Synthesis and Antileishmanial Activity of Indoloquinones Containing a Fused Benzothiazole Ring

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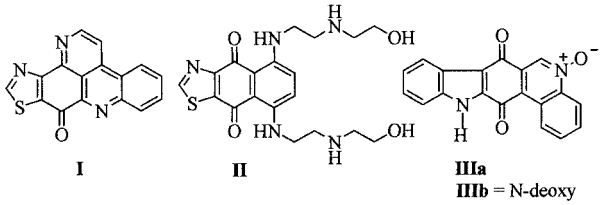
Pyrazinoindoloquinone **6** was synthesized by alkylation of ethyl 4,7-dimethoxyindole-2-carboxylate (**1**), followed by cyclization of the *N*-bromoethyl derivative **2b** in the presence of ammonia. Oxidative demethylation of **2b** and of the oxopyrazinoindole **3** with silver(II) oxide furnished quinones **5b** and **6**. Reduction of **3** with lithium aluminium hydride in dioxane provided **4**, which was oxidized to afford **7**. Quinones **5b**, **6**, and **7** were then treated in situ with the thiazole *o*-quinodimethane **9** to afford regioisomeric mixtures of the tetracyclic quinones **10** or the pentacyclic derivatives **11** or **12**. The structural assignment was made by 2D NMR ^{1}H-^{13}C HMBC correlation performed on the major regioisomer **10a**. In vitro antileishmanial assays showed that dimethoxyindole **2a** and quinones **12a** + **12b** possess good inhibitory activity against two *Leishmania* sp. without any cytotoxicity towards a THP-1 cell line.

Introduction

Many quinone derivatives exhibit various biological activities. Among them, kuanoniamine A (**I**),[1] a marine metabolite, contains a thiazole ring fused to a tetracyclic aromatic framework with a quinone-imine function. This compound inhibits the proliferation of KB cell lines in vitro with an IC_{50} of 1−2 μg/mL and possesses antiviral activities.[2] The synthetic naphthothiazoledione **II** also exhibits pharmacological properties,[3] and calotrixines **IIIa** and **IIIb**, novel angular pentacyclic metabolites containing indole moieties, were recently isolated and their antiplasmodial and anticancer activities described[4] (Scheme 1). Some heterocyclic quinones have also been found to be active in vitro against virulent strains of *Leishmania* sp.[5−8] Leishmaniasis is an important tropical disease that is the cause of considerable mortality world-wide. Resistance to treatment with antimony-based agents occurs frequently, while the use of amphotericin B and pentamidine, both second-line drugs, results in high toxicity at the effective therapeutic doses. For these reasons, the search for new antileishmanial compounds remains a priority, since no drug is totally active and safe. With this as a goal, we planned to explore the synthesis and antileishmanial activity of angular pentacyclic quinones possessing benzothiazole and indole rings as part of their structures.



Scheme 1. Examples of natural or synthetic heterocyclic quinones displaying biological activities

Synthesis

Our group had developed a mild method by which to obtain polycyclic heterocyclic quinones by Diels−Alder trapping of a thiazole *o*-quinodimethane (*o*-QDM) with naphthoquinones.[9] In this work, we have applied the *o*-QDM strategy to indoloquinone derivatives.

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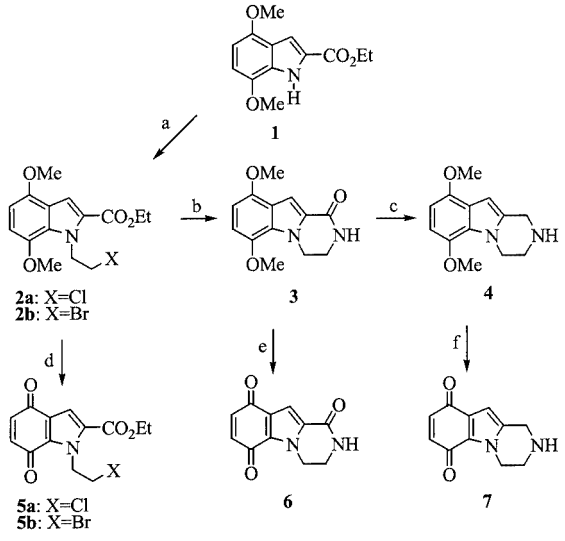
We first performed the synthesis of the new pyrazinoindoloquinones. Thus, dimethoxyindole **1**,[10] obtained in an improved yield (99%) from 2,5-dimethoxybenzaldehyde and ethyl azidoacetate in a Hemetsberger−Knittel reaction,[11] was treated at room temperature with 1,2-dichloroethane or 1,2-dibromoethane in the presence of sodium hydroxide to afford the alkylated indoles **2**. Attempts to cyclize **2a** into the oxopyrazinoindole **3** in the presence of ammonia failed. The synthesis of **3** was achieved in good yield, however, by treatment of compound **2b** with ammonia in an autoclave for 18 h at 90°C.[12] Reduction of **3** with lithium aluminium hydride in refluxing dioxane then quantitatively furnished the pyrazinoindole **4**. On the other hand, oxidative demethylation of the alkylated indoles **2** or the pyrazinoindole derivatives **3** or **4** with silver(II) oxide in the presence of 6 N nitric acid gave the corresponding quinones **5**−**7** in 35−99% yields (Scheme 2).

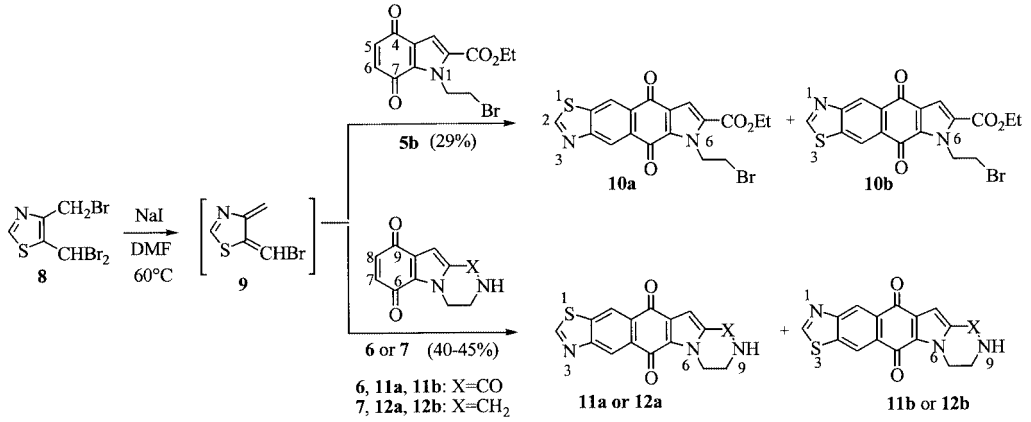


Scheme 2. Reagents and conditions: a) $ClCH_2CH_2Cl$ or $BrCH_2CH_2Br$, NaOH, TBAB, room temp., 18 h, 95%; b) NH_3, 90°C, 18 h, 74%; c) LAH, dioxane, reflux, 30 min, 85%; d) AgO, HNO_3, room temp., 4 min, 99%; e) AgO, HNO_3, −10°C, 2 min, 56%; f) AgO, HNO_3, room temp., 6 min, 35%

Then, 5-(bromomethylene)-4-methylene-4,5-dihydro-1,3-thiazole (**9**), generated in situ from 4-(bromomethyl)-5-(dibromomethyl)-1,3-thiazole (**8**) by treatment with sodium iodide in DMF,[9] was trapped with indoloquinones **5b**, **6**, and **7**. From quinone **5b**, the Diels−Alder reaction gave a mixture of the aromatized tetracyclic quinones **10a** and **10b** in 29% yield and in 1.7:1 ratio. The regioisomers **10a** and **10b** were separated by column chromatography with dichloromethane/ethyl acetate (19:1) as the eluent. Similarly, treatment of **9** with quinones **6** and **7** afforded mixtures of the pentacyclic quinones **11a** + **11b** (40% yield, ratio 1.4:1) and **12a** + **12b** (45% yield, ratio 1.8:1), respectively (Scheme 3). Because of their insolubility in almost all solvents, the regioisomers **11a** and **11b** could not be separated. However, they were identified from their ^1H and ^{13}C NMR spectra in deuterated trifluoroacetic acid. Separation of the regioisomers **12a** and **12b** after the trapping of *o*-QDM **9** with quinone **7** also failed.

Frontier molecular orbital (FMO) theory could be used to predict the regioselectivity of the Diels−Alder reactions. We calculated the HOMO and LUMO frontier molecular orbital coefficients for *o*-QDM **9** and quinones **5b**, **6**, and **7** by the density functional theory (DFT) method with the hybrid B3LYP functional,[13] by use of the GAUSSIAN 94 package[14] with a 6-31G* basis set (Table 1). The calculations indicate that for *o*-QDM **9** the largest orbital coefficients are located (for $2p_z$) at the carbon atom bearing the bromine atom, while for the quinones they are always situated at the carbon atom *para* to the nitrogen atom (C-5 or C-8). These results found are similar to those obtained by the semiempirical PM3 method. It appears from Table 2 that the major regioisomers should result from the attack of the brominated carbon atom of **9** at C-5 or C-8 of the corresponding quinone.

Assignment of regiochemistry was established by 2D NMR ^1H-^{13}C HMBC correlations performed on the major regioisomer **10a**. The spectral data of the latter are reported in Table 2. The characteristic long-range ^3J coupling of the benzothiazole nucleus[2] was used. Thus, the 2-H proton has two ^3J couplings with C-3a and C-10a. However, the former appears as a doublet typical of a cross-peak through the

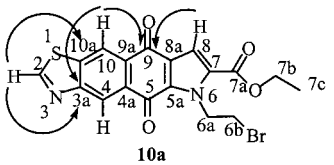


Scheme 3. Regioisomer ratios: **10a/10b** = 1.7:1; **11a/11b** = 1.4:1; **12a/12b** = 1:1.8

Table 1. Frontier molecular orbital coefficients

Compound	Carbon atom	DFT method 2pz	DFT method 3pz	PM3 method
9 (*Z*)	CHBr (HOMO)	0.305	0.231	0.441
	CH_2 (HOMO)	0.289	0.239	0.354
5b	C-5 (LUMO)	0.204	0.213	0.369
	C-6 (LUMO)	0.177	0.189	0.320
6	C-8 (LUMO)	0.228	0.239	0.371
	C-7 (LUMO)	0.198	0.213	0.323
7	C-8 (LUMO)	0.222	0.238	0.392
	C-7 (LUMO)	0.213	0.229	0.359

Table 2. 2D ^{1}H-^{13}C HMBC correlations for **10a** ($CDCl_3$, 500.13 and 125.78 MHz)



10a

Atom	^{13}C	^{1}H	HMBC [*J*(C,H)] ^1J	^2J	^3J	^4J	^5J
2	159.0	9.28	2-H				
3a	156.5			4-H	2-H, 10-H		
4	123.4	8.94	4-H			10-H	
4a	133.1			4-H	10-H		2-H
5	176.8				4-H	10-H	
5a	134.2				8-H, 6a-H		
6a	47.9	5.48	6a-H	6b-H			
6b	30.4	3.86	6b-H	6a-H			
7	128.0			8-H			
7a	160.7				8-H, 7b-H		
7b	62.1	4.46	7b-H	7c-H			
7c	14.6	1.47	7c-H	7b-H			
8	115.9	7.56	8-H				
8a	130.7			8-H			
9	179.8				10-H, 8-H	4-H	
9a	130.7			10-H	4-H	8-H	
10	122.3	8.86	10-H			2-H, 4-H	
10a	139.6			10-H	2-H, 4-H		

nitrogen atom of the thiazole ring ($^3J_{C3a\text{-}H2}$ = 15.3 Hz), while the latter gives an unresolved singlet indicating a coupling of < 5 Hz for $^3J_{C10a\text{-}H2}$. These data enabled us to identify both carbon atoms. Next, three ^3J couplings − 10-H with C-3a and C-9 and 8-H with C-9 − allowed the structure of the regioisomer **10a** to be assigned. The minor regioisomer **10b** should therefore have the opposite regiochemistry. Thus, the predicted HOMO−LUMO molecular orbital coefficients for *o*-QDM **9** and quinone **5b** agree with the observed regioselectivity. On the basis of the FMO predictions, we assigned regiochemistries similar to those of **10a** and **10b** to compounds **11a** and **12a** on one hand, and **11b** and **12b** on the other.

Biological Results

The in vitro antileishmanial activities of compounds **2a**, **2b**, **5a**, **5b**, **6**, **7**, **10a**, **10b**, and **12a** + **12b** were evaluated against promastigote forms of *Leishmania donovani* and *Leishmania major*. The potential toxicities of these derivatives against a THP-1 cell line were also determined. Pentamidine and amphotericin B were used as the reference drugs (Table 3). Except for **6**, **10a**, and **10b**, the tested compounds exhibit significant activity against both *Leishmania* sp. It is noteworthy that the dimethoxyindole derivatives **2a** and **2b** were found to be as active as their corresponding quinones **5a** and **5b**. These results suggest that the antiprotozooal activity of these compounds is not solely due to the presence of a quinone function. Moreover, **2a** and **2b** were found to be less cytotoxic than **5a** and **5b**. The mixture of regioisomers **12a** + **12b** show good inhibitory activity similar to that of pentamidine and amphotericin B, but without any cytotoxicity even at 0.3 μM, indicating a selectivity of the action against the *Leishmania* sp.

Conclusion

Starting with dimethoxyindole **1**, we have prepared the new pyrazinoindoloquinones **6** and **7** through an alkylation/cyclization procedure followed by an oxidative demethyl-

Table 3. In vitro inhibitory activity of the synthesized compounds against *Leishmania* sp. and their cytotoxicity against THP-1 cells; the values are the means ±S.D. of triplicate experiments

Compounds	*Leishmania donovani* IC_{50} [μM]	*Leishmania major* IC_{50} [μM]	THP-1 cell growth inhibition (%) 0.1 μM	0.3 μM
2a	0.029	0.027	0	0
2b	0.03	0.03	18.2	37.2
5a	0.03	0.06	58.4	90.7
5b	0.05	0.09	35	92.8
6	0.25	0.24	5	25.5
7	0.04	0.035	41	96.6
10a	NI	0.30	0	0
10b	NI	> 3	0	11.2
12a + **12b**	0.027	0.027	0	0
Pentamidine	0.006	0.03	81	85
Amphotericin B	0.02	0.005	71	75

ation. Trapping of the thiazole *o*-QDM **9** with these quinones provides the angular pentacyclic quinones **11** or **12**, containing indole and benzothiazole rings as part of their structures. The prepared quinones and dimethoxyindoles **2** were evaluated in vitro against two virulent strains of *Leishmania*. Among the tested compounds, dimethoxyindole **2a** and quinones **12a** + **12b** showed inhibitory activity similar to that of the reference compounds but without any cytotoxicity towards THP-1 cells. In view of the better selectivity of **12a** + **12b** with regard to that of the reference drugs, it is of interest to estimate the activity of **12a** and **12b** separately. Their separation and the evaluation of other indole derivatives are underway.

Experimental Section

General: Melting points were determined with a Stuart Scientific SMP3 apparatus and are not corrected. IR spectra were performed with a Bruker Model Vector 22 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with Bruker AM 300, AM 400, and AMX 500 spectrometers with tetramethylsilane as an internal reference. Coupling constants are given in Hz. Column chromatography was performed on Merck silica gel 60 (70−230 mesh). Elemental analyses were carried out on a FISONS EA 1108 CHNS-O analyzer.

Ethyl 4,7-Dimethoxy-1*H*-indole-2-carboxylate (1): A solution of ethyl 2-azido-3-(2,5-dimethoxyphenyl)-2-propenoate[10] (0.50 g, 1.81 mmol) in toluene (35 mL) was heated at reflux for 5 h. The reaction mixture was allowed to cool and the solvent was removed. The residue was purified by column chromatography on silica gel with dichloromethane as eluent to give compound **1** (0.45 g, 99%). M.p. 126−127°C (ref.[10] 127−127.5 °C).

Ethyl 1-(2-Chloroethyl)-4,7-dimethoxy-1*H*-indole-2-carboxylate (2a): A mixture of indole **1** (1.0 g, 4.02 mmol), 1,2-dichloroethane (10 mL), sodium hydroxide (9 N, 10 mL), and tetrabutylammonium bromide (0.05 g, 0.155 mmol) was stirred at room temperature for 30 h. The layers were separated and the aqueous fraction was extracted with dichloromethane. The combined organic layers were concentrated and the residue was purified by recrystallization from ethanol to afford compound **2a** (1.0 g, 80%). M.p. 101°C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 1710 (CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.40 (t, J = 7.1 Hz, 3 H, OCH_2CH_3), 3.83 (t, J = 7.3 Hz, 2 H, CH_2CH_2Cl), 3.91 (s, 3 H, OCH_3), 3.93 (s, 3 H, OCH_3), 4.36 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 5.18 (t, J = 7.3 Hz, 2 H, CH_2CH_2Cl), 6.36 (d, J = 8.3 Hz, 1 H, 5-H or 6-H), 6.63 (d, J = 8.3 Hz, 1 H, 6-H or 5-H), 7.40 (s, 1 H, 3-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.3, 43.5, 47.5, 55.5, 55.9, 60.6, 98.9,105.5, 109.2, 119.5, 126.9, 130.0, 142.2, 148.5, 161.7. $C_{15}H_{18}ClNO_4 \cdot 0.3H_2O$ (317.169): calcd. C 56.76, H 5.86, N 4.41, found C 56.84, H 6.03, N 4.14.

Ethyl 1-(2-Bromoethyl)-4,7-dimethoxy-1*H*-indole-2-carboxylate (2b): A mixture of indole **1** (1.0 g, 4.02 mmol), 1,2-dibromoethane (10 mL), sodium hydroxide (9 N, 10 mL), and tetrabutylammonium bromide (0.05 g, 0.155 mmol) was stirred at room temperature for 18 h. The layers were separated and the aqueous fraction was extracted with dichloromethane. The combined organic layers were concentrated and the residue was purified by recrystallization from ethanol to afford compound **2b** (1.36 g, 95%). M.p. 110−111°C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 1700 (CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.41 (t, J = 7.1 Hz, 3 H, OCH_2CH_3), 3.68 (t, J = 7.3 Hz, 2 H, CH_2CH_2Br), 3.90 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 4.36 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 5.20 (t, J = 7.3 Hz, 2 H, CH_2CH_2Br), 6.46 (dd, J = 8.3 Hz, 2 H, 5-H and 6-H), 7.40 (s, 1 H, 3-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.3, 31.1, 47.6, 55.5, 55.9, 60.6, 98.9,105.6, 109.2, 119.5, 126.8, 129.9, 142.2, 148.5, 161.7. $C_{15}H_{18}BrNO_4$ (356.216): calcd. C 50.58, H 5.09, N 3.93, found C 50.88, H 5.06, N 3.96.

6,9-Dimethoxy-3,4-dihydropyrazino[1,2-*a*]indol-1(2*H*)-one (3): A mixture of indole **2** (0.66 g, 1.86 mmol), ethanol (10 mL), and ammonia (40 mL) was heated at 90 °C in an autoclave for 18 h. The reaction mixture was concentrated and the residue was taken up in water (15 mL). The solid was collected by filtration, washed with water, and dried to give compound **3** (0.33 g, 74%). M.p. 169 °C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3280 (NH), 1700 (CO). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ [ppm] = 3.96 (t, J = 6.3 Hz, 2 H, 3-H), 4.01 (s, 3 H, OCH_3), 4.06 (s, 3 H, OCH_3), 5.17 (t, J = 6.3 Hz, 2 H, 4-H), 6.71 (dd, J = 8.4 Hz, 2 H, 7-H and 8-H), 7.44 (s, 1 H, 10-H), 8.21 (s, 1 H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.3, 31.1, 49.3, 56.1, 56.7, 63.7, 99.9, 106.9, 109.6, 120.7, 128.6, 143.7, 163.7, 149.7. $C_{13}H_{14}N_2O_3$ (246.266): calcd. C 63.60, H 5.73, N 11.38; found C 63.85, H 5.61, N 11.80.

6,9-Dimethoxy-1,2,3,4-tetrahydropyrazino[1,2-*a*]indole (4): Lithium aluminium hydride (0.10 g, 2.54 mmol) was added to a solution of indole **3** (0.20 g, 0.81 mmol) in dry dioxane (10 mL), and the reaction mixture was heated at reflux for 30 min. The solvent was removed and the residue was purified by column chromatography on silica gel with dichloromethane as the eluent to give compound **4** (0.16 g, 85%). M.p. 160 °C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3210 (NH). ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.38 (br. s, 1 H, NH), 3.86 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 3.97 (t, J = 6.3 Hz, 2 H, 3-H), 4.51 (t, J = 4.9 Hz, 2 H, 4-H), 4.67 (s, 2 H, 1-H), 6.44 (dd, J = 8.4 Hz, 2 H, 7-H and 8-H), 6.55 (s, 1 H, 10-H), ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 47.5, 56.2, 55.6, 55.7, 63.3, 98.8, 99.9, 102.7, 120.5, 127.6, 138.6, 142.1, 147.5. $C_{13}H_{16}N_2O_2$ (232.282): calcd. C 67.22, H 6.94, N 12.06; found C 67.48, H 6.98, N 12.26.

Ethyl 1-(2-Chloroethyl)-4,7-dioxo-4,7-dihydro-1*H*-indole-2-carboxylate (5a): Silver(II) oxide (0.12 g) and nitric acid (6N, 0.29 mL) were added at room temperature to a solution of indole **2** (0.10 g, 0.28 mmol) in THF (2.5 mL), and the reaction mixture was stirred for 4 min and was then quenched with water (1 mL) and extracted with dichloromethane. The solvent was removed and the residue was purified by column chromatography on silica gel with dichloromethane as eluent to give compound **5a** (0.074 g, 82%). M.p. 134 °C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 1710, 1690, 1650 (CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.39 (t, J = 7.3 Hz, 3 H, OCH_2CH_3), 3.84 (t, J = 6.2 Hz, 2 H, CH_2CH_2Cl), 4.36 (q, J = 7.3 Hz, 2 H, OCH_2CH_3), 5.24 (t, J = 6.2 Hz, 2 H, CH_2CH_2Cl), 6.67 (dd, J = 10.2 Hz, 2 H, 5-H and 6-H), 7.33 (s, 1 H, 3-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.8, 43.5, 47.8, 62.2, 115.4, 125.9, 129.7, 132.6, 138.0, 138.6, 160.9, 179.2, 182.9. $C_{13}H_{12}ClNO_4$ (281.695): calcd. C 55.43, H 4.29, N 4.97; found C 55.56, H 4.32, N 4.87.

Ethyl 1-(2-Bromoethyl)-4,7-dioxo-4,7-dihydro-1*H*-indole-2-carboxylate (5b): Silver(II) oxide (0.10 g) and nitric acid (6 N, 0.25 mL) were added at room temperature to a solution of indole **2** (0.10 g, 0.28 mmol) in THF (2 mL), and the reaction mixture was stirred for 4 min, quenched with water (1 mL), and extracted with dichloromethane. The solvent was removed and the residue was purified by column chromatography on silica gel with dichloromethane as eluent to give compound **5b** (0.090 g, 99%). M.p. 141−141.5 °C.

IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 1705, 1660 (CO). ^1H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.38 (t, J = 7.1 Hz, 3 H, OCH_2CH_3), 3.65 (t, 2 H, J = 6.7 Hz, N-CH_2CH_2Br), 4.35 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 5.24 (t, J = 6.7 Hz, 2 H, N-CH_2CH_2Br), 6.66 (dd, J = 10.3 Hz, 2 H, 5-H and 6-H), 7.30 (s, 1 H, 3-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.2, 29.9, 47.1, 61.6, 114.7, 125.2, 128.9, 131.7, 137.4, 137.9, 160.3, 178.5, 182.2. $C_{13}H_{12}BrNO_4$ (326.147): calcd. C 47.87, H 3.71, N 4.29; found C 47.88, H 3.56, N 4.42.

3,4-Dihydropyrazino[1,2-*a*]indol-1,6,9(2*H*)-trione (6): Silver(II) oxide (0.14 g) and nitric acid (6 N 0.35 mL) were added at −10 °C to a solution of indole **3** (0.10 g, 0.38 mmol) in THF/acetone (10:2) (4 mL), and the reaction mixture was stirred for 2 min and worked up and purified as described above, but with ethyl acetate as eluent, to give compound **6** (50 mg, 56%). M.p. 263−264°C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3145 (NH), 1670, 1666 (CO). ^1H NMR (300 MHz, [D_6]DMSO): δ [ppm] = 3.59 (t, J = 6.0 Hz, 2 H, 3-H), 4.51 (t, J = 6.0 Hz, 2 H, 4-H), 6.75 (dd, J = 10.5 Hz, 2 H, 7-H and 8-H), 6.95 (s, 1 H, 10-H), 8.33 (s, 1 H, NH). ^{13}C NMR (75 MHz, [D_6]DMSO): δ [ppm] = 39.0, 42.8, 108.1, 124.5, 128.9, 130.4, 137.4, 137.5, 158.4, 178.2, 182.2. $C_{11}H_8N_2O_3$ (216.196): calcd. C 61.11, H 3.77, N 12.96; found C 61.10, H 3.57, N 12.75.

1,2,3,4-Tetrahydropyrazino[1,2-*a*]indol-6,9-dione (7): Silver(II) oxide (0.06 g) and nitric acid (6 N, 0.35 mL) were added at room temperature to a solution of indole **4** (0.50 g, 0.21 mmol) in THF (1.5 mL) and the reaction mixture was stirred for 6 min and worked up and purified as described above, with ethyl acetate as eluent, to give compound **7** (15 mg, 35%). M.p. 149−149.5°C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3357 (NH), 1665, 1630 (CO). ^1H NMR (300 MHz, [D_6]acetone): δ [ppm] = 3.97 (q, J = 5.2 Hz, 2 H, 3-H), 4.50 (t, J = 5.2 Hz, 1 H, NH), 4.62 (t, J = 5.2 Hz, 2 H, 4-H), 4.79 (d, J = 5.2 Hz, 2 H, 1-H), 6.57 (s, 1 H, 10-H), 6.63 (s, 2 H, 7-H and 8-H). ^{13}C NMR (75 MHz, [D_6]acetone): δ [ppm] = 49.1, 56.4, 62.4, 107.5, 127.2, 130.3, 137.6, 138.7, 144.2, 178.7, 184.1. $C_{11}H_{10}N_2O_2$ (202.213): calcd. C 65.34, H 4.98, N 13.85, found C 65.31, H 5.12, N 13.82.

Generation of *o*-QDM 9 and Its Trapping with Quinones 5−7. General Procedure: A solution of 4-(bromomethyl)-5-(dibromomethyl)-1,3-thiazole (**8**)[9] (0.216 g, 0.6 mmol) in dry DMF (2.0 mL) was slowly added to a stirred and heated (60°C) solution of the corresponding quinone (0.5 mmol) and NaI (5 equiv.) in DMF (3 mL). Stirring and heating were maintained for 1 h. After cooling, the precipitate was filtered off and washed with water and then with ethyl acetate.

Ethyl 6-(2-Bromoethyl)-5,9-dioxo-6,9-dihydro-5*H*-indolo[6,5-*f*][1,3]-benzothiazole-7-carboxylate (10a) and Ethyl 6-(2-Bromoethyl)-5,9-dioxo-6,9-dihydro-5*H*-indolo[5,6-*f*][1,3]benzothiazole-7-carboxylate (10b): The crude mixture of compounds **10** was purified by column chromatography to afford 0.80 g (29%) of **10a** + **10b**. The regioisomers **10a** and **10b** were separated (dichloromethane/ethyl acetate, 19:1) to give **10a** (0.50 g, 18.3%) and **10b** (0.30 g, 10.7%). Compound **10a** (major regioisomer, R_f = 0.27): M.p. 241 °C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 1710, 1660 (CO). ^1H NMR (500.13 MHz, $CDCl_3$): δ [ppm] = 1.47 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 3.86 (t, J = 6.8 Hz, 2 H, CH_2-CH_2Br), 4.46 (q, J = 7.0 Hz, 2 H, OCH_2CH_3), 5.48 (t, J = 6.8 Hz, 2 H, CH_2-CH_2Br), 7.56 (s, 1 H, 8-H), 8.86 (s, 1 H, 10-H), 8.94 (s, 1 H, 4-H), 9.28 (s, 1 H, 2-H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ [ppm] = 14.6, 30.4, 47.9, 62.1, 115.9, 122.3, 123.4, 128.0, 130.7, 131.1, 134.2, 139.6, 156.5, 159.0, 160.7, 176.8, 179.8, $C_{18}H_{13}BrN_2O_4S$ (433.282): calcd. C 49.90, H 3.02, Br 18.44, N 6.47, S 7.40; found C 49.54, H 3.00, Br 18.32, N 6.37, S 7.41. Compound **10b** (minor regioisomer, R_f = 0.38): M.p. 204 °C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 1710, 1660 (CO). ^1H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.39 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 3.74 (t, J = 6.6 Hz, 2 H, CH_2CH_2Br), 4.37 (q, J = 7.0 Hz, 2 H, OCH_2CH_3), 5.40 (t, J = 6.6 Hz, 2 H, CH_2CH_2Br), 7.49 (s, 1 H, 8-H), 8.79 (s, 1 H, 4-H), 8.88 (s, 1 H, 10-H), 9.21 (s, 1 H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.2, 29.9, 47.5, 61.7, 115.6, 122.2, 122.7, 128.0, 130.3, 133.1, 132.1, 138.9, 156.2, 158.7, 176.2, 179.5. $C_{18}H_{13}BrN_2O_4S$ (433.282): calcd. C 49.90, H 3.02, Br 18.44, N 6.47, S 7.40; found C 49.54, H 3.00, Br 18.32, N 6.37, S 7.41.

8,9-Dihydropyrazino[1′,2′:1,2]indolo[6,5-*f*][1,3]benzothiazole-5,10,12(7*H*)-trione (11a) and 8,9-Dihydropyrazino[1′,2′:1,2]indolo-[5,6-*f*][1,3]benzothiazole-5,10,12(7*H*)-trione (11b): Compounds **11a** + **11b** were obtained as an inseparable mixture in the form of a pale green solid (0.65 g, 40%) in a 1.4:1 ratio. M.p. > 300 °C. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3200 (NH), 1660, 1650, 1620 (CO). ^1H NMR (300 MHz, CF_3CO_2D): **11a**: δ [ppm] = 4.18 (m, J = 6.0 Hz, 2 H, 8-H), 5.13 (t, J = 6.0 Hz, 2 H, 7-H), 7.90 (s, 1 H, 11-H), 9.37 (s, 1 H, 13-H), 9.43 (s, 1 H, 4-H), 10.69 (s, 1 H, 2-H); **11b**: δ [ppm] = 4.18 (m, J = 6.0 Hz, 2 H, 8-H), 5.13 (t, J = 6.0 Hz, 2 H, 7-H), 7.89 (s, 1 H, 11-H), 9.34 (s, 1 H, 4-H), 9.38 (s, 1 H, 13-H), 10.68 (s, 1 H, 2-H). ^{13}C NMR (75 MHz, CF_3CO_2D): **11a**: δ [ppm] = 42.1, 45.1, 116.2, 120.9, 122.3, 126.9, 130.1, 132.3, 135.1, 135.7, 137.4, 138.1, 145.6, 168.9, 177.1, 182.6; **11b**: δ [ppm] = 42.1, 45.1, 116.1, 120.7, 122.3, 126.5, 130.1, 132.3, 135.1, 135.7, 137.4, 138.1, 145.6, 168.9, 177.0, 182.4. $C_{16}H_9N_3O_3S$ (323.332): **11a** + **11b**: calcd. C 59.44, H 2.81, N 13.00, S 9.92; found C 59.40, H 2.90, N 12.97, S 9.90.

7,8,9,10-Tetrahydropyrazino[1′,2′:1,2]indolo[6,5-*f*][1,3]benzothiazole-5,12-dione (12a) and 7,8,9,10-Tetrahydropyrazino[1′,2′:1,2]indolo[5,6-*f*][1,3]benzothiazole-5,12-dione (12b): Compounds **12a** + **12b** were obtained as an inseparable mixture in the form of an orange solid (0.70 g, 45%) in a 1.8:1 ratio. IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3420 (NH), 1655, 1650 (CO). ^1H NMR (400 MHz, [D_6]DMSO): **12a**: δ [ppm] = 3.78 (m, 2 H, 8-H), 4.54 (t, J = 5.2 Hz, 2 H, 7-H), 4.65 (s, 2 H, 10-H), 6.68 (s, 1 H, 11-H), 8.61 (s, 1 H, 13-H), 8.88 (s, 1 H, 4-H), 9.66 (s, 1 H, 2-H); **12b**: δ [ppm] = 3.78 (m, 2 H, 8-H), 4.54 (t, J = 5.2 Hz, 2 H, 7-H), 4.66 (s, 2 H, 10-H), 6.69 (s, 1 H, 11-H), 8.56 (s, 1 H, 4-H), 8.91 (s, 1 H, 13-H), 9.66 (s, 1 H, 2-H). ^{13}C NMR (75 MHz, [D_6]DMSO): **12a**: δ [ppm] = 49.2, 55.9, 62.4, 66.7, 97.3, 108.1, 122.0, 127.0, 131.5, 133.7, 139.7, 146.2, 156.9, 162.5, 175.5, 180.5; **12b**: δ [ppm] = 49.2, 55.9, 62.4, 66.7, 97.3, 108.1, 122.0, 127.0, 131.5, 133.7, 146.2, 156.9, 162.5, 175.5, 180.5. $C_{16}H_{11}N_3O_2S$ (309.348): **12a** + **12b**: calcd. C 62.12, H 3.58, N 13.58, S 10.37; found C 62.10, H 3.68, N 13.55, S 10.30.

Antileishmanial Activity: Antileishmanial activity against promastigotes of *Leishmania donovani* MHOM/ET/67/L82 and *Leishmania major* MHOM/PT/92/CRE26: LV9 was assessed in 96-well plates (Falcon) at 27°C using the CellTiter 96®AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega) colorimetric method. 10^5 parasites per mL were resuspended in fresh medium in 100-μL wells. The compound was dissolved in DMSO and then diluted at the appropriate concentration in the standard culture medium [RPMI 1640 medium (sigma) containing 20% fetal calf serum]. Median inhibitory concentrations (IC_{50}s) were determined after 48 h culture time, the drug being tested in serial fourfold dilution from 0.01 to 1 μM and six replicate cultures being set up at each concentration.

Assays of Cytotoxicity: Assays of cytotoxicity of the drugs were conducted on a human myelomonocytic cell line THP-1 (European collection of animal cell culture number 88081201: Sophia-Antipolis, France). These non-adherent cells were suspended in RPMI

1640 medium (DAP, Vogelgrun, France) supplemented with 100 U/mL of penicillin, 100 μg/mL of streptomycin and 10% fetal calf serum (DAP). The growth of THP-1 cells was assessed in 96-well plates at 37 °C by the method described above for parasites.

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