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Synthesis and Antiprotozoal Evaluation of Benzothiazolopyrroloquinoxalinones, Analogues of Kuanoniamine A

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Abstract—Boc-aminoethylindoloquinone **8**, a key intermediate for the building of pentacyclic quinoneimines, analogues of kuanoniamine A, was synthesized by alkylation of 4,7-dimethoxyindole **3** with 1,2-dibromoethane followed by transformation into azide, reduction of the latter with trimethylphosphine in the presence of 2-(*tert*-butoxycarbonyloximino)-2-phenylacetonitrile and oxydative demethylation of the Boc-amine **6** with silver(II) oxide. Quinone **8** was then treated in situ with the thiazole *o*-quinodimethane **10** to afford a regioisomeric mixture of the tetracyclic quinones **11**. Treatment of the mixture with trifluoroacetic acid and molecular sieves 4-Å provided the corresponding quinoneimines **12**. Separation of the regioisomers was performed by preparative thin-layer chromatography on silica gel. The structural assignment was made by 2D ^{1}H-^{13}C HMBC correlations performed on the less polar regioisomer **12b**. In vitro anti-leishmanial assays showed that the tested compounds possess a good potency towards two *Leishmania* sp. as well as against a virulent strain of *Toxoplasma gondii* and without any cytotoxicity against THP-1 cells.

Introduction

During the last two decades, many polycyclic and aromatic alkaloids, isolated from plants or marine sources, show various biological properties like antifungal, antimicrobial, antiviral or cytotoxic activities. Most of these derivatives possess in common a benzonaphthyridinone skeleton fused to a benzene and (or) an heterocyclic ring.[1] Among them, kuanoniamine A[2] (Scheme 1), a marine metabolite containing a thiazole ring inhibits in vitro the proliferation of KB cells and possesses antiviral properties.[3] Recently, the synthesis of structurally related pyrrolothiazolobenzoquinolinones was reported.[4b] However, evaluation of their biological activities was limited by their low solubility in usual solvents.

In a previous paper, we described the synthesis and antiprotozoal activity of pyrazinoindoloquinones,[5a] naphthofuranquinones and naphthothiophenquinones[5b] containing a fused benzothiazole ring. In continuation of our work, we aimed to synthesize new pentacyclic analogues of kuanoniamine A having in their structure a pyrroloquinoxalinone moiety. A retrosynthetic pathway for these quinoneimines is described in Scheme 1. The Diels–Alder trapping of a thiazole *o*-quinodimethane (*o*-QDM) with an appropriate indoloquinone would lead to the tetracyclic quinones which could be cyclized into the target quinoneimines for estimation of their antiprotozoal activities against virulent strains of *Leishmania* sp. and *Toxoplasma gondii*.

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Results and Discussion

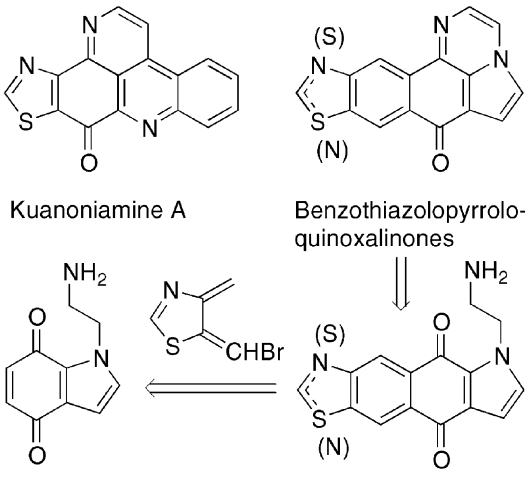
Chemistry

The Boc-aminoethylindoloquinone **8** is the key intermediate for the building of the pentacyclic framework. Its synthesis is described in Scheme 2. The preparation of 4,7-dimethoxyindole **3** from 2,5-dimethoxybenzaldehyde in 26–46% overall yields was previously reported.[4] Starting from the same aldehyde, we describe an efficient procedure which affords **3** in an improved yield (Scheme 2). Thus, treatment of 2,5-dimethoxybenzaldehyde with ethyl azidoacetate according to the Hemetberger–Knittel reaction[6] gave the intermediate ethyl 2-azido-3-(2,5-dimethoxyphenyl)-2-propenoate[7] which was cyclized into ethyl dimethoxyindole-2-carboxylate **1**.[5a,7] Hydrolysis of **1** with 10% sodium hydroxide followed by neutralisation with 6 M hydrochloric acid provided dimethoxyindole-2 carboxylic acid **2**. Then, decarboxylation of **2**, performed with copper and quinoline at 150–160 °C gave **3** in 63% overall yield calculated from 2,5-dimethoxybenzaldehyde.[8]

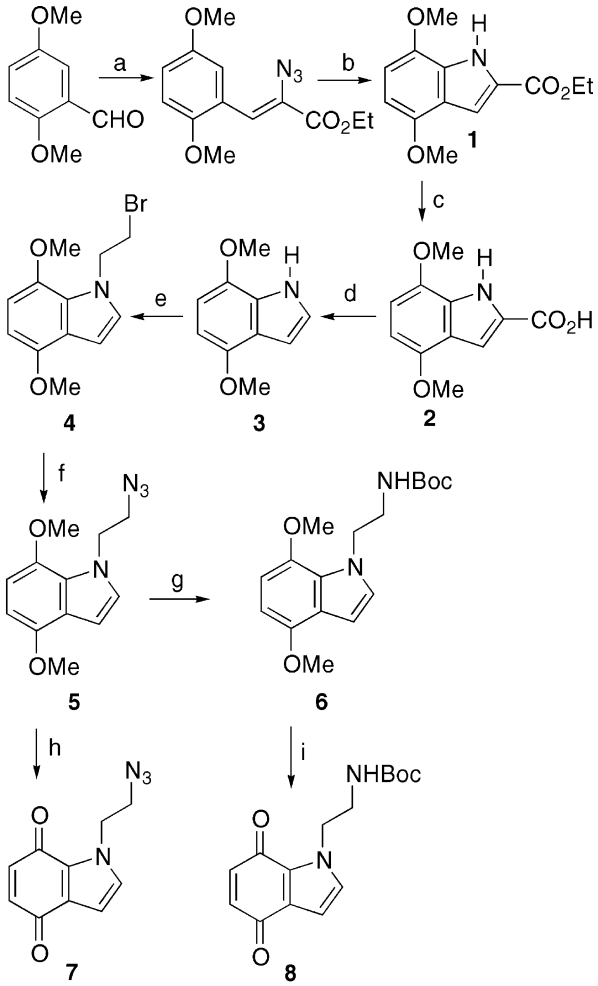
Treatment of **3** with 1,2-dibromoethane in the presence of benzyltributylammonium chloride (BTBAC) at reflux for 10 h afforded **4** which was transformed into **5** by the mean of sodium azide and BTBAC. Reaction of **5** with trimethylphosphine followed by addition of 2-(*tert*-butoxycarbonyloximino)-2-phenylacetonitrile (Boc-ON)[9] at −10 °C and stirring at room temperature for 2 h led to the desired Boc-amine **6**. Oxidative demethylation of the azide **5** and Boc-amine **6** with silver(II) oxide in the presence of 6 N nitric acid gave the corresponding quinones **7** and **8** in 81 and 82% yields, respectively.

Then, treatment of 4-(bromomethyl)-5-(dibromomethyl)-1,3-thiazole **9**[10] with sodium iodide in DMF generated 5-(bromomethylene)-4-methylene-4,5-dihydro-1,3-thiazole **10** which was trapped in situ with indoloquinone **8** (Scheme 3). The Diels–Alder reaction afforded directly the aromatized tetracyclic quinones **11a** and **11b** as an unseparable mixture of regioisomers with a ratio **11a**/**11b**: 52/48. Treatment of **11a** + **11b** with a mixture of dichloromethane/trifluoroacetic acid (4:1) at room temperature for 2 h and then, with molecular sieves (4Å) at reflux of absolute ethanol provided the corresponding quinoneimines **12a** and **12b** with the same ratio. Separation of the regioisomers was efficiently performed by preparative thin layer chromatography on silica gel using several successive elutions with a mixture of ethylacetate/chloroform (1:1).

To explain the weak regioselectivity observed for the trapping of *o*-QDM **10** with indoloquinone **8**, we calculate for the latter the LUMO molecular orbital coefficients by the density functional theory (DFT) method using the GAUSSIAN 98 package with a 3-21G* basis set. The calculations indicate that the larger orbital coefficient is located at 5-C (0.3066 for 5-C and 0.2743 for 6-C). On the other hand, the larger HOMO orbital coefficient for *o*-QDM **10** is situated at CHBr (0.305 for CHBr and 0.289 for CH_2). Therefore, the calculations predict a regioselective Diels–Alder reaction with **11a** as the major regioisomer. The fact that the cycloaddition reaction is weakly regioselective cannot be explained by FMO considerations.

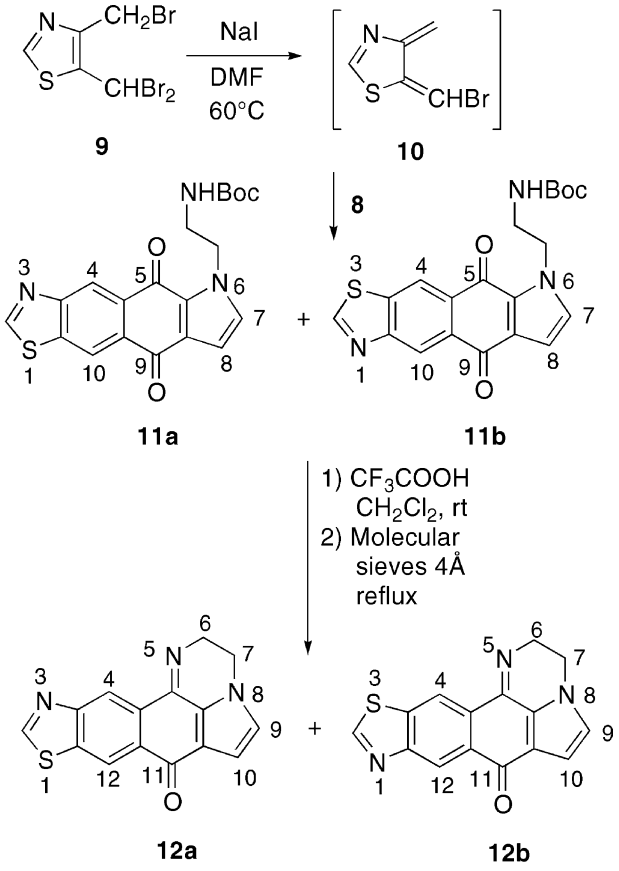


Scheme 1.



Scheme 2. Reagents and conditions: (a) Na, EtOH, $N_3CH_2CO_2Et$, −10 °C, rt, 2 h, 75%; (b) toluene, reflux, 5 h, 99%; (c) 10% NaOH, reflux, 1 h, 6 M HCl, 0 °C, 90%; (d) Cu, quinoline, N_2, 150–160 °C, 3 h, 95%; (e) Br–CH_2–CH_2–Br, NaOH, BTBAC, reflux, 10 h, 74%; (f) NaN_3, BTBAC, reflux, 20 h, 93%; (g) Me_3P, Boc-ON, −10 to 0 °C, 1 h, rt, 2 h, 76%; (h) AgO, HNO_3, rt, 15 min, 81%; (i) AgO, HNO_3, −10 °C, 1 min, 82%.

The structural assignment was made by 2D ^{1}H–^{13}C HMBC correlations performed on the less polar regioisomer of the quinonimine **12b** (Table 1). The use of the characteristic long-range ^{3}J coupling of the benzothiazole nucleus[3] let us to assign first 3a-C and 12a-C. Indeed, the ^{3}J coupling between 2-H and 12a-C appears as a doublet typical of a cross-peak through the nitrogen atom of the thiazole ring ($^{3}J_{12aC-2H} = 15$ Hz), while that between 2-H and 3a-C gives an unresolved singlet indicating a coupling of <5 Hz for $^{3}J_{3aC-2H}$. Then, three ^{3}J couplings: 4-H with 12a-C and 4b-C and 6-H with 4b-C allowed the structure of the regioisomer **12b**to be established. Finally, the two ^{3}J couplings: 12-H with 3a-C and 11-C confirmed this assignment.

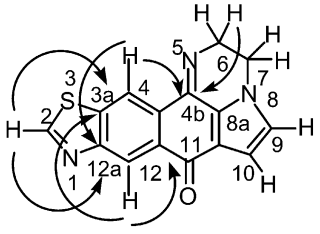


Scheme 3. Regioisomers ratio: **11a**/**11b** = 52/48; **12a**/**12b** = 52/48.

Biology

The in vitro antiprotozoal activities of compounds **1**–**8**, **11a** + **11b**, **12a** and **12b** were evaluated against promastigote forms of *Leishmania donovani* and *Leishmania major*, and against *T. gondii* (Table 2). The potential toxicities of these derivatives were also determined against an infected human myelomonocytic THP-1 cell line. Pen-

Table 1. 2D ^{1}H–^{13}C HMBC correlations for **12b** ($CDCl_3$, 500.13 and 125.78 MHz, δ ppm)



12b

Atom	^{13}C	^{1}H	HMBC [J(C,H)]			
			^{1}J	^{2}J	^{3}J	^{4}J
2	157.1	9.16	2-H			
3a	138.1				2-H, 12-H	
4	117.8	8.90	4-H			2-H
4a	130.8			4-H	12-H	
4b	151.8				4-H, 6-H	12-H
6	49.2	4.40	6-H	7-H		
7	42.5	4.19	7-H	6-H		
8a	129.0				7-H, 9-H, 10-H	6-H
9	126.0	6.90	9-H	10-H	7-H	
10	108.0	6.81	10-H	9-H		
11	179.8				12-H	4-H
11a	133.7			12-H	4-H	
12	123.5	9.06	12-H			
12a	155.4				2-H, 4-H	

Table 2. In vitro inhibitory activity against *Leishmania* sp. and *Toxoplasma gondii*, and cytotoxicity against THP-1 cells of the synthetized compounds[a]

Compd	*Leishmania donovani* IC_{50} (μM)	*Leishmania major* IC_{50} (μM)	*Toxoplasma gondii* IC_{50} (μM)	THP-1 cells IC_{50} (μM)
1	0.004	0.062	0.0011	0.096
2	0.0093	0.076	0.0007	0.011
3	0.0026	0.250	0.0007	0.392
4	0.0044	0.045	0.0021	0.011
5	0.0051	0.064	0.0034	0.099
6	0.007	0.008	0.0012	0.110
7	0.005	0.043	0.0031	0.817
8	0.0075	0.006	0.0021	0.081
11a + **11b**	0.0080	0.006	0.0008	0.075
12a	0.210	0.008	0.0051	0.615
12b	0.0060	0.007	0.0027	0.083
Pentamidine	0.0012	0.0018		0.0043
Pyrimethamine			0.0034	0.010
Spiramycin			0.0042	0.013
Sulfadiazine			0.0034	0.012

[a]The values are the means ± SD of triplicate experiments

tamidine for the anti-leishmanial assays and pyrimethamine, spiramycin, and sulfadiazine for anti-toxoplasmal tests, were used as the reference drugs.

Almost all the tested compounds are more active against *T. gondii* than the reference drugs and less toxic towards THP-1 cells excepted **2** and **4**. Moreover, compounds **1**, **2**, **4**–**8**, **11a** + **11b**, and **12b** exhibit significant activities against both strains of *Leishmania* sp. and *T. gondii*. Concerning the inhibitory activity towards *L. major*, it is noteworthy that the best compounds are those bearing a Boc-moiety **6**, **8**, **11a** + **11b** and the quinoneimine function (**12a** and **12b**). It seems, in these cases, that lipophilicity plays an important role in the antileishmanial activity. Among the quinoneimine regioisomers, we observed a large difference for their IC_{50} towards *L. donovani* and TPH-1 cells.

Conclusion

We have described an efficient procedure to obtain 4,7-dimethoxyindole in an improved yield. Then, alkylation of the latter followed by transformation into azide, reduction and oxidative demethylation afforded the new Boc-aminoethylindoloquinone **8**. A Diels–Alder trapping of the thiazole *o*-QDM **10** with quinone **8** gave the regioisomeric tetracyclic quinones **11**. Treatment of **11** with trifluoroacetic acid followed by addition of molecular sieves in refluxing ethanol provided the pentacyclic quinoneimines **12a** and **12b** containing benzothiazole and pyrroloquinoxaline rings as part of their structures. All the prepared compounds were evaluated in vitro against virulent strains of *Leishmania* sp. and *T. gondii*. Almost all of them show significant inhibitory activities against *L. donovani* and *T. gondii* with lower cytotoxicities against TPH-1 cells than the reference drugs. The best results towards *L. major* are observed with compounds bearing a Boc-moiety **6**, **8**, **11a** + **11b** and the pentacyclic quinoneimine **12b**.

Experimental

Chemical synthesis

Melting points were measured with a Stuart Scientific SMP3 apparatus and are uncorrected. IR spectra were recorded on a Bruker Vector 22 spectrophotometer using KBr discs. ^{1}H and ^{13}C NMR spectra were obtained on Bruker AM-200, AM-300 and DRX-500 spectrometers using tetramethylsilane as an internal reference. Column chromatography was performed on silica gel Merck 60 (70–230 mesh). Thin layer chromatography separations were performed on Merck Kieselgel 60 (70–230 mesh). Elemental analyses were carried out on a Fisons EA 1108 CHNS-O analyzer.

Compound **1** was prepared according to ref 5a. 4-(Bromomethyl)-5-(dibromomethyl)thiazole **9** was obtained by selective bromination of the commercially available 4,5-dimethylthiazole as previously reported.[10]

4,7-Dimethoxy-1*H*-indole-2-carboxylic acid (2). An aqueous solution of 10% sodium hydroxyde (45 mL) was added to indole **1** (3.0 g, 1.20 mmol). The mixture was heated to reflux for 1 h then, cooled in an ice-bath at 0 °C and neutralized with 6 M HCl. The precipitate was filtered, washed with water and dried. Compound **2** was obtained as a white solid (2.4 g, 90%). Mp 204–205 °C. IR (KBr) ν cm^{-1} 3370, 1550, 1525. ^{1}H NMR (DMSO-d_6, 200 MHz) δ: 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.38 (d, 1H, J = 8.3 Hz, 5-H or 6-H), 6.64 (d, 1H, J = 8.3 Hz, 6-H or 5-H), 7.02 (d, 1H, J = 2.2 Hz, 3-H), 11.70 (s, 1H, COOH), 12.80 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz) δ: 55.0, 55.5, 98.6, 104.3, 105.4, 119.4, 127.6, 129.0, 141.1, 147.6, 162.3. Anal. calcd for $C_{11}H_{11}NO_4$: C 59.73, H 5.01, N 6.33. Found: C 59.49, H 4.89, N 6.06.

4,7-Dimethoxy-1*H*-indole (3). A mixture of compound **2** (1.0 g, 4.52 mmol), quinoline (2.5 mL, 2.10 mmol) and copper (0.2 g) was heated under nitrogen at 150–160 °C for 3 h. After evaporation of the solvent, the residue was purified by column chromatography on silica gel with dichloromethane as eluent to give compound **3** as a white solid (0.76 g, 95%). Mp 118–119 °C (ref 11 119 °C). IR (KBr) ν cm^{-1} 3370, 1525, 1500. ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.95 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.42 (d, 1H, J = 8.3 Hz, 5-H or 6-H), 6.55 (d, 1H, J = 8.3 Hz, 6-H or 5-H), 6.66 (dd, 1H, J = 2.4 and 3.1 Hz, 3-H), 7.11 (t, 1H, J = 2.4 Hz, 2-H), 8.48 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 55.7, 98.8, 100.4, 101.5, 120.0, 122.6, 127.7, 141.2, 147.8.

1-(2-Bromoethyl)-4,7-dimethoxy-1*H*-indole (4). A mixture of indole **3** (100 mg, 0.56 mmol), 1,2-dibromoethane (1 mL), sodium hydroxide (70 mg, 1.75 mmol), and BTBAC (20 mg, 0.06 mmol) in dichloromethane (2 mL) was heated to reflux for 10 h. After filtration, the solid was washed with dichloromethane. The combined organic layers were evaporated and the residue was recrystallized from ethanol. Compound **4** was obtained as a white solid (118 mg, 74%). Mp 55–55.5 °C. IR (KBr) ν cm^{-1} 2950, 1525, 1500. ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.69 (t, 2H, J = 6.9 Hz, N-CH_2–$\underline{CH_2}$–Br), 3.92 (s, 6H, OCH_3), 4.70 (t, 2H, J = 6.9 Hz, N-$\underline{CH_2}$–CH_2–Br), 6.39 (d, 1H, J = 8.3 Hz, 5-H or 6-H), 6.53 (d, 1H, J = 8.3 Hz, 6-H or 5-H), 6.57 (d, 1H, J = 3.1, 3-H), 6.97 (d, 1H, J = 3.1 Hz, 2-H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 31.9, 50.8, 55.6, 55.8, 98.9, 99.1, 102.2, 121.9, 126.2, 128.2, 142.1, 147.8. Anal. calcd for $C_{12}H_{14}BrNO_2$: C 50.72, H 4.97, N 4.93. Found: C 50.57, H 5.17, N 4.94.

1-(2-Azidoethyl)-4-,7-dimethoxy-1*H*-indole (5). A mixture of indole **4** (250 mg, 0.88 mmol), sodium azide (4 eqiv), BTBAC (30 mg, 0.09 mmol) in toluene (5 mL) was heated to reflux for 20 h. After filtration, the solid was washed with dichloromethane. The combined organic layers were evaporated and the residue was purified by column chromatography on silica gel with dichloromethane as eluent. Compound **5** was obtained as a liquid (200 mg, 93%). IR (KBr) ν cm^{-1} 2100, 1525, 1500. ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.65 (t, 2H, J = 6.1 Hz, N-CH_2–$\underline{CH_2}$–N_3), 3.93 (s, 3H, OCH_3), 3.94 (s, 3H,

OCH_3), 4.53 (t, 2H, $J=6.1$ Hz, N–$\underline{CH_2}$–CH_2-N_3), 6.41 (d, 1H, $J=8.3$ Hz, 5-H or 6-H), 6.56 (d, 1H, $J=8.3$ Hz, 6-H or 5-H), 6.63 (d, 1H, $J=3.1$ Hz, 3-H), 6.96 (d, 1H, $J=3.1$ Hz, 2-H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 48.3, 52.7, 55.6, 98.8, 99.4, 102.2, 122.0, 126.3, 128.1, 142.2, 147.8. Anal. calcd for $C_{12}H_{14}N_4O_2$: C 58.53, H 5.73, N 22.75. Found: C 58.27, H 5.57, N 22.48.

1-(2-*tert*-Butylcarbamoylethyl)-4-,7-dimethoxy-1*H*-indole (6). Trimethylphosphine (0.84 mL, 1.05 equiv) was added under nitrogen to a stirred solution of indole **5** (200 mg, 0.813 mmol) in dry THF (8 mL). Stirring was continued at room temperature for 2 h. Then, a solution of Boc-ON (208 mg, 0.84 mmol) in dry THF (8 mL) was added to the reaction mixture cooled at −10 °C. Stirring was maintained at 0 °C for 1 h and then, at room temperature for 5 h. The mixture was extracted with ether (200 mL), washed with 2 M sodium hydroxide (3×50 mL), then, with water (2×50 mL) and dried over magnesium sulfate. Removing of the solvent left a residue which was purified by column chromatography on silica gel with dichloromethane as eluent. Compound **6** was obtained as an orange solid (197 mg, 76%). Mp 80 °C. IR (KBr) ν cm^{-1} 3442, 1714, 1697. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.41 (s, 9H, CH_3), 3.49 (m, 2H, $J=5.8$ Hz, N–CH_2–$\underline{CH_2}$–NHCOO–), 3.90 (s, 3H, OCH_3), 3.91 (s, 3H, $O\underline{CH_3}$), 4.47 (t, 2H, $J=5.8$ Hz, N-$\underline{CH_2}$–CH_2–NHCOO–), 4.60 (br s, 1H, NH), 6.37 (d, 1H, $J=8.4$ Hz, 5-H or 6-H), 6.51 (d, 1H, $J=8.4$ Hz, 6-H or 5-H), 6.54 (d, 1H, $J=2.9$ Hz, 3-H), 6.89 (d, 1H, $J=2.9$ Hz, 2-H). Anal. calcd for $C_{17}H_{24}N_2O_4$: C 63.73, H 7.55, N 8.74. Found: C 63.70, H 7.62, N 8.42.

1-(2-Azidoethyl)-1*H*-indole-4,7-dione (7). Silver(II) oxide (100 mg) and nitric acid (6 N, 0.25 mL) were added at room temperature to a solution of indole **5** (70 mg, 0.28 mmol) in THF (3 mL), and the reaction mixture was stirred for 15 min, quenched with water (0.5 mL), and extracted with dichloromethane. The organic phase was washed with 10% aqueous solution of sodium bicarbonate, the solvent was removed and the residue was purified by column chromatography on silica gel with dichloromethane as eluent. Compound **7** was obtained as a yellow liquid (50 mg, 81%). IR (KBr) ν cm^{-1} 2100, 1700, 1650, 1590. ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.66 (t, 2H, $J=5.4$ Hz, N–CH_2–$\underline{CH_2}$–N_3), 4.43 (t, 2H, $J=5.4$ Hz, N–$\underline{CH_2}$–CH_2–N_3), 6.49 (d, 1H, $J=10.2$ Hz, 5-H or 6-H), 6.55 (d, 1H, $J=10.2$ Hz, 6-H or 5-H), 6.56 (d, 1H, $J=2.7$ Hz, 3-H), 6.93 (d, 1H, $J=2.7$ Hz, 2-H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 48.3, 51.2, 107.5,127.4, 128.3, 130.6, 137.0, 137.1, 177.9, 183.0. Anal. calcd for $C_{10}H_8N_4O_2$: C 55.55, H 3.73, N 25.91. Found: C 55.61, H 3.78, N 25.89.

1-(2-*tert*-Butylcarbamoylethyl)-1*H*-indole-4,7-dione (8). Silver(II) oxide (55 mg) and nitric acid (6 M, 1 mL) were added at −10 °C to a solution of indole **6** (50 mg, 0.156 mmol) in THF (33 mL), and the reaction mixture was stirred for 1 min, quenched with water (0.5 mL) at −10 °C, and extracted with dichloromethane. The organic phase was washed with 10% aqueous solution of sodium bicarbonate, the solvent was removed and the residue was purified by column chromatography on silica gel with a mixture of dichloromethane/ethylacetate (3:1) as eluent. Compund **8** was obtained as a yellow solid (37 mg, 82%). Mp 145 °C. IR (KBr) ν cm^{-1} 3384, 1687, 1665, 1652. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.42 (s, 9H, CH_3), 3.51 (m, 2H, $J=6.0$ Hz, N–CH_2–$\underline{CH_2}$–NHCOO–), 4.47 (t, 2H, $J=6.0$ Hz, N–$\underline{CH_2}$–CH_2–NHCOO–), 4.70 (br s, 1H, NH), 6.58 (dd, 2H, $J=10.2$ Hz, 5-H and 6-H), 6.61 (d, 1H, $J=2.8$ Hz, 3-H), 6.88 (d, 1H, $J=2.8$ Hz, 2-H). HRMS Calcd. for $C_{15}H_{19}N_2O_4$: 291.13448. Found: 291.13407 (MH^+).

6-(2-*tert*-Butylcarbamoylethyl)-5,9-dioxo-6,9-dihydro-5*H*-indolo[6,5-*f*][1,3]benzothiazole (11a) and 6-(2-*tert*-butylcarbamoylethyl)-5,9-dioxo-6,9-dihydro-5*H*-indolo[5,6-*f*][1,3]benzothiazole (11b). A solution of 4-(bromomethyl)-5-(dibromomethyl)thiazole **9**[9] (0.216 g, 0.6 mmol) in dry DMF (2.0 mL) was slowly added to a stirred and heated solution, at 60 °C, of quinone **8** (145 mg, 0.5 mmol) and NaI (5 equiv) in DMF (3 mL). Stirring and heating were maintained for 2 h. After cooling, water (50 mL) and 10% aqueous solution of sodium bisulfite were added to eliminate excess of iodine. Then, the mixture was extracted with ethyl acetate (2×30 mL), washed with water (2×30 mL) and dried over magnesium sulfate. Removing of the solvent left a residue which was purified by column chromatography on silica gel with dichloromethane/ethyl acetate (1:1) as eluent to afford an unseparable mixture of **11a** + **11b** as a yellow solid (119 mg, 61%; ratio **11a**/**11b**: 52/48). Mp 122 °C. IR (KBr) ν cm^{-1} 3367, 1720, 1681, 1650. ^{1}H NMR ($CDCl_3$, 300 MHz): **11a** δ: 1.42 (s, 9H, CH_3), 3.47–3.64 (m, 2H, N–CH_2–$\underline{CH_2}$–NHCOO–), 4.50–4.70 (m, 2H, N-$\underline{CH_2}$–CH_2–NHCOO–), 4.93 (br s, 1H, NH), 6.76 (d, 1H, $J=2.7$ Hz, 8-H), 6.97 (d, 1H, $J=2.7$ Hz, 7-H), 8.73 (s, 1H, 4-H), 8.77 (s, 1H, 10-H), 9.17 (d, 1H, 2-H). **11b** δ: 1.42 (s, 9H, CH_3), 3.47–3.64 (m, 2H, N–CH_2–$\underline{CH_2}$–NHCOO–), 4.50–4.70 (m, 2H, N–$\underline{CH_2}$–CH_2–NHCOO–), 5.07 (br s, 1H, NH), 6.77 (d, 1H, $J=2.7$ Hz, 8-H), 6.98 (d, 1H, $J=2.7$ Hz, 7-H), 8.71 (s, 1H, 4-H), 8.83 (s, 1H, 10-H), 9.19 (d, 1H, 2-H). Anal. calcd for $C_{20}H_{19}N_3O_4S$: C 60.44, H 4.82, N 10.57, S 8.07. Found: C 60.43, H 4.80, N 10.60, S 8.08.

6,7-Dihydrobenzothiazolo[6,5-*h*]pyrrolo[1,2,3-*de*]quinoxaline-11-one (12a) and 6,7-dihydrobenzothiazolo[5,6-*h*]pyrrolo[1,2,3-*de*]quinoxaline-11-one (12b). A mixture of quinones **11a** + **11b** (100 mg, 0.25 mmol) was added under stirring at room temperature to a solution of dichloromethane/trifluoroacetic acid (4:1) (10 mL). Stirring was maintained for 2 h. The reaction mixture was concentrated under vacuum, absolute ethanol (10 mL) and molecular sieves (4 Å) were added. Then, the mixture was heated to reflux under nitrogen for 8 h. Removing of the solvent afforded a mixture of quinoneimines **12a** + **12b** as a yellow solid (22 mg, 31%; ratio **12a**/**12b**: 52/48 from the ^{1}H NMR spectrum of the mixture). Separation of the regioisomers was realized by preparative thin-layer chromatography on silica gel with 10 successive elutions with ethyl acetate: chloroform (1:1) as eluent. **12a**: $R_f=0.43$ (chloroform/ethanol, 19:1). Mp >300 °C. IR (KBr) ν cm^{-1} 1649. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.18 (t, 2H, $J=6.7$ Hz, 7-H), 4.41 (t, 2H, 6.7 Hz, 6-H), 6.79 (d, 1H, $J=2.8$ Hz, 10-H), 6.89

(d, 1H, $J=2.8$ Hz, 9-H), 8.94 (s, 1H, 12-H), 9.0 (s, 1H, 4-H), 9.18 (s, 1H, 2-H).12b: $R_f=0.49$ (chloroform/ethanol, 19:1). Mp $>300\,^{\circ}C$. IR (KBr) ν cm^{-1} 1656. ^1H NMR ($CDCl_3$, 300 MHz) δ: 4.17 (t, 2H, $J=6.8$ Hz, 7-H), 4.38 (t, 2H, 6.8 Hz, 6-H), 6.79 (d, 1H, $J=2.8$ Hz, 10-H), 6.88 (d, 1H, $J=2.8$ Hz, 9-H), 8.87 (s, 1H, 4-H), 9.03 (s, 1H, 12-H), 9.14 (s, 1H, 2-H). HRMS Calcd. for $C_{15}H_9N_3OS$: 279.04663. Found: 279.04664.

Anti-leishmanial activity against promastigotes of *L. donovani* MHOM/ET/67/L82 and *L. major* MHOM/PT/92/CRE26: LV9 was assessed in 96-well plates (Falcon) at 27 °C using CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega), colorimetric method. 10^5 parasites were resuspended in fresh medium/well. 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}) were determined after 48 h of culture, the drug being tested as a serial 4-fold dilution from 0.01 to 1 μM and six replicate cultures being set up at each concentration.

Growth inhibition of *T. gondii*: the virulent RH strain of *T. gondii* was maintained in culture with the human myelomonocytic cell line THP-1 (ECACC number 88081201, Sophia-Antipolis, France) as previously described.[12] Three different experiments were performed in quadruplicate.

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 using the method described above for parasites.

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