Synthesis and in Vitro Antiprotozoal Activities of Water-Soluble, Inexpensive 3,7-Bis(dialkylamino)phenoxazin-5-ium Derivatives

Jian-Feng Ge,[†,‡] Chika Arai,[†] Marcel Kaiser,[§] Sergio Wittlin,[§] Reto Brun,[§] and Masataka Ihara*[,†]

Institute of Medicinal Chemistry, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan, Department of Material and Chemistry, Soochow University, Suzhou 215006, China, and Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland

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3,7-Bis(dialkylamino)phenoxazinium salts were synthesized and evaluated for in vitro activities against *Plasmodium falciparum*, *Trypanosoma cruzi, T. brucei rhodesiense*, and *Leishmania donovani*. Notably, the compounds showed potent antiprotozoal activities, especially against *P. falciparum* and *T. cruzi*. The compounds with alkyl side chains less than three carbons in length possessed good activities with high selective indices.

Introduction

Tropical diseases that result from infection by parasitic protozoa, such as malaria, Chagas disease, leishmaniasis, and African trypanosomiasis, are serious threats to public health. Malaria, which is caused by the *Plasmodium* parasites, exists in 100 countries. Over 300 million patients are infected with malaria, and two children are killed by the parasite each minute.[1] In many parts of the world, the *Plasmodium* parasites have developed resistance to a number of antimalarial medicines.[2]

Chagas disease, which is caused by *Trypanosoma cruzi*, is mainly endemic in South and Latin America. Chagas disease is a very serious public health problem in several countries, with 18 million people known to be infected with the parasite and an additional 100 million at risk of infection. Benznidazole is used as a medicine for Chagas disease, but the drug has low efficacy and high acute toxicity. Actually, there is no effective treatment for the prevalent chronic form of Chagas disease.[3]

Over 10 million patients are infected by leishmaniasis.[4] Pentostam, amphotericin B, and miltefosine are used to treat leishmaniasis, but these are highly toxic or very expensive compounds. African trypanosomiasis has reappeared in several areas over the past 30 years. Most medicines for this disease, such as melarsoprol, have been used for 60 years but have substantial side effects.[5,6] Developing countries have many patients infected by parasitic protozoa. Thus, the identification of effective, low-toxicity, and inexpensive antiprotozoal candidates is an important challenge for chemists and pharmacists.

In our previous attempts to develop synthetic antimalarial compounds, we reported that rhodacyanine and phenoxazinium derivatives exhibit strong in vitro activities against *P. falciparum*.[7–11] Phenoxazinium derivatives showed good efficacies by oral administration.[11] However, the development of 3,7-bis(dialkylamino)phenoxazinium derivatives as medicines is difficult because these compounds typically are purified with the aid of zinc chloride.[12] Therefore, the reactions of *m*-aminophenols and *p*-nitrosoanilines in perchloric acid gave phenoxaziniums in very poor yields (2−40%).[11] Recently, we found that symmetric and asymmetric 3,7-bis(dialkylamino)phenoxazinium derivatives with high purity could be obtained by chromatography followed by crystallization.[13] In the present study, 3,7-bis(dialkylamino)phenoxazinium derivatives were synthesized and tested against various parasites in in vitro assays. Remarkably, some of the compounds showed potent activities against the chloroquine- and pyrimethamine-resistant strain of *P. falciparum* and against *T. cruzi*.

Results and Discussion

Chemistry. The synthesis of intermediates of 3,7-bis(dialkylamino)phenoxaziniums is illustrated in Scheme 1. 3-(Pyrrolidin-1-yl)phenol **3b** and 3-(piperidin-1-yl)phenol **3c** were obtained by cleavage of the corresponding ethers **2b**,**c**,[14] which can be prepared easily by the palladium-catalyzed reaction[15] or by the reaction of 3-methoxyaniline **4** with dibromoalkane.[16] *N,N*-Dipropylaminophenol **3a** could be prepared easily by N-alkylation of 3-aminophenol,[17] and *N,N*-dialkylaminophenols **3f**−**h** were obtained in the same manner. *N,N*-Dialkyl-3-methoxy-4-nitrosoanilines **4a**−**e** were prepared by the reaction of **2a**−**e** with sodium nitrate in the presence of hydrochloric acid. 3-Methoxy-*N,N*-dipropylaniline **2a** and 4-(3-methoxyphenyl)morpholine **2d** were obtained by the reactions of the corresponding *N,N*-dialkylaminophenols and methyl *p*-toluenesulfonate in the presence of sodium hydroxide in acetonitrile.[13]

3,7-Bis(dialkylamino)phenoxazinium salts **5a**−**s** were afforded by the reaction of *N,N*-dialkylaminophenols **3a**−**h** and *N,N*-dialkyl-3-methoxy-4-nitrosoanilines **4a**−**e** in acidic solutions (Scheme 2).[13] **5a**−**s** were purified by chromatography with a short silica gel column and eluted with a $CHCl_3$/MeOH gradient from 10:1 to 10:3 (v/v). After evaporation of the solvents, the remaining crude products were crystallized by treatment with ethyl alcohol and then with ethyl acetate to remove impurities.

Biology. Antiprotozoal and cytotoxic activities of the 3,7-bis(dialkylamino)phenoxazinium salts are shown in Table 1. Comparison of the IC_{50} values of **5d**, **5h**, **5i**, and **5j** with the IC_{50} values of the other compounds suggests that the presence of a morpholino group in **5d**, **5h**, **5i**, and **5j** might increase the polarity and hydrophilicity of those compounds, thereby decreasing their activities. However, the introduction of a morpholino group substantially reduced the toxicity, which suggests that increased polarity and hydrophilicity result in decreased toxicity. This phenomenon was indicated by the high toxicity and low selectivity of **5p**−**s**, in which long alkyl chains led to increased lipophilicity of the compounds.

* To whom correspondence should be addressed. Phone and fax: +81-03-5498-6391. E-mail: m-ihara@hoshi.ac.jp.
† Hoshi University.
‡ Soochow University.
§ Swiss Tropical Institute.

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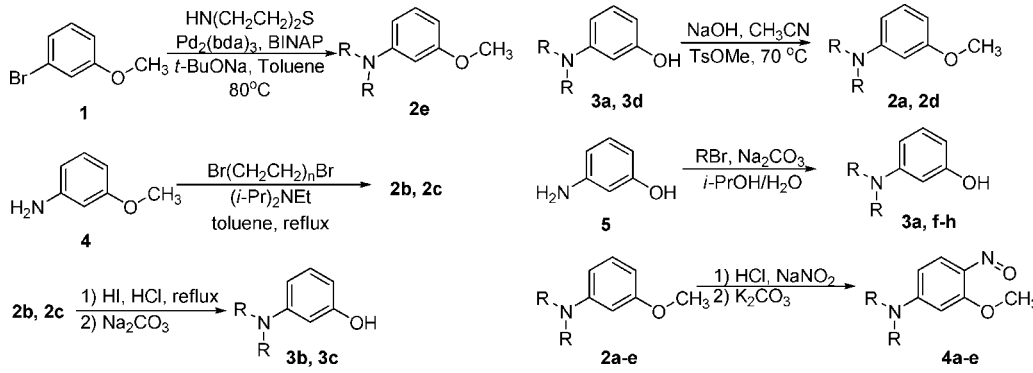
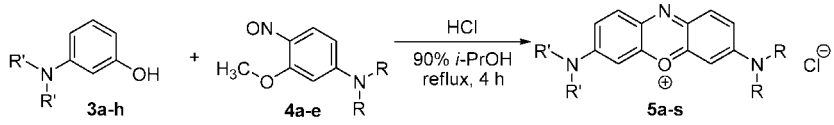
Scheme 1. Synthesis of *N,N*-Dialkylaminophenol and *N,N*-Dialkyl-3-methoxy-4-nitrosoaniline



a: R= *n*-C_3H_7; **b**: R, R= $(CH_2)_4$; **c**: R, R= $(CH_2)_5$; **d**: R, R= $(CH_2)_2O(CH_2)_2$; **e**:$(CH_2)_2S(CH_2)_2$; **f**: R= *n*-C_4H_9; **g**: R= *n*-C_5H_{11}; **h**: R=*n*-C_6H_{13}

Scheme 2. Synthesis of 3,7-Bis(dialkylamino)phenoxazinium Salts



Entry	R'	R	3a-g	4a-e	5a-s	yield (%)
1	R'= *n*-C_3H_7	R= *n*-C_3H_7	**3a**	**4a**	**5a**	60
2	R', R'= $(CH_2)_4$	R, R= $(CH_2)_4$	**3b**	**4b**	**5b**	63
3	R', R'=$(CH_2)_5$	R, R=$(CH_2)_5$	**3c**	**4c**	**5c**	60
4	R, R=$(CH_2)_2O(CH_2)_2$	R, R=$(CH_2)_2O(CH_2)_2$	**3d**	**4d**	**5d**	51
5	R'= CH_3	R, R= $(CH_2)_4$	**3h**	**4b**	**5e**	70
6	R'= C_2H_5	R, R= $(CH_2)_4$	**3i**	**4b**	**5f**	84
7	R'= *n*-C_3H_7	R, R= $(CH_2)_4$	**3a**	**4b**	**5g**	63
8	R'= CH_3	R, R=$(CH_2)_2O(CH_2)_2$	**3h**	**4d**	**5h**	40
9	R'= C_2H_5	R, R=$(CH_2)_2O(CH_2)_2$	**3i**	**4d**	**5i**	58
10	R'= *n*-C_3H_7	R, R=$(CH_2)_2O(CH_2)_2$	**3a**	**4d**	**5j**	35
11	R'= CH_3	R, R=$(CH_2)_2S(CH_2)_2$	**3h**	**4e**	**5k**	44
12	R'= C_2H_5	R, R=$(CH_2)_2S(CH_2)_2$	**3i**	**4e**	**5l**	68
13	R'= *n*-C_3H_7	R, R=$(CH_2)_2S(CH_2)_2$	**3a**	**4e**	**5m**	55
14	R'= CH_3	R, R=$(CH_2)_5$	**3i**	**4c**	**5n**	27
15	R'= C_2H_5	R, R=$(CH_2)_5$	**3j**	**4c**	**5o**	28
16	R'= *n*-C_3H_7	R, R=$(CH_2)_5$	**3a**	**4c**	**5p**	51
17	R'= *n*-C_4H_9	R, R=$(CH_2)_5$	**3f**	**4c**	**5q**	50
18	R'= *n*-C_5H_{11}	R, R=$(CH_2)_5$	**3g**	**4c**	**5r**	54
19	R'= *n*-C_6H_{13}	R, R=$(CH_2)_5$	**3h**	**4c**	**5s**	51

Compounds with a short alkyl chain (e.g., methyl or ethyl) had high selectivity, low toxicity, and high activity. However, when the number of carbon atoms exceeded three (propyl or longer chains), the selectivity decreased and the toxicity increased dramatically. Taking into consideration activity and toxicity, the compounds with a short alkyl chain (methyl and ethyl) are good antiprotozoal candidates, whereas the introduction of long alkyl chains or morpholino groups is not recommended. Symmetrical and asymmetrical structures did not have different activities, but a bulky wing structure affected toxicity and efficacy. We hypothesize that the active center of these drugs might be the central tricyclic moiety and the flattened structure is essential. Thus, the introduction of bulky groups might increase toxicity or decrease activity.

Most 3,7-bis(dialkylamino)phenoxaziniums, with the exceptions of **5d**, **5h**, **5i**, and **5p**−**s**, showed good activities (in the nanomolar region) against *P. falciparum*. Compounds **5j**−**l**, **5n**, and **5o** had good selective indices. Phenoxazinium derivatives had particularly potent activity against *T. cruzi*. Nine compounds including basic blue 3, **5a**−**c**, **5f**, **5g**, and **5l**−**o** showed good activities against *T. cruzi*, with IC_{50} of 0.036−0.174 μM and selectivity of 32−185. In the in vitro assays against *T. brucei*

Table 1. Antiprotozoal and Cytotoxic Activities (IC_{50}, μM) of 3,7-Bis(dialkylamino)phenoxazinium salts (**5a**−**s**)[a]

	P. falc. K1		*T. cruzi*		*T.b. rhod.*		*L. don.*, axenic		
compd	IC_{50}	SI[b]	IC_{50}	SI[b]	IC_{50}	SI[b]	IC_{50}	SI[b]	cytotox L6 IC_{50}
standard[c]	0.148	NT[d]	0.866	NT[d]	0.006	NT[d]	0.280	NT[d]	00.02
basic blue 3[e]	0.003	2410.0	0.036	185.4	0.228	29.4	0.214	31.3	06.70
5a	0.002	165.0	0.017	23.6	0.023	17.4	0.002	165.0	00.40
5b	0.003	1400.0	0.121	32.6	0.160	24.6	0.037	107.7	03.93
5c	0.005	1050.0	0.081	67.7	0.154	35.6	1.136	4.8	05.47
5d	0.431	66.5	7.090	4.0	8.735	3.3	5.182	5.5	28.62
5e	0.006	2166.5	0.258	51.0	0.091	144.4	1.710	7.7	13.14
5f	0.006	1390.0	0.056	139.0	0.168	46.3	0.654	11.9	07.77
5g	0.003	355.0	0.036	25.4	0.016	59.2	0.016	59.2	00.92
5h	0.023	2153.9	5.060	9.8	2.594	19.2	1.055	47.2	49.83
5i	0.029	1363.6	2.942	13.6	4.065	9.9	16.315	2.5	40.12
5j	0.005	3883.0	0.406	47.6	0.871	22.2	0.754	25.6	19.32
5k	0.006	4022.5	0.608	36.6	5.471	4.1	14.369	1.5	22.23
5l	0.008	2822.0	0.154	141.1	1.613	13.5	1.005	21.6	21.71
5m	0.007	976.7	0.163	43.1	1.510	4.6	1.170	6.0	07.01
5n	0.003	7740.0	0.174	129.0	0.843	26.7	1.655	13.6	22.51
5o	0.003	2390.0	0.048	132.8	0.245	26.3	1.439	4.5	06.43
5p	0.008	105.3	0.023	35.1	0.238	3.3	0.098	8.1	00.79
5q	0.007	12.3	0.058	1.5	0.269	0.3	0.002	37.0	00.09
5r	0.002	63.0	0.015	9.0	0.022	6.3	0.002	63.0	00.14
5s	0.004	48.0	0.021	9.6	0.014	13.7	0.014	13.7	00.20

[a] Values indicate the inhibitory concentration of a compound or standard in μM that is necessary to achieve 50% growth inhibition (IC_{50}). Data shown are values from two replicate experiments. [b] Selectivity index = (IC_{50} for L6)/(IC_{50} for *P. falciparum, T.b. rhodesiense, T. cruzi,* or *L. donovani*). [c] Standards: chloroquine (*P. falciparum*), melarsoprol (*T.b. rhodesiense*), benznidazole (*T. cruzi*), miltefosine (*L. donovani*), and podophyllotoxin (L6 cells, cytotoxicity). [d] Not tested. [e] 3,7-Bis(diethylamino)phenoxazinium chloride, 99% purity.[13]

rhodense and *Leishmania donovani*, only **5b** showed reasonable activity against *L. donovani*.

Conclusion

3,7-Bis(dialkylamino)phenoxazinium salts were synthesized and evaluated for effectiveness against various parasitic protozoa by in vitro assays. A number of derivatives showed substantial activities against the chloroquine- and pyrimethamine-resistant strain of *P. falciparum* as well as against *T. cruzi*.

Experimental Section

Chemistry. General Details. Starting materials were obtained from Wako and Aldrich Company and used as received. Melting points were determined on a Yanaco apparatus and are uncorrected. NMR spectra were recorded on a Jeol-270, Bruker-400, or Vaian-500 spectrometer. TMS was used as an internal standard for ^{1}H NMR, and the solvent peak was used as an internal standard for ^{13}C NMR. Absorption spectra were taken on Jasco V-550 UV−vis spectrophotometer. IR spectra were determined on a JASCO FT/IR-4100. LC−MS (ESI^+) spectra were recorded with a Shimadzu LCMS-2010EV spectroscope. The elemental analysis was performed with Yanano CHN Corder MT-5 element analyzer.

General Method of Preparation of 3,7-Bis(Dialkylamino)phenoxazin-5-iums 5a, 5c, 5e, 5g−s. A mixture of *N,N*-dialkylaminophenol (**3a**−**g**, 1 mmol) and 90% *i*-PrOH (20 mL) was stirred at 70 °C in a 50 mL two-neck bottle with distilling apparatus filled with argon. A suspended solution of *N,N*-dialyl-3-methoxy-4-nitrosoaniline (**4a**−**e**, 1 mmol) and acid (1 mmol) in 90% *i*-PrOH (20 mL) was injected with syringe into the above mixture in four portions during 45 min. The temperature rose to reflux. When about 20 mL of the solvent was distilled out, 20 mL of 90% *i*-PrOH was added to the reaction mixture. This procedure was repeated three times during 3−4 h. The dark-blue solution was evaporated, and the residue was purified by column chromatography with silica gel, eluting with $CHCl_3$/MeOH from 10:1 to 10:3 (v/v). The dark-blue solution was evaporated. To a solution of the residue in EtOH or MeOH (2 mL) was added AcOEt (20 mL). After ultrasonication for 10 min, the mixture was filtrated. The powder was washed by AcOEt and Et_2O and then dried in vacuum.

3,7-Di(piperidin-1-yl)phenoxazin-5-ium Chloride (5c). Yield 60%, mp >250 °C. IR ν (neat, cm^{-1}): 2933, 2856, 1595, 1488, 1399, 1157. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 659 (4.99), 265 (4.44). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 1.82 (br, 12H), 3.87 (br, 8H), 7.05 (d, J = 2.6 Hz, 2H), 7.47 (dd, J = 9.6, 2.6 Hz, 2H), 7.68 (d, J = 9.6 Hz, 2H). ^{13}C NMR (68 MHz, CD_3OD) δ_{ppm}: 25.2, 27.6, 50.9, 97.9, 118.6, 135.4, 135.7, 150.9, 157.9. MS (ESI^+), *m/z*: 348.1 [M − Cl^-]$^+$. Anal. Calcd. for $C_{22}H_{26}ClN_3O\cdot 2.25H_2O$: C, 62.25; H, 7.24; N, 9.90. Found: C, 62.08; H, 7.21; N, 9.83.

3-(Dimethylamino)-7-(pyrrolidin-1-yl)phenoxazin-5-ium Chloride (5e). Yield 70%, mp >250 °C. IR ν (neat, cm^{-1}): 2975, 2873, 1603, 1489, 1397, 1151. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 647 (5.02), 261 (4.49). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 2.15−2.20 (m, 4H), 3.41 (s, 6H), 3.73−3.82 (m, 4H), 6.83 (d, J = 2.5 Hz, 1H), 6.94 (d, J = 2.6 Hz, 1H), 7.29 (dd, J = 9.5, 2.5 Hz, 1H), 7.37 (dd, J = 9.6, 2.6 Hz, 1H), 7.79 (d, J = 9.6 Hz, 1H), 7.80 (d, J = 9.5 Hz, 1H). ^{13}C NMR (68 MHz, CD_3OD) δ_{ppm}: 26.0, 26.3, 41.7, 51.0, 51.3, 97.5, 98.1, 118.1, 119.9, 134.7, 135.0, 135.4, 136.0, 150.1, 150.3, 156.7, 158.9. MS (ESI^+), *m/z*: 294.1 [M − Cl^-] $^+$. Anal. Calcd for $C_{18}H_{20}ClN_3O\cdot 1.25H_2O$: C, 61.36; H, 6.44; N, 11.93. Found: C, 61.50; H, 6.26; N, 11.68.

3-(Dipropylamino)-7-(pyrrolidin-1-yl)phenoxazin-5-ium Chloride (5g). Yield 63%, mp >250 °C. IR ν (neat, cm^{-1}): 2964, 2873, 1599, 1488, 1398, 1149. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 653 (5.10), 262 (4.52). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 1.06 (t, J = 7.3 Hz, 6H), 1.72−1.86 (m, 4H), 2.15−2.20 (m, 4H), 3.64−3.70 (m, 8H), 6.77 (br, 1H), 6.89 (d, J = 2.2 Hz, 1H), 7.23 (dd, J = 9.4, 1.4 Hz, 1H), 7.34 (dd, J = 9.7, 2.5 Hz, 1H), 7.69−7.75 (m, 2H). ^{13}C NMR (68 MHz, CD_3OD) δ_{ppm}: 11.4, 22.1, 26.0, 26.3, 51.0, 51.2, 55.0, 97.6, 98.0, 118.4, 119.7, 135.0, 135.2, 135.3, 136.0, 150.4, 150.5, 156.6, 157.9. MS (ESI^+), *m/z*: 350.1 [M − Cl^-]$^+$. Anal. Calcd for $C_{22}H_{28}ClN_3O\cdot 2.25H_2O$: C, 61.96; H, 7.68; N, 9.85. Found: C, 61.83; H, 7.45; N, 9.68.

3-(Dimethylamino)-7-morpholinophenoxazin-5-ium Chloride (5h). Yield 40%, mp >250 °C. IR ν (neat, cm^{-1}): 3032, 2869, 1603, 1490, 1400, 1159. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 648 (4.92), 263 (4.39). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 3.44 (s, 6H), 3.83−3.90 (m, 8H), 6.91 (d, J = 2.6 Hz, 1H), 7.09 (d, J = 2.6 Hz, 1H), 7.41 (dd, J = 9.6, 2.6 Hz, 1H), 7.48 (dd, J = 9.6, 2.6 Hz, 1H), 7.70−7.76 (m, 2H). ^{13}C NMR (68 MHz, CD_3OD)

δ_{ppm}: 42.1, 49.2, 67.5, 97.7, 98.2, 118.0, 119.5, 135.0, 135.2, 135.4, 136.8, 150.47, 150.5, 158.4, 159.7. MS (ESI^+), *m/z*: 310.1 [M − Cl^-]$^+$. Anal. Calcd for $C_{18}H_{20}ClN_3O_2 \cdot 2.75H_2O$: C, 54.68; H, 6.50; N, 10.63. Found: C, 54.62; H, 6.37; N, 10.48.

3-(Diethylamino)-7-morpholinophenoxazin-5-ium Chloride (5i). Yield 58%, mp >250 °C. IR ν (neat, cm^{-1}): 2977 (alkyl-CH), 1597, 1488, 1400, 1153. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 651 (5.06), 263 (4.51). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 1.38 (t, J = 7.2 Hz, 6H), 3.78−3.86 (m, 12H), 7.00 (d, J = 2.6 Hz, 1H), 7.12 (d, J = 2.6 Hz, 1H), 7.44−7.51 (m, 2H), 7.78 (d, J = 9.5 Hz, 1H), 7.79 (d, J = 9.5 Hz, 1H). ^{13}C NMR (68 MHz, CD_3OD) δ_{ppm}: 12.9, 46.4, 47.7, 65.9, 96.2, 96.9, 117.0, 118.5, 133.1, 133.6, 134.3, 135.2, 148.6, 149.1, 156.1, 156.3. MS (ESI^+), *m/z*: 338.1 [M − Cl^-]$^+$. Anal. Calcd for $C_{20}H_{24}ClN_3O_2 \cdot 1.25H_2O$: C, 60.60; H, 6.74; N, 10.60. Found: C, 60.33; H, 6.63; N, 10.49.

3-(Dipropylamino)-7-morpholinophenoxazin-5-ium Chloride (5j). Yield 35%, mp 133−135 °C. IR ν (neat, cm^{-1}): 2964, 2873, 1595, 1490, 1400, 1151. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 654 (5.01), 264 (4.47). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 1.06 (t, J = 7.3 Hz, 6H), 1.74−1.88 (m, 4H), 3.73 (t, J = 7.7 Hz, 4H), 1.86 (br, 8H), 6.97 (d, J = 2.6 Hz, 1H), 7.12 (d, J = 2.5 Hz, 1H), 7.46 (dd, J = 9.7, 2.6 Hz, 1H), 7.49 (dd, J = 9.7, 2.6 Hz, 1H), 7.82 (br, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{ppm}: 10.8, 20.7, 47.7, 53.3, 65.9, 96.4, 96.8, 117.0, 118.7, 133.2, 133.6, 134.2, 135.1, 148.6, 149.0, 156.3, 156.6. MS (ESI^+), *m/z*: 366.1 [M − Cl^-]$^+$. Anal. Calcd for $C_{22}H_{28}ClN_3O_2 \cdot 1.25H_2O$: C, 62.25; H, 7.24; N, 9.90. Found: C, 62.09; H, 7.41; N, 9.64.

3-(Dimethylamino)-7-thiomorpholinophenoxazin-5-ium Chloride (5k). Yield 44%, mp >250 °C. IR ν (neat, cm^{-1}): 3031, 1598, 1490, 1397, 1148. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 651 (4.94), 239 (4.44). ^{1}H NMR (400 MHz, CD_3OD) δ_{ppm}: 2.80−2.82 (m, 4H), 3.36 (s, 6H), 4.43 (br, 4H), 6.95 (br, 1H), 7.22 (br, 1H), 7.47−7.59 (m, 2H), 7.81 (br, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{ppm}: 26.9, 41.6, 51.1, 96.4, 97.3, 117.3, 118.6, 133.2, 133.8, 133.9, 135.2, 148.7, 148.9, 155.7, 157.6. MS (ESI^+), *m/z*: 326.0 [M − Cl^-]$^+$. Anal. Calcd for $C_{18}H_{20}ClN_3OS \cdot 3H_2O$: C, 51.98; H, 6.30; N, 10.10. Found: C, 51.70; H, 6.12; N, 9.97.

3-(Diethylamino)-7-thiomorpholinophenoxazin-5-ium Chloride (5l). Yield 68%, mp 145−147 °C. IR ν (neat, cm^{-1}): 2975, 1594, 1489, 1399, 1151. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 654 (5.06), 239 (4.59). ^{1}H NMR (400 MHz, CD_3OD) δ_{ppm}: 1.27 (t, J = 7.1 Hz, 6H), 2.80−2.81 (m, 4H), 3.78−3.84 (m, 4H), 4.19−4.20 (m, 4H), 6.98 (d, J = 2.3 Hz, 1H), 7.20 (d, J = 2.3 Hz, 1H), 7.51 (dd, J = 9.7, 2.4 Hz, 1H), 7.58 (dd, J = 9.6, 2.5 Hz, 1H), 7.80−7.82 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{ppm}: 12.8, 26.9, 46.4, 51.1, 96.1, 97.3, 117.2, 118.5, 133.1, 133.8, 134.3, 135.2, 148.8, 149.1, 155.6, 156.1. MS (ESI^+), *m/z*: 354.0 [M − Cl^-]$^+$. Anal. Calcd for $C_{20}H_{24}ClN_3OS \cdot H_2O$: C, 58.88; H, 6.42; N, 10.30. Found: C, 58.62; H, 6.67; N, 10.15.

3-(Dipropylamino)-7-thiomorpholinophenoxazin-5-ium Chloride (5m). Yield 55%, mp 126−127 °C. IR ν (neat, cm^{-1}): 2964, 2873, 1593, 1490, 1399, 1150. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 658 (4.98), 264 (4.44). ^{1}H NMR (400 MHz, CD_3OD) δ_{ppm}: 1.06 (t, J = 7.4 Hz, 6H), 1.77−1.86 (m, 4H), 2.83−2.87 (m, 4H), 3.70−3.78 (m, 4H), 4.18−4.22 (m, 4H), 6.96 (d, J = 2.6 Hz, 1H), 7.12 (d, J = 2.6 Hz, 1H), 7.43−7.51 (m, 2H), 7.78 (br, 1H), 7.81 (br, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ_{ppm}: 10.8, 20.5, 26.8, 51.1, 53.3, 96.3, 97.3, 117.2, 118.7, 133.2, 133.8, 134.2, 135.2, 148.8, 149.0, 155.6, 156.6. MS (ESI^+), *m/z*: 382.1 [M − Cl^-] $^+$. Anal. Calcd for $C_{22}H_{28}ClN_3OS \cdot 2.25H_2O$: C, 57.63; H, 7.14; N, 9.16. Found: C, 57.59; H, 7.06; N, 9.01.

3-(Dimethylamino)-7-(piperidin-1-yl)phenoxazin-5-ium Chloride (5n). Yield 27%, mp >250 °C. IR ν (neat, cm^{-1}): 2940, 2862, 1600, 1490, 1397, 1153. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 652 (5.04), 263 (4.50). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 1.83 (br, 6H), 3.41 (s, 6H), 3.90 (br, 4H), 6.93 (d, J = 2.5 Hz, 1H), 7.13 (d, J = 2.3 Hz, 1H), 7.37 (dd, J = 9.5, 2.6 Hz, 1H), 7.53 (dd, J = 9.7, 2.6 Hz, 1H), 7.77 (br, 2H). ^{13}C NMR (68 MHz, CD_3OD) δ_{ppm}: 25.1, 27.7, 41.7, 51.0, 97.5, 97.9, 118.3, 118.9, 135.0, 135.2, 135.5, 135.8, 150.2, 151.0, 158.0, 159.0. MS (ESI^+), *m/z*: 308.1 [M − Cl^-] $^+$. Anal. Calcd for $C_{19}H_{22}ClN_3O \cdot 1.5H_2O$: C, 61.53; H, 6.79; N, 11.33. Found: C, 61.85; H, 6.61; N, 11.14.

3-(Diethylamino)-7-(piperidin-1-yl)phenoxazin-5-ium Chloride (5o). Yield 28%, mp >250 °C. IR ν (neat, cm^{-1}): 2936, 2862, 1596, 1490, 1401, 1155. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 655 (5.05), 263 (4.51). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 1.36 (t, J = 7.2 Hz, 6H), 1.83 (br, 6H), 3.77 (q, J = 7.0, 4H), 3.88 (br, 4H), 6.92 (d, J = 2.7 Hz, 1H), 7.08 (d, J = 2.6 Hz, 1H), 7.36 (dd, J = 9.7, 2.7 Hz, 1H), 7.49 (dd, J = 9.7, 2.7 Hz, 1H), 7.71 (d, J = 9.7 Hz, 1H), 7.73 (d, J = 9.6 Hz, 1H). ^{13}C NMR (68 MHz, DMSO-d_6) δ_{ppm}: 13.1, 25.2, 27.6, 47.7, 50.9, 97.4, 97.9, 118.5, 118.7, 135.46, 135.5, 135.6, 150.7, 151.0, 157.6, 158.0. MS (ESI^+), *m/z*: 336.1 [M − Cl^-]$^+$. Anal. Calcd for $C_{21}H_{26}ClN_3O \cdot H_2O$: C, 64.69; H, 7.24; N, 10.78. Found: C, 64.94; H, 7.34; N, 10.76.

3-(Dipropylamino)-7-(piperidin-1-yl)phenoxazin-5-ium Chloride (5p). Yield 51%, mp 154−155 °C. IR ν (neat, cm^{-1}): 2936, 2873, 1595, 1490, 1400, 1153. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 657 (5.00), 264 (4.48). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 1.05 (t, J = 7.4 Hz, 6H), 1.72−1.86 (m, 10H), 3.65−3.71 (m, 4H), 3.89 (br, 4H), 6.91 (d, J = 2.6 Hz, 1H), 7.11 (d, J = 2.6 Hz, 1H), 7.37 (dd, J = 9.7, 2.6 Hz, 1H), 7.52 (dd, J = 9.7, 2.6 Hz, 1H), 7.76 (br, 1H), 7.79 (br, 1H). ^{13}C NMR (68 MHz, CD_3OD) δ_{ppm}: 11.4, 22.1, 25.2, 27.6, 50.9, 55.0, 97.6, 97.9, 118.6, 118.7, 135.3, 135.5, 135.8, 150.7, 151.0, 158.0, 158.1. MS (ESI^+), *m/z*: 364.1 [M − Cl^-]$^+$. Anal. Calcd for $C_{23}H_{30}ClN_3O \cdot 2H_2O$: C, 63.36; H, 7.86; N, 9.64. Found: C, 63.39; H, 7.62; N, 9.42.

3-(Dibutylamino)-7-(piperidin-1-yl)phenoxazin-5-ium Chloride (5q). Yield 50%, mp 60−61 °C. IR ν (neat, cm^{-1}): 2931, 2858, 1595, 1490, 1400, 1153. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 659 (5.03), 264 (4.48). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 1.03 (t, J = 7.3 Hz, 6H), 1.42−1.55 (m, 4H), 1.69−1.83 (m, 10H), 3.68−3.74 (m, 4H), 3.89 (br, 4H), 6.87 (d, J = 2.3 Hz, 1H), 7.11 (d, J = 2.1 Hz, 1H), 7.34 (dd, J = 9.6, 2.6 Hz, 1H), 7.51 (dd, J = 9.6, 2.6 Hz, 1H), 7.73 (br, 1H), 7.76 (br, 1H). ^{13}C NMR (68 MHz, CD_3OD) δ_{ppm}: 14.2, 21.1, 25.2, 27.6, 30.9, 50.9, 53.3, 97.5, 97.9, 118.6, 118.7, 135.3, 135.5, 135.6, 135.8, 150.7, 151.1, 157.9, 158.1. MS (ESI^+), *m/z*: 392.2 [M − Cl^-]$^+$. Anal. Calcd for $C_{25}H_{34}ClN_3O \cdot 0.75H_2O$: C, 68.01; H, 8.10; N, 9.52. Found: C, 67.99; H, 8.28; N, 9.36.

3-(Dipentylamino)-7-(piperidin-1-yl)phenoxazin-5-ium Chloride (5r). Yield 54%, mp 83−84 °C. IR ν (neat, cm^{-1}): 2933, 2859, 1595, 1490, 1399, 1153. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 659 (5.06), 264 (4.51). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 0.94−1.00 (m, 6H), 1.40−1.47 (m, 8H), 1.71−1.83 (m, 10H), 3.67−3.73 (m, 4H), 3.89 (br, 4H), 6.87 (d, J = 2.6 Hz, 1H), 7.11 (d, J = 2.5 Hz, 1H), 7.34 (dd, J = 9.6, 2.5 Hz, 1H), 7.51 (dd, J = 9.6, 2.6 Hz, 1H), 7.74 (d, J = 9.6 Hz, 1H), 7.75 (d, J = 9.5 Hz, 1H). ^{13}C NMR (68 MHz, CD_3OD) δ_{ppm}: 14.4, 23.6, 25.2, 27.6, 28.5, 30.0, 50.9, 53.5, 97.5, 98.0, 118.6, 118.7, 135.4, 135.5, 135.6, 135.8, 150.7, 151.1, 157.9, 158.1. MS (ESI^+), *m/z*: 420.2 [M − Cl^-]$^+$. Anal. Calcd for $C_{27}H_{38}ClN_3O \cdot 1.75H_2O$: C, 66.51; H, 8.58; N, 8.62. Found: C, 66.23; H, 8.50; N, 8.55.

3-(Dihexylamino)-7-(piperidin-1-yl)phenoxazin-5-ium Chloride (5s). Yield 51%, mp 90−91 °C. IR ν (neat, cm^{-1}): 2954, 2869, 1595, 1490, 1399, 1152. UV−vis ($CHCl_3$), λ (nm) (log ε/L mol^{-1} cm^{-1}): 658 (4.84), 264 (4.29). ^{1}H NMR (270 MHz, CD_3OD) δ_{ppm}: 0.94−0.97 (m, 6H), 1.38−1.49 (m, 12H), 1.70−1.83 (m, 10H), 3.67−3.73 (m, 4H), 3.89 (br, 4H), 6.87 (d, J = 2.5 Hz, 1H), 7.11 (d, J = 2.6 Hz, 1H), 7.34 (dd, J = 9.6, 2.5 Hz, 1H), 7.51 (dd, J = 9.7, 2.6 Hz, 1H), 7.74 (d, J = 9.7 Hz, 1H), 7.75 (d, J = 9.6 Hz, 1H). ^{13}C NMR (68 MHz, CD_3OD) δ_{ppm}: 14.4, 23.7, 25.2, 27.5, 27.6, 28.8, 32.8, 50.9, 53.5, 97.5, 98.0, 118.6, 118.7, 135.4, 135.5, 135.6, 135.8, 150.7, 151.1, 157.9, 158.1. MS (ESI^+), *m/z*: 448.3 [M − Cl^-]$^+$. Anal. Calcd for $C_{29}H_{42}ClN_3O \cdot 2.75H_2O$: C, 65.27; H, 8.97; N, 7.87. Found: C, 65.05; H, 8.88; N, 7.90.

Antiprotozoal Activity. General Procedure for Preparing the Stock Drug Solutions. Stock drug solutions were prepared in 100% DMSO at 10 mg/mL and heated or sonicated if necessary to dissolve the sample. For the assay, the compound was further diluted in serum-free culture medium and finally to the appropriate concentration in complete medium without hypoxanthine. The

DMSO concentration in the wells with the highest drug concentration did not exceed 1%.

In Vitro Assay. *P. falciparum* K1 (resistant to chloroquine and pyrimethamine), *T. cruzi* (Tulahuen C2C4), *L. donovani* (MHOM-ET-67/L82), and *T. b. rhodesiense* (STIB900) were used for the in vitro assay. The cytotoxicity was assessed with rat skeletal myoblasts (L6 cells). IC_{50} values were obtained by the earlier described methods.[18] The following compounds were used as reference standards: chloroquine (*P. falciparum*), melarsoprol (*T.b. rhodesiense*), benznidazole (*T. cruzi*), miltefosine (*L. donovani*), and podophyllotoxin (L6 cells, cytotoxicity).

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Supporting Information Available: Additional experimental details and combustion data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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