd, *J* = 10.5, 5.9 Hz, H-8) and a methoxy group at δ_H 3.43 (3H, s, OCH_3-8) were detected instead of the methylene on C-8. The HMBC correlations between OCH_3-8 and C-8 and between H-8 and C-9 led to the unequivocal location of the second methoxy group at C-8. It is worth noting that an isomer of **2**, bearing a methoxy group in C-7 instead of C-8 and named chamaedrone, was isolated from *Melochia chamaedrys* (Malvaceae).[15] In addition, in the same study, two enantiomers of **2**, (5*S*,8*R*) and (5*S*,8*S*), were produced semisynthetically from antidesmone. Unfortunately, neither NMR spectra nor optical rotation data were provided for these two enantiomers, precluding the determination of the relative configuration at C-8. To establish the absolute configuration, the ECD spectrum of **2** was measured and compared with calculated spectra of the two possible stereoisomers, (5*S*,8*S*) and (5*S*,8*R*). The

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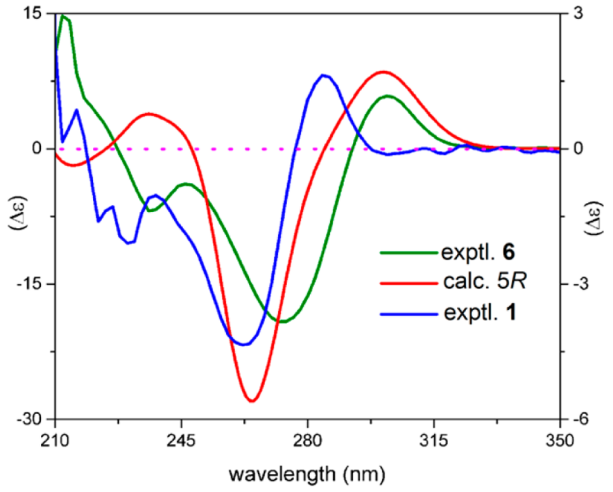

Figure 2. Experimental ECD spectra of **1** and **6** and comparison with the calculated spectrum for the (5*R*)-stereoisomer. The calculation was achieved with TDDFT at the B3LYP/6-31** level in MeOH.

experimental spectrum showed two positive CEs at 226 and 265 nm and one negative CE at 285 nm (Figure 3). These

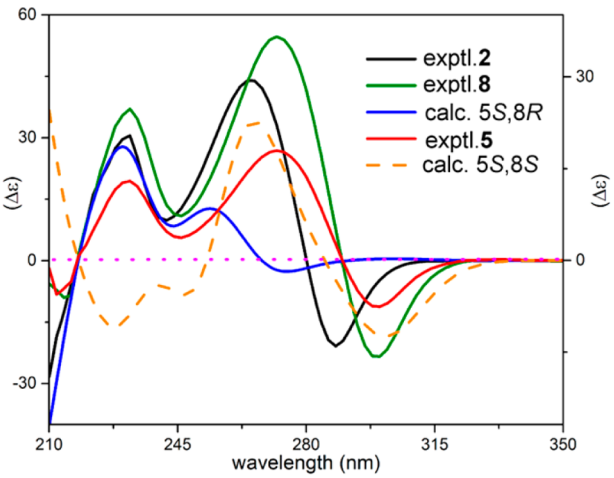

Figure 3. Experimental ECD spectra of **2**, **5**, and **8** and comparison with calculated spectra for the two possible [(5*S*,8*R*) and (5*S*,8*S*)] stereoisomers.

features in the UV and ECD spectra are mainly due to $\pi \rightarrow \pi^*$ transitions of the quinoline chromophore. The calculated spectrum for the (5*S*,8*R*)-stereoisomer showed two positive CEs at 225 and 260 nm together with a negative CE at 280 nm. In contrast, the calculated ECD data of the (5*S*,8*S*)-stereoisomer showed a CE of an opposite sign at 225 nm when compared to the experimental spectrum. Hence, the good match between the experimental spectrum and the calculated data for the (5*S*,8*R*)-stereoisomer led to the conclusion that the absolute configuration of **2** is (5*S*,8*R*). Compound **2** was identified as 3,8-dimethoxy-2-methyl-5-octyl-5,6,7,8-tetrahydroquinolin-4(1*H*)-one and named waltherione E.

Compound **3** (waltherione F) exhibited a pseudomolecular ion peak at *m*/*z* 332.2220 $[M + H]^+$ (calcd for $C_{20}H_{30}NO_3$, 332.2226), indicating a molecular formula of $C_{20}H_{29}NO_3$ and implying two additional degrees of unsaturation than **2**. The NMR data confirmed that instead of a cyclohexene ring fused to a 4-pyridinone moiety as for **2**, a benzene ring was found in **3**. Indeed, in the ^1H and ^{13}C NMR spectra two aromatic protons at δ_H 6.93 (1H, d, *J* = 8.0 Hz, H-6) and 7.03 (1H, d, *J* = 8.0 Hz, H-7) were observed instead of two methylene protons in **2** and two quaternary aromatic carbons at δ_C 136.7 (C-5) and 147.9 (C-8) instead of the two methine carbons in **2** (Table 2). Compound **3** was thus identified as 3,8-dimethoxy-2-methyl-5-octylquinolin-4(1*H*)-one.

Compound **4** (waltherione G) showed an $[M + H]^+$ ion at *m*/*z* 370.2378 (calcd for $C_{23}H_{32}NO_3$, 370.2382), corresponding to the molecular formula $C_{23}H_{31}NO_3$. When compared with **1**, the ^1H NMR spectrum of **4** displayed five aromatic protons at δ_H 7.23 (2H, t, *J* = 7.6 Hz, H-18, 20), 7.16 (2H, d, *J* = 7.6 Hz, H-17, 21), and 7.13 (1H, t, *J* = 7.6 Hz, H-19) and an additional methoxy group at δ_H 3.99, whereas the CH_3-18 signal of **1** was missing. The same conclusion was made from the ^{13}C NMR spectrum of **4**, where eight methylene signals were detected between δ_C 18.2 and 36.9 instead of 10 for compound **1**. The long-range heteronuclear correlations between H-17/H-21 and C-15 (δ_C 36.9) and between H_2-14 (δ_H 1.64) and C-16 (δ_H 144.0) could be used to position unambiguously the phenyl group at the terminal position of the *n*-pentyl side chain (Figure 4). The additional methoxy group was positioned on the

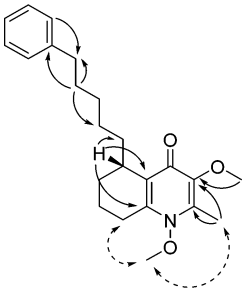

Figure 4. Key NOESY (dashed arrows) and HMBC (plain arrows) correlations of **4**.

nitrogen atom, based on the NOE correlations observed between this methyl and both CH_3-2 (δ_H 2.46) and H-8 (δ_H 2.74 and 3.01).

The ECD spectrum of compound **4** showed two positive CEs at 232 and 265 nm and one negative CE at 295 nm. As compared to compound **1**, the spectrum was opposite in sign. Given that **4** has one chiral center, the opposite configuration at C-5 was expected. The calculated ECD spectrum of the (5*S*)-stereoisomer matched with the experimental data (Figure 5) and confirmed the absolute configuration at C-5 as *S*. Therefore, the structure of **4** could be assigned as 1,3-dimethoxy-2-methyl-5-(5-phenylpentyl)-5,6,7,8-tetrahydroquinolin-4(1*H*)-one.

The alkaloid **5** (waltherione H) was found to be similar to **4** except that an additional methoxy group was present as indicated by the molecular formula, $C_{24}H_{34}NO_4$, deduced from the ion *m*/*z* 400.2483 $[M + H]^+$ instead of $C_{23}H_{32}NO_3$ for **4**. The third methoxy group at δ_H 3.48 was positioned at C-8 (δ_C 71.9) based on the HMBC correlation. The absolute configurations of C-5 and C-8 were assigned as (5*S*,8*R*) by comparison of the experimental and calculated ECD spectra (Figure 3). Alkaloid **5** was thus identified as 1,3,8-trimethoxy-2-methyl-5-(5-phenylpentyl)-5,6,7,8-tetrahydroquinolin-4(1*H*)-one. Compounds **4** and **5** are closely related to melovilone (3,7,8-trimethoxy-2-methyl-5-(5-phenylpentyl)quinolin-4(1*H*)-one), an alkaloid isolated previously from *Melochia tomentosa*.[16]

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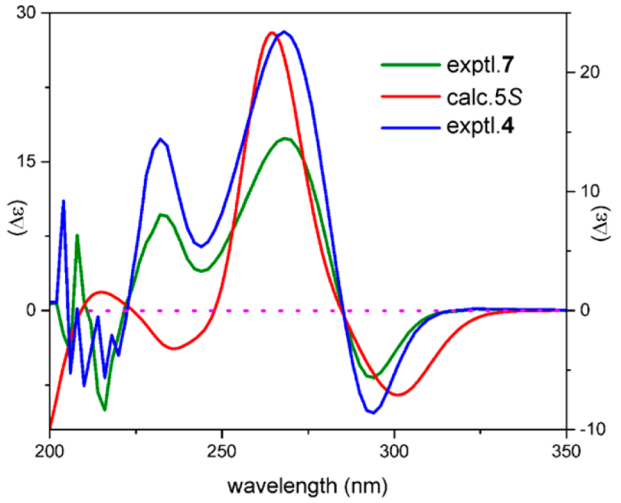

Figure 5. Experimental ECD spectra of **4** and **7** and comparison with the calculated spectrum for the (5*S*)-stereoisomer.

Compound **6** showed a pseudomolecular ion peak at *m/z* 352.2483 $[M + H]^+$ (calcd for $C_{20}H_{34}NO_4$, 352.2488), indicating a molecular formula of $C_{20}H_{33}NO_4$. The structure of **6** was close to that of **1** except that the methyl on C-2 was replaced by a hydroxymethyl at δ_H 4.76 and that an additional methoxy group was at δ_H 4.12 attached to the nitrogen atom. The NOE observed between CH_2OH and both OCH_3-1 and OCH_3-3 was used to position unambiguously the hydroxymethyl on C-2 and the methoxy on the nitrogen atom. The ECD spectrum of **6** (Figure 2) was identical to that of compound **1**, and the configuration at C-5 was determined as (5*R*). Alkaloid **6** was identified as 2-(hydroxymethyl)-1,3-dimethoxy-5-octyl-5,6,7,8-tetrahydroquinolin-4(1*H*)-one and named waltherione I. The latter is similar to vanessine, a tetrahydroquinoline alkaloid isolated previously from *Waltheria douradinha*.[17] However, the absolute configuration (5*R*) of **6** determined by ECD was found to be opposite of that suggested for vanessine (5*S*).

Compound **7** was found to share the same molecular formula, $C_{20}H_{34}NO_3$, as **2**, suggesting that they are isomeric compounds. The ^{1}H and HSQC spectra of **7** indicated the presence of 10 methylene groups between δ_C 17.5 and 33.4 (one more than **2**). Like for compounds **4** and **5**, the methoxy group at δ_H 3.99 (3H, s, OCH_3-1) was positioned on the nitrogen atom, as it gave no HMBC cross-peak and showed a NOE with the methyl at δ_H 2.47 (3H, s, CH_3-2). All these data led to the structure for **7** as 1,3-dimethoxy-2-methyl-5-octyl-5,6,7,8-tetrahydroquinolin-4(1*H*)-one (waltherione J). The absolute configuration at C-5 was assigned as (5*S*) (Figure 5), identical to isomer **2**.

The HRESIMS of compound **8** led to the molecular formula $C_{21}H_{35}NO_4$. In comparison to **7**, the ^{1}H spectrum displayed an additional methoxy group at δ_H 3.48 (3H, s, OCH_3-8). A long-range correlation between C-8 and OCH_3-8 was used to locate the latter without ambiguity. Compound **8** (waltherione K) was identified as 3,8-trimethoxy-2-methyl-5-octyl-5,6,7,8-tetrahydroquinolin-4(1*H*)-one. The ECD spectrum of alkaloid **8** was similar to that of **2**, with two positive CEs at 225 and 250 nm

Table 1. ^{1}H NMR Data of Compounds 1−9 (500 MHz, in CD_3OD except for 2 in $CDCl_3$)

	δ_H (*J* in Hz)								
position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
5	2.91, m	2.88, m		2.95, m	2.92, m	2.98, dt (10.6, 3.1)	2.95, m	2.91, m	3.03, m
6	1.91, brd (13.4)	1.92, m	6.93, d (8.0)	1.86, m	1.87, m	1.92, m	1.91, m	1.87, m	1.95, m
	1.54, m	1.58, dtd (13.4, 7.1, 3.5)		1.50, m	1.70, m	1.55, m	1.52, m	1.68, m	1.52, m
7	1.82, m	2.07, dt (9.7, 3.5)	7.03, d (8.0)	1.80, m	2.25, d (12.0)	1.84, m	1.80, m	1.87, m	1.90, m
	1.77, m	1.70, m			1.35, m				
8	2.62, m	4.23, dd (10.5, 5.9)		3.01, m	4.67, s	2.77, d (10.4)	3.02, m	4.67, s	2.63, t (7.8)
				2.74, m			2.75, m		
11	1.75, m	1.78, m	3.27, m	1.76, m	1.99, m	1.72, m	1.72, m	1.98, m	1.69, m
	1.26, m	1.29, m		1.25, m	1.35, m	1.29, m	1.25, m	1.39, m	
12	1.41, m	1.38, m	1.59, m	1.44, m	1.33, m	1.41, m	1.41, m	1.30, m	1.30, m
13	1.34, m	1.24, m	1.32, m	1.39, m	1.33, m	1.32, m	1.32, m	1.29, m	1.30, m
14	1.34, m	1.24, m	1.30, m	1.64, m	1.61, m	1.32, m	1.32, m	1.29, m	1.40, m
15	1.34, m	1.24, m	1.30, m	2.60, t (7.7)	2.58, t (7.7)	1.32, m	1.32, m	1.29, m	2.63, t (7.7)
16	1.34, m	1.24, m	1.30, m			1.32, m	1.32, m	1.29, m	
17	1.32, m	1.22, m	1.30, m	7.16, d (7.6)	7.15, d (7.2)	1.32, m	1.32, m	1.29, m	7.17, d (7.3)
18	0.90, t (6.5)	0.84, t (6.8)	0.89, t (6.2)	7.23, t (7.6)	7.22, t (7.6)	0.90, t (6.7)	0.90, t (6.5)	0.89, t (6.6)	7.24, t (7.6)
19				7.13, t (7.6)	7.12, t (7.2)				7.14, t (7.3)
20				7.23, t (7.6)	7.22, t (7.6)				7.24, t (7.6)
21				7.16, d (7.6)	7.15, d (7.2)				7.17, d (7.3)
OCH_3-1				3.99, s	4.16, s	4.12, s	3.99, s	4.16, s	
CH_3-2	2.30, s	2.30, s	2.48, s	2.46, s	2.49, s		2.47, s	2.49, s	2.47, s
CH_2OH-2						4.76, s			
OCH_3-3	3.76, s	3.79, s	3.78, s	3.77, s	3.77, s	3.85, s	3.77, s	3.78, s	3.83, s
OCH_3-4									3.99, s
OCH_3-8		3.43, s	4.00, s		3.48, s			3.48, s	

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Table 2. ^{13}C NMR Data of Compounds 1–9 (125 MHz, in CD_3OD except for 2 in $CDCl_3$)

	δ_C, type								
position	1	2	3	4	5	6	7	8	9
2	140.4, C	137.5, C	142.8, C	141.0, C	140.5, C	[a]	140.9, C	140.5, C	141.2, C
3	145.6, C	144.8, C	143.2, C	145.4, C	144.7, C	146.2, C	145.3, C	145.4, C	145.7, C
4	174.0, C	172.9, C	175.9, C	172.6, C	[a]	[a]	172.6, C	[a]	152.0, C
5	32.3, CH	31.2, CH	136.7, C	32.7, CH	33.5, CH	32.8, CH	32.5, CH	32.5, CH	32.9, CH
6	25.8, CH_2	22.8, CH_2	125.4, CH	25.7, CH_2	22.6, CH_2	[a]	25.2, CH_2	24.6, CH_2	24.8, CH_2
7	18.0, CH_2	22.4, CH_2	110.6, CH	18.2, CH_2	[a]	[a]	17.5, CH_2	22.7, CH_2	17.2, CH_2
8	27.6, CH_2	75.3, CH	147.9, C	24.9, CH_2	71.9, CH	24.9, CH_2	24.2, CH_2	72.3, CH	36.9, CH_2
9	144.8, C	140.7, C	132.4, C	145.1, C	[a]	[a]	[a]	144.5, C	142.5, C
10	129.4, C	129.1, C	125.0, C	129.7, C	[a]	[a]	[a]	[a]	127.8, C
11	33.7, CH_2	32.6, CH_2	36.4, CH_2	33.5, CH_2	34.9, CH_2	33.1, CH_2	33.4, CH_2	35.1, CH_2	32.2, CH_2
12	29.0, CH_2	28.4, CH_2	33.7, CH_2	28.8, CH_2	27.4, CH_2	28.2, CH_2	28.8, CH_2	27.4, CH_2	30.9, CH_2
13	30.8, CH_2	29.4, CH_2	30.8, CH_2	30.5, CH_2	30.2, CH_2	30.9, CH_2	30.7, CH_2	30.4, CH_2	30.9, CH_2
14	31.0, CH_2	29.9, CH_2	30.9, CH_2	32.8, CH_2	32.5, CH_2	30.8, CH_2	30.8, CH_2	31.0, CH_2	30.0, CH_2
15	30.5, CH_2	29.8, CH_2	30.6, CH_2	36.9, CH_2	36.6, CH_2	30.5, CH_2	30.3, CH_2	30.7, CH_2	36.8, CH_2
16	33.1, CH_2	32.0, CH_2	33.1, CH_2	144.0, C	142.5, C	*a*	32.9, CH_2	33.0, CH_2	142.4, C
17	23.7, CH_2	22.8, CH_2	23.7, CH_2	129.2, CH	129.0, CH	23.8, CH_2	23.6, CH_2	23.7, CH_2	129.5, CH
18	14.4, CH_3	14.2, CH_3	14.4, CH_3	129.2, CH	129.0, CH	14.4, CH_3	14.3, CH_3	14.4, CH_3	129.5, CH
19				126.6, CH	126.3, CH				126.8, CH
20				129.2, CH	129.0, CH				129.5, CH
21				129.2, CH	129.0, CH				129.5, CH
OCH_3-1				66.1, CH_3	67.7, CH_3	67.7, CH_3	66.2, CH_3	67.3, CH_3	
CH_3-2	13.5, CH_3	14.4, CH_3	14.1, CH_3	10.4, CH_3	10.1, CH_3		10.2, CH_3	14.4, CH_3	11.7, CH_3
CH_2OH-2						54.4, CH_2			
OCH_3-3	60.3, CH_3	59.6, CH_3	60.2, CH_3	60.3, CH_3	60.1, CH_3	61.3, CH_3	60.1, CH_3	60.1, CH_3	61.6, CH_3
OCH_3-4									61.4, CH_3
OCH_3-8		56.1, CH_3	56.6, CH_3		56.1, CH_3			56.2, CH_3	

[a]Signal too weak to be measured.

and a negative CE at 300 nm. It was concluded that the absolute configuration for **8** is (5*S*,8*R*) (Figure 3).

The molecular formula of compound **9**, $C_{23}H_{32}NO_3$, determined from the ion *m*/*z* 370.2375 $[M + H]^+$ was identical to that of **4**. The NMR spectra of **4** and **9** exhibited strong similarities except that the carbonyl at δ_C 172.6 in **4** was replaced by a quaternary sp^2 carbon at δ_C 152.0 and the methoxy group at δ_H 3.99 (OCH_3-4) correlated with this carbon. The NOE correlations from OCH_3-4 to OCH_3-3 and H-5 and from OCH_3-3 to CH_3-2 were used to confirm this hypothesis (Figure 6).

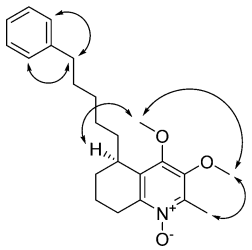

Figure 6. Key NOESY correlations of **9**.

However, the molecular formula of **9** indicated the presence of three oxygen atoms, but only two methoxy signals were observed in the ^1H NMR spectrum. The only possibility for the location of the remaining oxygen was on the nitrogen atom. The ECD spectrum of **9** showed two positive CEs at 208 and 256 nm along with a negative CE at 222 nm. These were ascribed to a $\pi \rightarrow \pi^*$ transition of the tetrahydroquinolin-1-oxide moiety. The calculated ECD spectrum of the (5*R*)-stereoisomer (Figure 7) showed an excellent fit with the experimental data. Thus, the absolute configuration at C-5 was established as *R*. Compound **9** (waltherione L) was identified as 3,4-dimethoxy-2-methyl-5-(5-phenylpentyl)-5,6,7,8-tetrahydroquinolin-1-oxide.

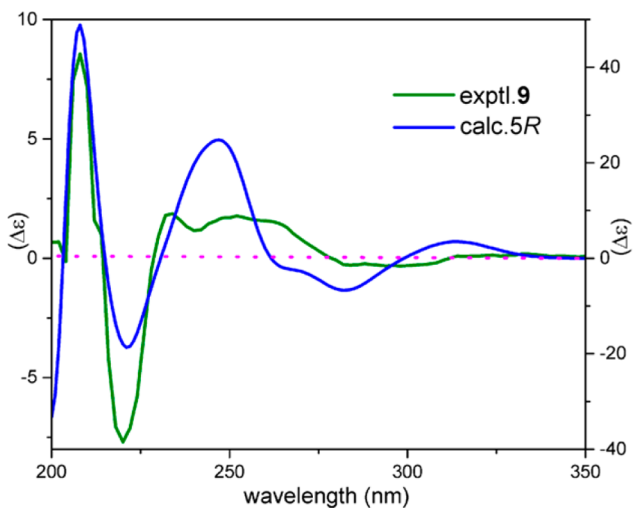

Figure 7. Comparison of the experimental ECD spectrum of **9** with calculated data for the (5*R*)-stereoisomer.

For compound **10**, the molecular formula $C_{19}H_{29}NO_3$, suggested by HRESIMS, and the NMR, UV, and IR data were consistent with literature values[18] of antidesmone, a

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Table 3. Antitrypanosomal and Cytotoxic Activity (IC_{50} in μM[a]) of Alkaloids 1−10

compound	*T. b. brucei*	*T. b. rhodesiense*	*T. cruzi*	cytotoxicity	SI[b] for *T. cruzi*
1	>100	32.6	0.49	0.75	1.5
2	80.3 ± 3.57	38.5	0.23	0.91	4.0
3	31.0 ± 3.60	38.6	1.1	25.7	22.7
4	24.1 ± 2.28	30.4	0.02	0.64	33.8
5	ND[c]	35.9	0.04	0.26	6.5
6	>100	87.9	2.2	22.1	10.1
7	>100	53.3	0.10	0.19	1.9
8	>100	45.8	0.04	0.07	1.8
9	ND	55.7	3.1	41.1	13.5
10	>100	14.3[d]	0.17[d]	184.3[d]	1084.3[d]
suramin	0.31 ± 0.05[e]				
melarsoprol		0.01			
benznidazole			2.9		
podophyllotoxin				0.02	

[a]Results are the means of two independent assays, except for *T. b. brucei*, where the assays were performed in triplicates and expressed ± SD. [b]SI (selectivity index) = IC_{50} cytotoxicity/IC_{50} antitrypanosomal activity. [c]ND: not determined due to lack of product. [d]Values from the literature.[20] [e]Values from the literature.[20,21]

tetrahydroquinoline alkaloid isolated initially from *Antidesma membranaceum* (Euphorbiaceae).[19]

The dichloromethane extract of the roots of *W. indica* showed a strong in vitro inhibitory activity against *T. cruzi* (IC_{50} = 0.74 μg/mL) and *T. b. brucei* (2.3% survival at 20 μg/mL) and to a lesser extent against *T. b. rhodesiense* (IC_{50} = 17.4 μg/mL). In addition, cytotoxicity toward mouse skeletal L-6 cells was considered as weak, with an IC_{50} = 24.0 μg/mL. On the basis of these encouraging results, the isolated alkaloids were tested in vitro for their antitrypanosomal and cytotoxic activities, and the data obtained are summarized in Table 3.

Potent growth inhibition toward the amastigote form of *T. cruzi* (Tulahuen C4 strain) was measured for this series of alkaloids. Indeed, their IC_{50} values were lower than the reference drug benznidazole (IC_{50} = 2.9 μM) for all compounds except compound **9**. The results showed that alkaloids **4** (IC_{50} = 0.02 μM) and **5** and **8** (IC_{50} = 0.04 μM each) displayed the most potent antichagasic activity. However, the cytotoxicity against L6 cells was high. From these results, it appears that a methoxy group bound to the nitrogen atom (compounds **4**−**8**) increased lethality toward *T. cruzi*, suggesting that a nitrogen atom bearing a methoxy group is important for such activity, but further investigations must be performed to confirm this hypothesis. The configuration (5*R*) (compounds **1**, **6**, **9**) seems to result in a decrease of activity. Moreover, the presence of an *N*-oxide function (alkaloid **9**) may be detrimental for *T. cruzi* inhibitory activity. A comparison between the IC_{50} of *T. b.* ssp. and *T. cruzi* highlighted a selective toxicity toward the latter, which is a prerequisite for a potential new drug. It may be noted that the activity encountered with the crude CH_2Cl_2 extract on *T. b. brucei* (STIB 427 strain) cannot be attributed entirely to the isolated pure compounds. Cytotoxicity was observed against mouse skeletal L-6 cells, and the selectivity indices were lower than the reference value of 50 required to be considered as a hit,[22] except for antidesmone **10**. The latter was patented in 2003 for its potential as an antiprotozoal drug.[20] The good activity and selectivity toward *T. cruzi* encourage further investigations to elucidate the mechanism(s) of action and to look for similar structures with reduced toxicity as possible antichagasic drugs.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation was measured on a JASCO P-1030 (Easton, MD, USA) polarimeter (EtOH, *c* in g/100 mL). UV spectra were recorded on a PerkinElmer Lambda-25 UV−vis spectrophotometer (Wellesley, MA, USA) in MeOH. IR spectra were measured on a PerkinElmer Spectrum 100. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 MHz NMR (Palo Alto, CA, USA) instrument. Chemical shifts are reported in parts per million (δ) using the residual CD_3OD signal (δ_H 3.31; δ_C 49.0) or the $CDCl_3$ signal (δ_H 7.26; δ_C 77.2) as internal standards for ^{1}H and ^{13}C NMR, respectively, and coupling constants (*J*) are reported in Hz. HRMS spectra were obtained on a Q Exactive Plus Hybrid quadripole-orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA) using electrospray in the positive mode. The spray voltage was set at 3.5 kV; the sheath gas flow rate (N_2), 50 units; the capillary temperature, 320 °C; the S lens RF level, 50; and the probe heater temperature, 425 °C. UHPLC was performed on an Ultimate 3000 UPLC System (Thermo Scientific) with a Kinetex core shell C_{18} column (2.6 μm; 100 × 3.0 mm i.d.; Phenomenex). Medium-pressure liquid chromatography was performed using a Shimadzu LC10AD pump equipped with a Knauer UV detector and an MPLC glass column (460 × 70 mm i.d.) loaded with ZEOprep C_{18} as the stationary phase (15−25 μm, Zeochem AG, Uetikon am See, Switzerland). Semipreparative chromatography was performed on an ARMEN Spot System (Saint-Avé, France) with a Kinetex Axia Core−Shell C_{18} column (5 μm, 250 × 21.2 mm; Phenomenex).

ECD Computational Details. Conformational analysis was performed with MacroModel 9.1 software (Schrödinger LLC, New York) using the OPLS 2005 (Optimized Potential for Liquid Simulations) force field in H_2O. Conformers occurring within a 2 kcal/mol energy window from the global minimum were chosen for geometrical optimization and energy calculation using density function theory (DFT) with the B3LYP functional and the 6-31G** basis set in the gas phase with the Gaussian 09 program.[23] Vibrational analysis was done at the same level to confirm minima. TD-DFT/B3LYP/6-31G** was conducted in MeOH using the SCRF method, with the CPCM model. ECD curves were constructed on the basis of rotatory strength dipole velocity (R_{vel}), and dipole lengths (R_{len}) were calculated with a half-band of 0.2 eV using SpecDis v1.61.[24]

Plant Material. The roots of *Waltheria indica* were collected between June 2012 and February 2013 in Zinder (Niger). Their identification was confirmed by Didier Roguet (Botanical Garden of Geneva). Voucher specimens are deposited at the Botanical Garden of Geneva (no. 19003).

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Extraction and Isolation. Air-dried roots (3.0 kg) were powdered and extracted with 3 L of CH_2Cl_2 (3 × 24 h) at room temperature. After filtration, the CH_2Cl_2 extract was evaporated to dryness. The residue (2.3 g) was mixed with 8 g of the ZEOprep C_{18} stationary phase and introduced into an MPLC column by dry injection. Fractionation was carried out with a 460 × 70 mm i.d. column, ZEOprep C_{18}, 15−25 μm, as the stationary phase with MeOH and H_2O containing 0.1% formic acid in an optimized gradient mode: 10% to 60% (in 31 h), a plateau at 60% (for 30 h), and finally 60% to 100% (in 24 h) MeOH. The flow rate was set to 6 mL/min, and the UV detection was performed at 220 nm. The MPLC separation yielded 320 fractions, which were individually analyzed by UHPLC-MS and gathered in 90 fractions. Fraction 49 yielded **1** (12.7 mg), fraction 61 yielded **2** (36.9 mg), and fraction 76 yielded **3** (9.3 mg). Fractions 65, 66, 69, 73, and 79 were selected for further purification. The final fractionation steps were performed by semipreparative HPLC using a Kinetex Axia Core−Shell C_{18} column (5 μm, 250 × 21.2 mm; Phenomenex) using MeCN/H_2O/0.1% formic acid as solvents for the isocratic elution. The flow rate was set to 25 mL/min, and UV absorbance was at 220 nm. Fraction 65 (166.0 mg) yielded **4** (84.0 mg) and **10** (10.1 mg) (46% MeCN), fraction 66 (30.3 mg) yielded **4** (6.8 mg) and **5** (1.6 mg) (46% MeCN), fraction 69 (10.0 mg) yielded **6** (1.5 mg) (46% MeCN), fraction 73 (30.0 mg) yielded **7** (3.2 mg) (51% MeCN), and fraction 79 (10.7 mg) yielded **9** (1.7 mg) (55% MeCN).

*8-Deoxoantidesmone (**1**):* pale yellow oil; $[\alpha]_D^{22}$ −10 (*c* 0.05, EtOH); UV (MeOH) λ_{max} (log ε) 219 (3.4), 272 (2.0) nm; ECD (MeOH, *c* 0.16 mM, 0.1 cm) $[\theta]_{261}$ = −27 489, $[\theta]_{285}$ = +10 237; IR ($CHCl_3$) ν_{max} 2924, 2851, 1617, 1500 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 306.2428 $[M + H]^+$ (calcd for $C_{19}H_{32}NO_2$, 306.2433).

*Waltherione E (**2**):* pale yellow oil; $[\alpha]_D^{22}$ +61 (*c* 0.1, EtOH); UV (MeOH) λ_{max} (log ε) 220 (4.6), 273 (2.8) nm; ECD (MeOH, *c* 0.17 mM, 0.1 cm) $[\theta]_{226}$ = +214 875, $[\theta]_{265}$ = +309 309, $[\theta]_{285}$ = −141 518; IR ($CHCl_3$) ν_{max} 2923, 2853, 1617, 1504, 1268, 1228, 1099 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 336.2534 $[M + H]^+$ (calcd for $C_{20}H_{34}NO_3$, 336.2539).

*Waltherione F (**3**):* pale yellow oil; $[\alpha]_D^{22}$ +2 (*c* 0.05, EtOH); UV (MeOH) λ_{max} (log ε) 235 (2.3), 332 (7.4) nm; IR ($CHCl_3$) ν_{max} 3261, 2925, 2854, 1714, 1625, 1576, 1529, 1459, 1259, 1237 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 332.2220 $[M + H]^+$ (calcd for $C_{20}H_{30}NO_3$, 332.2226).

*Waltherione G (**4**):* pale yellow oil; **2**: colorless oil; $[\alpha]_D^{22}$ +33 (*c* 0.1, EtOH); UV (MeOH) λ_{max} (log ε) 208 (5.4), 280 (3.1) nm; ECD (MeOH, *c* 0.15 mM, 0.1 cm) $[\theta]_{232}$ = +95 660, $[\theta]_{265}$ = +154 243, $[\theta]_{295}$ = 56 323; IR ($CHCl_3$) ν_{max} 2930, 2855, 1615, 1563, 1452, 1276, 1128 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 370.2378 $[M + H]^+$ (calcd for $C_{23}H_{32}NO_3$, 370.2382).

*Waltherione H (**5**):* pale yellow oil; UV (MeOH) λ_{max} (log ε) 205 (4.7), 284 (3.2) nm; ECD (MeOH, *c* 0.15 mM, 0.1 cm) $[\theta]_{231}$ = +85 561, $[\theta]_{273}$ = +125 543, $[\theta]_{300}$ = −52 933; IR ($CHCl_3$) ν_{max} 2929, 2855, 1603, 1569, 1453, 1276 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 400.2483 $[M + H]^+$ (calcd for $C_{24}H_{34}NO_4$, 400.2488).

*Waltherione I (**6**):* pale yellow oil; UV (MeOH) λ_{max} (log ε) 205 (5.3), 223 (2.2), 284 (1.3) nm; ECD (MeOH, *c* 0.14 mM, 0.1 cm) $[\theta]_{271}$ = −133 366, $[\theta]_{300}$ = +39 348; IR ($CHCl_3$) ν_{max} 3257, 2925, 2855, 1601, 1547, 1457, 1277 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 352.24831 $[M + H]^+$ (calcd for $C_{20}H_{34}NO_4$ 352.24879).

*Waltherione J (**7**):* pale yellow oil; UV (MeOH) λ_{max} (log ε) 207 (5.7), 221 (2.5), 281 (1.5) nm; ECD (MeOH, *c* 0.15 mM, 0.1 cm) $[\theta]_{233}$ = +64 370, $[\theta]_{268}$ = +116 139, $[\theta]_{300}$ = −44 876; IR ($CHCl_3$) ν_{max} 2923, 2853, 1607, 1564, 1458, 1276 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 336.2534 $[M + H]^+$ (calcd for $C_{20}H_{34}NO_3$, 336.2539).

*Waltherione K (**8**):* pale yellow oil; UV (MeOH) λ_{max} (log ε) 208 (5.7), 284 (1.4) nm; ECD (MeOH, *c* 0.14 mM, 0.1 cm) $[\theta]_{232}$ = +2 448 934, $[\theta]_{268}$ = +364 776, $[\theta]_{300}$ = −156 693; IR ($CHCl_3$) ν_{max} 2924, 2855, 1605, 1570, 1453, 1276 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 366.2640 $[M + H]^+$ (calcd for $C_{21}H_{36}NO_4$, 366.2644).

*Waltherione L (**9**):* pale yellow oil; UV (MeOH) λ_{max} (log ε) 207 (5.4), 264 (1.1), 331 (0.7) nm; ECD (MeOH, *c* 0.13 mM, 0.1 cm) $[\theta]_{208}$ = +63 380, $[\theta]_{222}$ = −54 802, $[\theta]_{233}$ = +13 370, $[\theta]_{256}$ = +12 098; IR ($CHCl_3$) ν_{max} 2931, 2855, 1585, 1486, 1456, 1276 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 370.2375 $[M + H]^+$ (calcd for $C_{23}H_{32}NO_3$, 370.2382).

*Antidesmone (**10**):* pale yellow oil; HRESIMS *m*/*z* 320.2222 $[M + H]^+$ (calcd for $C_{19}H_{30}NO_3$, 320.2226); for other spectroscopic data, see Bringmann et al.[18]

Antitrypanosomal and Cytotoxicity Assays. The in vitro activities against *T. b. rhodesiense* and *T. cruzi* as well as cytotoxicity assessment in L6 cells were determined as reported elsewhere.[25] The following strains and parasite forms were used: *T. b. rhodesiense*, STIB900, trypomastigote forms; *T. cruzi*, Tuluhaen C2C4 (LacZ), amastigotes and L6 cells, rat skeletal myoblasts. Results are expressed in μg/mL for plant extracts and in μM for pure compounds.

***Trypanosoma brucei brucei* Assay.** The New York single marker (NYSM) cell line of bloodstream form of *T. b. brucei* was cultured in HMI-9 medium supplemented with 10% FCS and used as a wild type. IC_{50} values were measured using the AlamarBlue assay[26] in a 96-well plate in a final volume of 100 μL and a final cell concentration of 0.5 × 10^5 cells/mL. After 46 h of incubation with the compounds at different dilutions, the cells were incubated two more hours with the AlamarBlue reagent. The fluorescence was detected by excitation at 530 nm and recording the emission at 584 nm with a fluorescence scanner (SynergyMx, Biotek). IC_{50} values were calculated by nonlinear fitting to the sigmoidal dose−response curve using Prism 6.0 (GraphPad Software, San Diego, CA, USA). The assays were performed in triplicates.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra of compounds **1** to **9** are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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