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Pyridine-4(1*H*)-one Alkaloids from *Waltheria indica* as Antitrypanosomatid Agents

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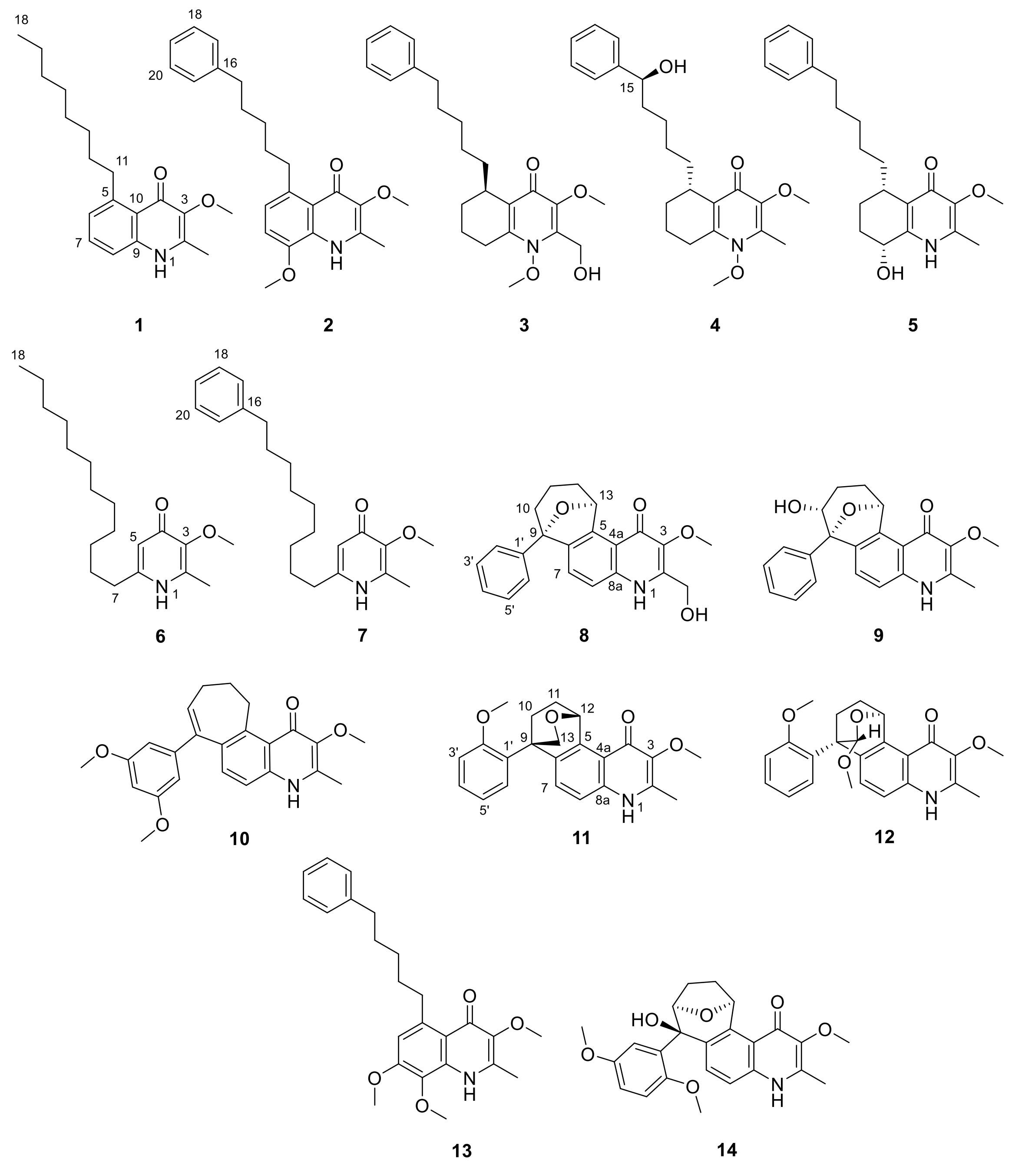
ABSTRACT: Twelve new pyridine-4(1*H*)-one derivatives, namely 8-demethoxywaltherione F (**1**), waltheriones R−V (**2**, **6**, **7**, **10**, and **11**), 1-methoxywaltherione O (**3**), (*S*)-15-hydroxywaltherione G (**4**), (8*R*)-8-hydroxywaltherione M (**5**), (9*S*,13*S*)-2-hydroxymethylwaltherione C and (9*S*,10*S*,13*S*)-10-hydroxywaltherione C (**8** and **9**), (*S*)-13-methoxywaltherione V (**12**), as well as melovinone (**13**) and 5´-methoxywaltherione A (**14**) were isolated from the CH_2Cl_2 extract of the aerial parts of *Waltheria indica.* Their chemical structures were determined by means of a comprehensive analysis including ^{1}H NMR, DEPTQ, HSQC, HMBC, ^{1}H-^{1}H COSY, ROESY and HRESIMS data. The absolute configurations were assigned via comparison of the experimental and calculated ECD data. In addition, the isolated constituents as well as the known waltheriones M−Q were evaluated for their in vitro antitrypanosomal activity. Compounds **2**, **5**, **7** as well as waltheriones M, P, and Q showed potent growth inhibition towards *Trypanosoma cruzi* with IC_{50} values of 2.1, 0.8, 2.1, 1.3, 0.5 and 0.1 μM, respectively, and selectivity indices of >12, >33, >13, 5, 25, and 14. These findings further demonstrate that the waltheriones are a promising class of anti-chagasic compounds worthy of further investigations.

Trypanosomiases are an important group of human and animal diseases caused by parasitic protozoa of the genus *Trypanosoma*. *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) and *T. b. gambiense* cause human African trypanosomiasis, also known as sleeping sickness, in sub-Saharan Africa, whereas in Central and Latin America *T. cruzi* is responsible for American trypanosomiasis or Chagas disease. For both diseases, drugs exist but are not always effective and cause severe side effects. It is therefore urgent to find new and more potent molecules to cure these diseases, which affect mostly poor populations.

As part of our search for compounds with antitrypanosomal activity from *Waltheria indica*,[1, 2] alkaloids present in small amounts in the plant were targeted. Twelve new compounds, namely 7 quinoline-4(1*H*)-ones **1**, **2**, **8**−**12**, three 5,6,7,8-tetrahydroquinoline-4(1*H*)-ones **3**−**5**, and two pyridine-4(1*H*)-ones **6** and **7**, as well as melovinone (**13**) and 5´-methoxywaltherione A (**14**) were isolated from the CH_2Cl_2 extract of the aerial parts of *W. indica.* Compounds **1**−**14** and five known waltheriones (M−Q)[3] were screened for their in vitro growth inhibition of *T. cruzi*.



1 2 3 4 5

6 7 8 9

10 11 12

13 14

RESULTS AND DISCUSSION

The aerial parts of *W. indica* were extracted using CH_2Cl_2 at room temperature, and the resulting extract was fractionated by flash chromatography. Further purifications of the fractions were carried out by semi-preparative HPLC and yielded 12 new compounds **1−12**, as well as melovinone (**13**) and 5´-methoxywaltherione A (**14**).

NMR data (Tables 1 and 2), 2D NMR experiments and comparison with the literature[1,3] suggested pyridine-4(1*H*)-one-type structures for compounds **1−14**.

Table 1. **^{1}H NMR Data of Compounds 1−12 (600 MHz, in Methanol-d_4)**

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	δ_H (mult., *J* in Hz)											
5			2.97, m	2.95, m	2.85, m	6.27, s	6.27, s					
6	7.01, d (7.2)	6.92, d (8.1)	1.54, m	1.60, m 1.34, m	1.92, d (13.9) 1.67, m							
7	7.43, dd (8.4, 7.2)	7.02, d (8.1)	*a*	2.25, m	2.00, m 1.82, m	2.55, t (7.7)	2.54, t (7.7)	7.11, d (8.4)	7.42, m	7.15, d (8.7)	7.54, d (8.9)	6.88, d (8.9)
8	7.31, d (8.4)		2.76, m	*a*	4.54, dd (10.1, 6.2)	1.64, m	1.64, m	7.62, d (8.4)	7.46, d (8.4)	7.30, d (8.7)	7.39, d (8.9)	7.27, m
10								2.13, m	4.28, dd (10.0, 5.8)	6.61, t (7.3)	2.35, ddd (13.5, 7.4, 3.0) 2.21, ddd (13.5, 11.2, 3.0)	2.77, t (10.5) 1.28, m
11	3.36, m	3.27, m	1.33, m	1.29, m	1.33, m	1.30, m	1.33, m	1.66, m 1.00, m	1.88, m 0.74, m	1.89, m	1.97, m 1.84, m	2.47, m 1.65, m
12	1.62, m	1.63, m	1.33, m	1.29, m	1.33, m	1.30, m	1.33, m	2.05, m 1.86, m	2.11, m 1.93, m	2.41, m	5.43, t (4.9)	7.27, m
13	1.42, m	1.43, m	1.33, m	1.29, m	1.33, m	1.30, m	1.33, m	6.31, d (2.6)	6.20, d (3.9)	*a*	4.31, s	5.33, s
14	1.30, m	1.65, m	1.64, m	1.78, m	1.65, m	1.30, m	1.60, m					
15	1.30, m	2.58, t (7.3)	2.61, t (7.6)	4.60, t (6.6)	2.61, t (7.7)	1.30, m	2.59, t (8.1)					
16	1.28, m					1.31, m						
17	1.30, m	7.14, d (7.6)	7.16, d (7.5)	7.32, m	7.16, d (7.2)	1.29, m	7.14, m					
18	0.90, m	7.21, t (7.6)	7.23, t (7.5)	7.30, m	7.23, t (7.2)	0.90, t (7.0)	7.23, t (7.6)					
19		7.11, m	7.12, t (7.5)	7.23, t (7.0)	7.12, t (7.3)		7.13, m					
20		7.21, t (7.6)	7.23, t (7.5)	7.30, m	7.23, t (7.2)		7.23, t (7.6)					
21		7.14, d (7.6)	7.16, d (7.5)	7.32, m	7.16, d (7.2)		7.14, m					
2´								7.56, d (8.4)	7.73, m	6.39, d (2.3)		
3´								7.41, t (8.4)	7.40, m		6.94, d (8.4)	7.12, d (8.3)
4´								7.33, t (7.3)	7.34, t (7.3)	6.42, t (2.3)	7.19, td (7.6, 2.3)	7.42, td (7.8, 3.9)

5´								7.41, t (8.4)	7.40, m		6.76, m	7.08, t (7.8)
6´								7.56, dd (8.3, 1.3)	7.73, m	6.39, d (2.3)	6.74, m	7.42, td (7.8, 3.9)
MeO-1			4.12, s	3.99, s								
Me-2	2.44, s	2.48, s		2.46, s	2.34, s	2.33, s	2.32, s		2.50, s	2.47, s	2.51, s	2.47, s
CH_2OH-2			4.76, s					4.86, s				
MeO-3	3.78, s	3.78, s	3.85, s	3.77, s	3.75, s	3.76, s	3.75, s	3.86, s	3.84, s	3.83, s	3.84, s	3.82, s
MeO-8		4.00, s										
MeO-13												3.23, s
MeO-2´											3.66, s	3.70, s
MeO-3´										3.73, s		
MeO-5´										3.73, s		

[a] Signal too weak to be observed.

Table 2. **^{13}C NMR Data of Compounds 1−12 (150 MHz, in Methanol-d_4)**

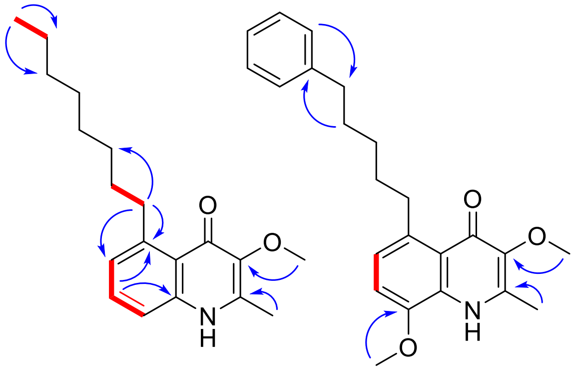
position	1	2	3	4	5	6	7	8	9	10	11	12
	δ_C, type											
2	142.7, C	142.6, C	141.9, C	140.7, C	140.8, C	142.0, C	142.3, C	147.0, C	145.3, C	142.7, C	143.8, C	143.9, C
3	142.4, C	143.1, C	146.1, C	145.2, C	146.2, C	146.3, C	146.5, C	140.3, C	142.5, C	142.7, C	143.4, C	142.4, C
4	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	175.8, C	*a*
4a								121.3, C	121.5, C	124.3, C	124.7, C	120.5, C
5	145.9, C	136.5, C	32.6, CH	32.2, CH	32.5, CH	115.5, CH	115.7, CH	142.3, C	140.2, C	144.5, C	140.2, C	138.9, C
6	125.9, CH	125.2, CH	*a*	26.0, CH_2	24.1, CH_2	151.4, C	151.7, C	141.6, C	138.8, C	137.4, C	139.1, C	138.8, C
7	131.4, CH	110.4, CH	*a*	35.2, CH_2	28.4, CH_2	33.6, CH_2	33.8, CH_2	124.5, CH	127.5, CH	132.8, CH	134.4, CH	129.0, CH
8	116.7, CH	147.7, C	*a*	*a*	67.6, CH	30.0, CH_2	30.3, CH_2	118.8, CH	118.1, CH	116.0, CH	118.6, CH	116.9, CH
8a								139.6, C	143.9, CH	140.3, C	140.3, C	141.5, C
9	141.5, C	132.3, C	*a*	*a*	145.8, C	30.4, CH_2	30.5, CH_2	86.5, C	89.0, C	143.6, C	51.1, C	48.1, C
10	124.2, C	124.9, C	*a*	*a*	*a*	30.4, CH_2	30.5, CH_2	32.9, CH_2	69.6, CH	130.7, CH	26.1, CH_2	22.6, CH_2
11	36.6, CH_2	36.0, CH_2	30.4, CH_2	29.8, CH_2	30.4, CH_2	30.4, CH_2	30.5, CH_2	18.4, CH_2	28.8, CH_2	26.4, CH_2	27.9, CH_2	26.1, CH_2
12	33.4, CH_2	33.1, CH_2	30.4, CH_2	29.8, CH_2	30.4, CH_2	30.4, CH_2	30.5, CH_2	26.6, CH_2	27.7, CH_2	38.2, CH_2	66.1, CH	67.6, CH
13	30.8, CH_2	30.1, CH_2	30.4, CH_2	29.8, CH_2	30.4, CH_2	30.4, CH_2	30.5, CH_2	81.2, CH	80.7, CH	29.0, CH_2	68.8, CH_2	101.8, CH
14	30.3, CH_2	32.7, CH_2	32.6, CH_2	39.9, CH_2	32.8, CH_2	30.4, CH_2	32.8, CH_2					
15	30.3, CH_2	36.8, CH_2	36.7, CH_2	74.8, CH	36.9, CH_2	30.4, CH_2	36.9, CH_2					
16	32.9, CH_2	144.0, C	143.9, C	146.2, C	144.0, C	32.9, CH_2	144.1, C					
17	23.5, CH_2	129.1, CH	129.2, CH	126.8, CH	129.4, CH	23.5, CH_2	129.4, CH					
18	14.2, CH_3	129.0, CH	129.0, CH	128.9, CH	129.2, CH	14.2, CH_3	129.2, CH					
19		126.3, CH	126.4, CH	127.8, CH	126.6, CH		126.6, CH					
20		129.0, CH	129.0, CH	128.9, CH	129.2, CH		129.2, CH					
21		129.1, CH	129.2, CH	126.8, CH	129.4, CH		129.4, CH					
1´								143.5, C	141.5, C	145.5, C	135.0, C	128.5, C
2´								126.8, CH	129.2, CH	107.0, CH	159.2, C	159.2, C
3´								129.1, CH	129.0, CH	162.1, C	113.2, CH	112.5, CH
4´								128.6, CH	129.0, CH	99.9, CH	129.1, CH	130.1, CH
5´								129.1, CH	129.0, CH	162.1, C	121.1, CH	122.3, CH
6´								126.8, CH	129.2, CH	107.0, CH	132.3, CH	130.4, CH

position	1	2	3	4	5	6	7	8	9	10	11	12
	δ_C, type											
MeO-1			67.5, CH_3	65.8, CH_3								
Me-2	14.0, CH_3	13.8, CH_3		9.9, CH_3	13.5, CH_3	13.3, CH_3	13.6, CH_3		14.6, CH_3	14.0, CH_3	14.3, CH_3	14.4, CH_3
CH_2OH-2			54.2, CH_2					57.1, CH_2				
MeO-3	60.0, CH_3	60.0, CH_3	61.0, CH_3	59.8, CH_3	60.1, CH_3	59.9, CH_3	60.1, CH_3	60.2, CH_3	60.4, CH_3	60.1, CH_3	60.4, CH_3	60.4, CH_3
MeO-8		56.3, CH_3										
MeO-13												56.3, CH_3
MeO-2´											55.5, CH_3	55.3, CH_3
MeO-3´										55.5, CH_3		
MeO-5´										55.5, CH_3		

[a] Signal too weak to be observed.

Compound **1** was obtained as a pale yellow oil with the molecular formula $C_{19}H_{27}NO_2$ established by HRESIMS data (*m/z* 302.2113 $[M+H]^+$, calcd for $C_{19}H_{28}NO_2$, 302.2115). The UV spectrum showed absorptions at 240 and 328 nm, suggesting a quinolinone skeleton similar to waltherione F.[1] The HSQC and HMBC spectra showed the presence of three sp^2 carbons linked to a heteroatom at δ_C 142.7, 142.4, and 141.5 (C-2, C-3, and C-9, respectively), two quaternary aromatic carbons at δ_C 145.9 and 124.2 (C-5 and C-10, respectively), three aromatic methines at δ_C 125.9, 131.4 and 116.7 (C-6, C-7, and C-8, respectively), a methoxy group at δ_C 60.0 (MeO-3), seven methylenes at δ_C 36.6, 33.4, 30.8, 30.3, 30.3, 32.9 and 23.5 (C-11 − C-17, respectively), and two methyl groups at δ_C 14.0 and 14.2 (Me-2 and C-18, respectively). ^{1}H-^{1}H COSY correlations between H-6 (δ_H 7.01), H-7 (δ_H 7.43) and H-8 (δ_H 7.31), and the HMBC correlations from MeO-3 (δ_H 3.78) to C-3, Me-2 (δ_H 2.44) to C-2, H_2-11 (δ_H 3.36) to C-5, C-6, and C-13, the *n*-

terminal methyl group H_3-18 (δ_H 0.90) to C-17 and C-16 led to the conclusion that **1** is 3-methoxy-2-methyl-5-octylquinolin-4(1*H*)-one (Figure 1).



1 **2**

Figure 1. Key ^{1}H-^{1}H COSY (bold red lines) and HMBC (blue arrows) correlations of **1** and **2**.

Compound **1** shows close similarities with waltherione F, previously isolated from the same plant, because both compounds have a benzene ring fused to the 4-pyridinone moiety. Only the presence of a methoxy group at C-8 distinguishes waltherione F from **1**. Therefore, the alkaloid (**1**) was trivially named 8-demethoxywaltherione F.

The HRESIMS data of compound **2** exhibited a protonated molecule $[M+H]^+$ at *m/z* 366.2064 (calcd for $C_{23}H_{28}NO_3$, 366.2064), corresponding to the molecular formula $C_{23}H_{27}NO_3$. When compared to **1**, the NMR data of **2** indicated five aromatic protons at δ_H 7.21 (2H, t, J = 7.6 Hz, H-18, 20), 7.14 (2H, d, J = 7.6 Hz, H-17, 21) and 7.11 (1H, m, H-19) in the terminal position of the *n*-pentyl chain, instead of the *n*-terminal methyl group H-18 signal in **1**. A second difference was the presence of an additional methoxy group at δ_H 4.00, which correlated with C-8 in the HMBC spectrum (Figure 1). The structure of waltherione R (**2**) was thus identified as 3,8-dimethoxy-2-methyl-5-(5-phenylpentyl)quinolin-4(1*H*)-one.

Compound **3** showed a protonated molecule $[M+H]^+$ at *m/z* 386.2327 (calcd for $C_{23}H_{32}NO_4$, 386.2326), indicating a molecular formula $C_{23}H_{31}NO_4$. The structure of **3** is close to waltherione O[3] (Figure S1, Supporting Information). Indeed, both compounds shared a C-2 hydroxymethyl group at δ_H 4.76, a cyclohexene ring fused to a 4-pyridinone moiety, a methine at δ_H 2.97, and an *n*-pentyl side chain with a phenyl group in the terminal position. However, an additional methoxy group was detected at δ_H 4.12 for **3**. The ROE association observed between CH_2OH and both MeO-1 and MeO-3 were used to unambiguously position the methoxy group at the nitrogen atom. The absolute configuration at C-5 was established by comparison of the experimental and reported[3] ECD spectra. The ECD spectrum of **3** showed sequential negative and positive Cotton effects (CE) at 260 and 290 nm (Figure 2A), which was similar to the ECD spectrum of waltherione O, and the absolute configuration at C-5 was therefore defined as (*R*). Thus, the structure of 1-methoxywaltherione O (**3**) was defined as (*R*)-2-hydroxymethyl-1,3-dimethoxy-(5-phenylpentyl)-5,6,7,8-tetrahydroquinolin-4(1*H*)-one.

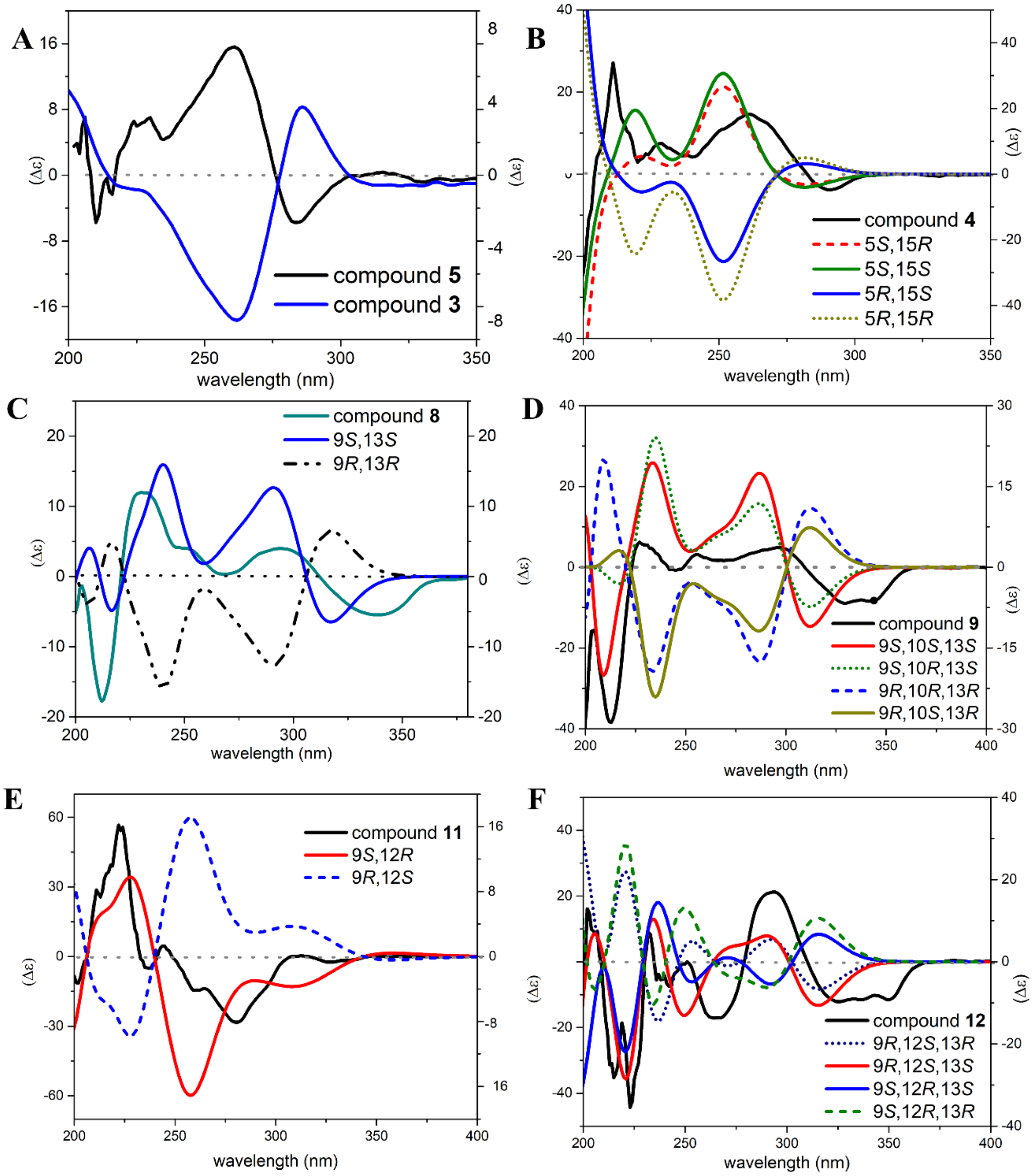
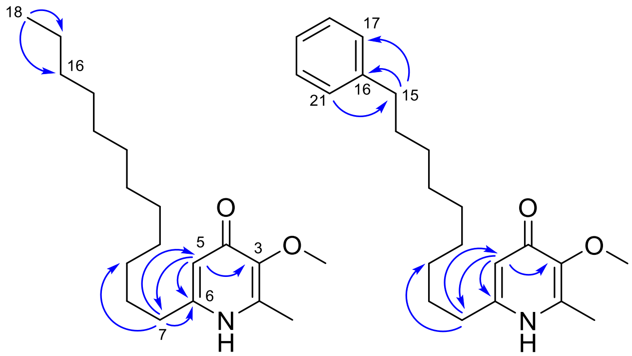


Figure 2. Experimental and TDDFT simulated ECD spectra of compounds **3**–**5**, **8**, **9**, **11**, and **12**.

Compound **4** shares the same HRESIMS protonated molecule $[M+H]^+$ at *m/z* 386.2324 than **3**. Instead of a C-2 hydroxymethyl group in **3**, the NMR data of **4** indicated a hydroxy group at C-15 at δ_C 74.8. This site of hydroxylation was unambiguously located by the HMBC correlation between the deshielded H-15 at δ_H 4.60 and the aromatic carbons C-17/C-21 at δ_C 126.8. The position of the hydroxy group was further confirmed by the ROE association observed between H-15 and H-17/H-21 at δ_H 7.32. The absolute configuration was assigned as (5*S*,15*S*) based on the comparison of calculated and experimental ECD spectra (Figure 2B). In the latter, compound **4** showed two negative CEs around 290 and 200 nm along with three sequential positive CEs around 260, 240, and 210 nm. The positive CE observed at 259 nm implies a (5*S*) configuration.[1] This compound was characterized as (5*S*,15*S*)-(5-hydroxy-5-phenylpentyl)-1,3-dimethoxy-2-methyl-5,6,7,8-tetrahydroquinolin-4(1*H*)-one and named (*S*)-15-hydroxywaltherione G.

The NMR data of compound **5** were similar to those of waltherione M[3] (Figure S1, Supporting Information). The only difference was the chemical shift of the methine C-8 at δ_C 67.6, indicating the presence of an oxygen atom instead of a methylene at δ_C 27.6 for waltherione M. This observation was in agreement with the molecular formula $C_{22}H_{29}NO_3$ deduced from the HRESIMS protonated molecule $[M+H]^+$ at *m/z* 356.2217 (calcd for $C_{22}H_{30}NO_3$, 356.2225). The comparison of the literature data of waltheriones E, H, K, N, and Q[1,3] and the ECD spectrum of **5** (Figure 2A) exhibiting positive CEs at 230 and 262 nm and a negative CE at 285 nm revealed the (5*S*,8*R*) absolute configuration. This compound was identified as 8-hydroxy-3-methoxy-2-methyl-5-(5-phenylpentyl)-5,6,7,8-tetrahydroquinolin-4(1*H*)-one and named (*R*)-8-hydroxywaltherione M.

Compound **6** exhibited an HRESIMS protonated molecule $[M+H]^+$ at *m/z* 308.2584 (calcd for $C_{19}H_{34}NO_2$, 308.2584), indicating a molecular formula $C_{19}H_{33}NO_2$. In comparison to 8-deoxoantidesmone ($C_{19}H_{31}NO_2$) previously isolated from the same plant,[1] two additional indices of hydrogen deficiency were observed. The NMR data of **6** revealed a linear dodecyl aliphatic side chain at C-6 instead of a cyclohexene ring fused to a 4-pyridinone moiety for 8-deoxoantidesmone. Indeed, the ^{1}H and HSQC spectra exhibited an olefinic proton at δ_H 6.27 (1H, s, H-5) correlating in HMBC with C-3, C-6, and C-7 at δ_C 146.3, 151.4 and 33.6, respectively (Figure 3). Likewise, the methylene protons H$_2$-7 at δ_H 2.55 correlated with C-5 at δ_C 115.5, C-6, and C-9 at δ_C 30.4. A signal at δ_H 0.90 (3H, t, J = 7.0 Hz, H-18) corresponding to the *n*-terminal methyl showed long-range correlation with C-16 and C-17 at δ_C 32.9 and 23.5, respectively. Waltherione S (**6**) was thus identified as 6-dodecyl-3-methoxy-2-methylpyridin-4(1*H*)-one.



6 **7**

Figure 3. Key HMBC (blue arrows) correlations of **6** and **7**.

Compound **7** showed NMR data close to those of waltherione S (**6**), except that in the terminal position of the *n*-alkyl side chain a phenyl group was present. HMBC correlations from the methylene protons H$_2$-15 at δ_H 2.59 (2H, t, J = 8.1 Hz) to C-16 and C-17/C-21 (δ_C 144.1 and 129.4, respectively), and between H-17/H-21 at δ_H 7.14 (2H, m) to C-15 (δ_C 36.9) allowed unambiguous assignment of the ω-position of the phenyl moiety (Figure 3). The integrations of the methylene groups in the ^{1}H NMR spectrum indicated a nonyl side chain, which was in agreement with the molecular formula

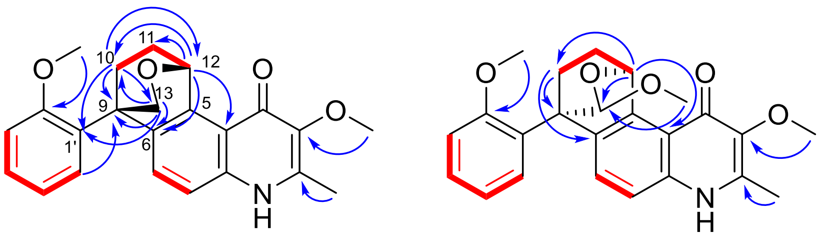
($C_{22}H_{31}NO_2$) deduced from the HRESIMS protonated molecule $[M+H]^+$ at *m/z* 342.2430 (calcd for $C_{22}H_{32}NO_2$, 342.2428). Therefore, Waltherione T (**7**) was identified as 3-methoxy-2-methyl-6-(9-phenylnonyl)pyridin-4(1*H*)-one.

Comparison of the observed and reported[4] NMR data of compound **8** indicated the presence of a 4-quinolinone moiety fused to a bicyclic ether with an attached phenyl ring as described for waltherione C. The chemical shifts at δ_C 86.5 and 81.2 of C-9 and C-13, respectively, suggested the presence of an ether bridge between these two carbons. However, no C-2 methyl group was observed in the HMBC experiment but instead hydroxymethyl protons at δ_H 4.86 correlated to C-2 and C-3 at δ_C 147.0 and 140.3, respectively. The molecular formula of $C_{22}H_{21}NO_4$ deduced from the HRESIMS ion at *m/z* 364.1543 $[M+H]^+$ (calcd for $C_{22}H_{22}NO_4$, 364.1544) confirmed the presence of an additional oxygen atom in comparison to waltherione C. Comparison of the TDDFT simulated spectra of the (9*S*,13*S*) and (9*R*,13*R*) enantiomers with the experimental ECD spectrum defined the absolute configuration as (9*S*,13*S*) (Figure 2C). These configurations are opposite to those described for waltherione C.[4] Consequently, compound **8** was identified as (9*S*,13*S*)-2-hydroxymethylwaltherione C.

Compound **9** is a regioisomer of **8** and shares the same HRESIMS protonated molecule $[M+H]^+$ at *m/z* 364.1543 (calcd for $C_{22}H_{22}NO_4$, 364.1544). The difference between these two compounds lies in the position of the hydroxy group. ROE correlations between the deshielded H-10 at δ_H 4.28 (1H, dd, *J* = 10.0, 5.8 Hz), H_{eq}-11 at δ_H 1.88 (1H, m), H_{ax}-11 at δ_H 0.74 (1H, m) and H-2´/H-6´ at δ_H 7.73 (2H, m) located the hydroxy group at C-10 (δ_C 69.6) in compound **9**. Four stereoisomers are probable for **9**, their simulated ECD spectra are shown in Figure 2D. The two stereoisomers (9*S*,10*S*,13*S* and 9*S*,10*R*,13*S*) revealed a better fit with experimental data, with some differences around 210 nm. Considering the ROE correlation between H-10 and H_{eq}-12 at δ_H 2.11 (1H, m) and the simulated ECD spectra, the absolute configurations of C-10 bearing the hydroxy group, as well as C-9 and C-13 were defined as (9*S*,10*S*,13*S*) (Figure 2D). Compound **9** was identified as (9*S*,10*S*,13*S*)-10-hydroxywaltherione C.

NMR data indicated that compound **10** was characterized by a 3-methoxy-2-methyl-quinoline-4(1*H*)-one skeleton with a cycloheptyl moiety attached to C-5/C-6 and a phenyl ring at C-9, as for compounds **8** and **9**. However, no oxygen atom was present on the cycloheptyl moiety but instead a double bond in C-9/C-10 as suggested by the chemical shifts at δ_C 143.6 and 130.7, respectively. Three aromatic protons at δ_H 6.39 (2H, d, *J* = 2.3 Hz, H-2´ and H-6´), and at δ_H 6.42 (1H, t, *J* = 2.3 Hz, H-4´) indicated that the phenyl ring is *m*-disubstituted. A signal at δ_H 3.73 integrating for six protons (two methoxy groups) and HMBC correlations completed the elucidation of the structure. It was named waltherione U and is the 3´,5´-dimethoxy derivative of melochinone, an alkaloid previously isolated from *Melochia tomentosa*.[5] Both *M. tomentosa* and *W. indica* are Malvaceae of the New World.

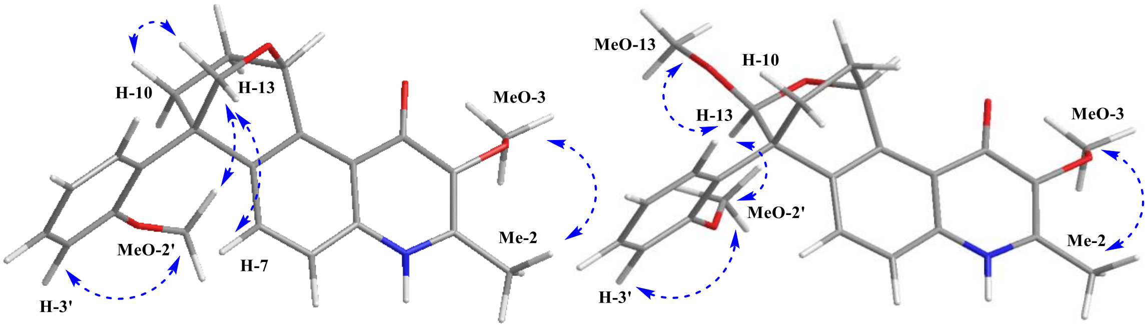
Compound **11** showed close structural similarities with compounds **8**–**10**, sharing a 3-methoxy-2-methyl-quinolin-4(1*H*)-one nucleus and a phenyl ring at C-9. However, instead of a cycloheptyl moiety fused at C-5/C-6, a cyclohexyl carrying an oxymethano bridge was identified. Indeed, in the DEPTQ and HSQC spectra, two methylene groups CH_2-10 and CH_2-11 at δ_C 26.1 and 27.9, respectively, an oxygenated CH-12 at δ_C 66.1, and a quaternary carbon C-9 at δ_C 51.1 were detected. HMBC and ^{1}H-^{1}H COSY correlations (Figure 4) showed that the cyclohexyl moiety was fused at C-5/C-6 to the quinoline-4(1*H*)-one nucleus and the phenyl ring linked at C-9. In addition, the HMBC correlations confirmed the location of the oxymethano group between C-9 and C-12. In particular, the signal of the methylene H$_2$-13 at δ_H 4.31 (2H, s) correlated with C-6, C-9, C-10, and C-1´ at δ_C 139.1, 51.1, 26.1, and 135.0, respectively. The relative configuration of **11** was inferred from ROESY data. A ROE correlation between H$_2$-10 and H$_2$-13 suggested that the tetrahydropyran ring from C-9 to C-13 adopted a boat conformation to which the aromatic ring of the quinolinone skeleton was fused diaxially (Figure 5). Additional ROE correlations between H-7, H$_2$-13 and MeO-2´ of the equatorial phenyl ring indicated a gauche conformation to one another, and placed these three elements in the same axial plane of the molecule. Moreover, MeO-2´ at δ_H 3.66 (3H, s) correlating with H-3´ at δ_H 6.94 (1H, d, J = 8.4 Hz) indicated that the phenyl ring was *o*-substituted. The absolute configurations of C-9 and C-12 were defined by ECD data as (9*S*) and (12*R*) (Figure 2E). This compound was defined as (9*S*,12*R*)-3-methoxy-9-(2´-methoxyphenyl)-2-methyl-9,10,11,12-tetrahydro-12,9-(oxymethano)benzo[*f*] quinolin-4(1*H*)-one and named waltherione V.



11 **12**

Figure 4. Key ^{1}H-^{1}H COSY (bold red lines) and HMBC (blue arrows) correlations of **11** and **12**.

The NMR data of compound **12** were compared with those of **11** and a difference at C-13 was obvious. Instead of a methylene group as for **11**, a methine bearing two oxygen atoms was detected at δ_C 101.8 in **12**. In the HMBC spectrum, a methoxy group at δ_H 3.23 (3H, s) correlated with C-13 implying that this group is attached to C-13 (Figure 4). The HRESIMS protonated molecule $[M+H]^+$ at *m/z* 408.1807 (calcd for $C_{24}H_{26}NO_5$, 408.1811) confirmed the presence of an additional methoxy group for **12** compared to **11**. ROE correlations between H-13, MeO-13 and MeO-2´, and the absence of correlation between H-10 and H-13 indicated that H-13 is equatorially oriented (Figure 5). This observation was confirmed by the (9*R*,12*S*,13*S*) absolute configurations determined by comparison of experimental and calculated ECD spectra (Figure 2F). Thus, (*S*)-13-methoxywaltherione V (**12**) was identified as (9*R*,12*S*,13*S*)-3,13-dimethoxy-9-(2´-methoxyphenyl)-2-methyl-9,10,11,12-tetrahydro-12,9-(oxymethano)benzo[*f*]quinolin-4(1*H*)-one and named.



11 **12**

Figure 5. Key ROE correlations (blue dashed arrows) of **11** and **12**.

The molecular formula $C_{19}H_{29}NO_3$ suggested by HRESIMS and NMR data of compound **13** were in accordance with those of melovinone, an alkaloid previously isolated from *Melochia tomentosa*.[6] Melovinone (**13**) is closely related to waltherione R (**2**). The only difference is an additional methoxy group at C-7 in melovinone.

The NMR data of compound **14** showed close similarities to those of waltherione A.[7] The only difference is a second methoxy group on the phenyl ring. Comparison of the observed and reported physical data[8, 9] confirmed that alkaloid **14** is 5´-methoxywaltherione A.

Compounds **1**−**14** as well as waltheriones M−Q (Figure S1, Supporting Information), previously isolated and characterized from the same plant[3] but not tested against the etiological agent of Chagas disease, were evaluated for their in vitro growth inhibition of the amastigote form of *T. cruzi* (Tulahuen C4 strain). The cytotoxicity towards uninfected host cells, i.e. rat skeletal L6 cells, was also assessed and the data are shown in Table 5.

Table 3. **Antitrypanosomal and Cytotoxic Activities (IC_{50} in μM) of Alkaloids 1−14 and Waltheriones M−Q**

compound	*T. cruzi*	Cytotoxicity[a]	SI[b]
1	10.0[c]	15.0	1.5
2	2.1[c]	>26	>12.5
3	3.9[c]	>26	>6
4	7.5 ± 0.5[d]	>26	>3.5
5	0.8 ± 0.1[d]	>28	>33
6	5.0[c]	>32	>6
7	2.1[c]	>29	>13
8	14.7 ± 0.6[d]	>22	>1.5
9	>20[c]	>22	>1
10	13.1[c]	>25	>1.5
11	19.0 ± 2.1[d]	>26	>1
12	18.7 ± 1.4[d]	>24	>1
13	12.9[c]	>23	>1.5

14	14.7[c]	>25	>1
Waltherione M	1.3 ± 0.2[d]	6.5	5.0
Waltherione N	6.5 ± 0.8[d]	26.2	4.0
Waltherione O	7.0 ± 0.3[d]	>28	>4
Waltherione P	0.5 ± 0.1[d]	12.0	24.5
Waltherione Q	0.1 ± 0.0[d]	1.8	13.8
Benznidazole[e]	2.9[c]	>345	>119
Podophyllotoxin[e]		0.02	

[a] Tested against rat skeletal L6 cells
[b] SI (selectivity index) = IC_{50} cytotoxicity/IC_{50} antitrypanosomal activity
[c] Results are the mean of two independent assays
[d] Results are the mean of three independent assays ± SD
[e] Positive controls

Compounds **2**, **5**, **7**, and waltheriones M, P, and Q showed IC_{50} values lower than the reference drug benznidazole ($IC_{50} = 2.9\ \mu M$). They carry an *n*-alkyl side chain (pentyl or nonyl for compound **7**) with a terminal phenyl ring and a 3-methoxy-2-methylpyridin-4(1*H*)-one moiety. The (5*S*) absolute configuration of compounds **5**, waltheriones M, P, and Q increased the activity against *T. cruzi*. This observation is in accordance with previous results.[1] Compounds **8**−**12** and **14** are waltheriones with cyclization of the aliphatic side chain between C-6 and C-9, which was detrimental for the antitrypanosomal activity. Thus, the best compounds had submicromolar IC_{50} values against intracellular *T. cruzi* and selectivity indices above 10, underlining the potential of the waltheriones as anti-chagasic agents.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation was measured on a Jasco (Easton, MD, USA) P-1030 polarimeter (MeOH, *c* in g/100 mL). UV spectra were recorded on a Perkin-Elmer Lambda-25 UV-vis spectrophotometer (Wellesley, MA, USA) in MeOH. ECD spectra were recorded on a JASCO J-815 CD spectrometer in MeOH. NMR spectra were recorded on a Bruker Avance III HD 600 MHz NMR spectrometer equipped with a QCI 5 mm Cryoprobe and a SampleJet automated sample changer (Bruker BioSpin, Rheinstetten, Germany). Chemical shifts are reported in parts per million (δ) using the residual methanol-d_4 signals (δ_H 3.31; δ_C 49.0) as internal standards for ^{1}H and ^{13}C NMR experiments, respectively. HRMS data were obtained on a Q Exactive Focus Hybrid quadrupole-orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA) using electrospray ionization in the positive-ion mode. The spray voltage was set at 3.5 kV; the sheath gas flow rate (N_2) at 50 units; the capillary temperature was 320 °C; the S lens RF level at 50 and the probe heater temperature at 425 °C. UHPLC was performed on an Acquity UPLC I-class System (Waters, Milford, MA, USA). The separation was performed on an Acquity BEH C_{18} UPLC column (150 × 2.1 mm i.d.; 1.7 μm, Waters), using a gradient (MeCN and H_2O both containing 0.1% formic acid) from 5 to 98% MeCN in 30 min, followed by an isocratic washing step at 98% MeCN for 3 min. After the washing step, the column was re-equilibrated with 5% MeCN for 5 min prior to the next injection. The flow rate was set to 0.4 mL/min, the column temperature to 40 °C, and the injection volume was 1 μL. Fractionation was performed on an Armen Spot preparative chromatographic system (Interchim, Montluçon, France) equipped with a quaternary pump, a UV detector, and a fraction collector. Semi-preparative chromatography was performed on an Armen Spot System with a Kinetex Axia Core-Shell C18 column (250 × 21.2 mm, 5 μm; Phenomenex, Torrance, CA, USA).

ECD Computational Details. Conformational analysis of compounds **3**−**5**, **8**, **9**, **11** and **12** was carried out using MacroModel 9.1 software (Schrödinger, LLC, New York, NY, USA) by applying the OPLS-2005 force field in H_2O. The selected conformers were subjected to geometrical optimization using the density function theory (DFT) with the CAM-B3LYP functional and the 6-31G** basis-set as implemented with the Gaussian 09 program package.[10] Vibrational analysis was performed at the same level to confirm stability of the minima. Time-dependent density function theory calculations at the TD-DFT/B3LYP/6-31G** level in MeOH using the "self-consistent reaction field" method with the conductor-like polarizable calculation model were employed to calculate excitation energy (denoted by wavelength in nm) and rotatory strength in dipole velocity (R_{vel}) and dipole length (R_{len}) forms. ECD curves were calculated based on rotatory strengths using a half bandwidth of 0.3 eV using SpecDis version 1.61.[11]

Plant Material. The aerial parts of *W. indica* were collected between June 2012 and February 2013 in Zinder (Niger). The identification was confirmed by Didier Roguet (Geneva Botanical Gardens). A voucher specimen is deposited at the Geneva Botanical Gardens (no. 19003).

Extraction and Isolation. The CH_2Cl_2 extract (1.2 g) of the aerial parts was fractionated by flash chromatography as previously described.[3] The flow rate was set to 25 mL/min and UV absorbance was at 220 nm. The separation yielded 200 fractions, which were individually analyzed by UHPLC-MS. Fractions 26-28, 31-32, 40-41, 52-54, 85-88, 112-114, 121-122, 135-138, 154-156, 159-160, and 176-178 were selected for further purification. The final fractionation step was performed by semi-preparative HPLC with a Kinetex Axia Core-Shell C_{18} column (250 × 21.2 mm, 5 μm; Phenomenex) using MeOH/H_2O/0.1% formic acid as solvents for a gradient elution of 40 to 100% MeOH in 80 min. The flow rate was set to 18 mL/min and UV absorbance was at 220 nm. Fractions 26-28 (4.3 mg) gave **8** (0.5 mg), fractions 31-32 (20.1 mg) gave **14** (3.7 mg), fractions 40-41 (2.2 mg) gave **12** (0.4 mg), fractions 52-53 (3.0 mg) gave **11** (1.0 mg), fraction 54 (2.0 mg) gave **9** (0.6 mg), fractions 85-86 (3.5 mg) gave **5** (0.8 mg), fractions 87-88 (2.0 mg) gave **3** (0.3 mg), fractions 112-113 (2.9 mg) gave **7** (0.8 mg), fractions 113-114 (2.2 mg) gave **10** (0.5 mg), fractions 121-122 (2.0 mg) gave **4** (0.3 mg), fractions 135-138 (2.5 mg) gave **6** (0.4 mg), fractions 154-156 (1.9 mg) gave **2** (0.3 mg), fractions 159-160 (2.1 mg) gave **1** (0.5 mg), and fractions 176-178 (3.0 mg) gave **13** (0.4 mg).

*8-Demethoxywaltherione F (**1**)*: pale yellow oil; UV (MeOH) λ_{max} (log ε) 220 (3.0), 240 (3.5), 328 (1.6) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 302.2113 $[M+H]^+$ (calcd for $C_{19}H_{28}NO_2$, 302.2115).

*Waltherione R (**2**)*: pale yellow oil; UV (MeOH) λ_{max} (log ε) 243 (3.4), 332 (2.6) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 366.2064 $[M+H]^+$ (calcd for $C_{23}H_{28}NO_3$, 366.2064).

*1-Methoxywaltherione O (**3**)*: pale yellow oil; $[a]_D^{22}$ not measured due to lack of material; UV (MeOH) λ_{max} (log ε) 211 (3.4), 268 (2.0) nm; ECD (0.3 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 285 (+8.02) and 262 (-18.14) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 386.2327 $[M+H]^+$ (calcd for $C_{23}H_{32}NO_4$, 386.2326).

*15-Hydroxywaltherione G (**4**)*: pale yellow oil; $[a]_D^{22}$ not measured due to lack of material; UV (MeOH) λ_{max} (log ε) 235 (3.8), 263 (3.5) nm; ECD (0.3 mM, MeOH) λ_{max}

($\Delta\varepsilon$) 290 (-3.89), 259 (+14.73), 229 (+7.48), and 262 (-18.02) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 386.2324 [M+H]$^+$ (calcd for $C_{23}H_{32}NO_4$, 386.2326).

*8-Hydroxywaltherione M (**5**)*: pale yellow oil; $[a]_D^{22}$ not measured due to lack of material; UV (MeOH) $\lambda_{\max}$ (log ε) 210 (3.6), 264 (1.6) nm; ECD (0.3 mM, MeOH) $\lambda_{\max}$ ($\Delta\varepsilon$) 285 (-5.24),262 (+15.88) and 230 (+7.85) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 356.2223 [M+H]$^+$ (calcd for $C_{22}H_{30}NO_3$, 356.2225).

*Waltherione S (**6**)*: pale yellow oil; UV (MeOH) $\lambda_{\max}$ (log ε) 212 (3.4), 224 (3.5), 264 (1.6) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 308.2584 [M+H]$^+$ (calcd for $C_{19}H_{34}NO_2$, 308.2584).

*Waltherione T (**7**)*: pale yellow oil; UV (MeOH) $\lambda_{\max}$ (log ε) 194 (4.0), 206 (3.7), 263 (2.0) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 342.2430 [M+H]$^+$ (calcd for $C_{22}H_{32}NO_2$, 342.2428).

*2-Hydroxymethylwaltherione C (**8**)*: pale yellow oil; $[a]_D^{22}$ not measured due to lack of material; UV (MeOH) $\lambda_{\max}$ (log ε) 223 (3.8), 245 (3.8), 340 (1.6) nm; ECD (0.3 mM, MeOH) $\lambda_{\max}$ ($\Delta\varepsilon$) 338 (-5.32), 295 (+4.77), 250 (+4.81), 232 (+12.29), and 213 (-18.04) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 364.1543 [M+H]$^+$ (calcd for $C_{22}H_{22}NO_4$, 364.1544).

*10-Hydroxywaltherione C (**9**)*: pale yellow oil; $[a]_D^{22}$ not measured due to lack of material; UV (MeOH) $\lambda_{\max}$ (log ε) 220 (4.6), 262 (4.6) nm; ECD (0.3 mM, MeOH) $\lambda_{\max}$ ($\Delta\varepsilon$) 334 (-9.07), 299 (+5.83), 256 (+4.04), 230 (+7.92), and 213 (-39.01) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 364.1543 $[M+H]^+$ (calcd for $C_{22}H_{22}NO_4$, 364.1544).

*Waltherione U (**10**)*: pale yellow oil; UV (MeOH) λ_{max} (log ε) 226 (3.8), 336 (1.8) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 392.1856 $[M + H]^+$ (calcd for $C_{24}H_{26}NO_4$, 392.1857).

*Waltherione V (**11**)*: pale yellow oil; $[a]_D^{22}$ +9 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.0), 251 (3.9), 327 (2.4) nm; ECD (0.3 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 280 (-29.50), 261 (-17.81), 243 (+4.03), 236 (+58.01), and 223 (+24.80) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 378.1701 $[M+H]^+$ (calcd for $C_{23}H_{24}NO_4$, 378.1700).

*13-Methoxywaltherione V (**12**)*: pale yellow oil; $[a]_D^{22}$ not measured due to lack of material; UV (MeOH) λ_{max} (log ε) 246 (3.6), 330 (1.9) nm; ECD (0.3 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 333 (-10.48), 295 (+22.31), 265 (-18.84), 233 (-43.03), 223 (-35.21), and 215 (+18.74) nm; IR not measured due to lack of material; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 408.1807 $[M+H]^+$ (calcd for $C_{24}H_{26}NO_5$, 408.1806).

Antitrypanosomal and Cytotoxicity Assays. The in vitro activity against *T. cruzi* as well as cytotoxicity assessment in L6 cells were determined as reported elsewhere.[12] The strain was *T. cruzi*, Tulahuen C2C4 (LacZ), amastigote forms grown in rat L6 skeletal myoblasts. Results are expressed in μM for pure compounds.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://www.pubs.acs.org/doi/

Structures of the waltheriones M−Q

HRMS, MS/MS, UV, 1D and 2D NMR spectra for compounds **1**−**12**

Calculation data for ECD curves for compounds **4**, **8**, **9**, **11** and **12**.

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Notes

The authors declare no competing financial interest.

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Abstract graphic