Supporting Information

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, Tokyo 142-8501, Japan, Department of Material and Chemistry, Soochow University, Suzhou 215006, China, and Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel Switzerland

Experiments

Chemical Experiments

General: Compounds **1**, **3d**, **3j**, **3j** are commercial available. Compounds **2a**, **2b**, **2c**, **2d**, **3a**, **3b**, **3d**, **4a**, **4b**, **4d**, **5a**, **5b**, **5d**, **5f** were obtained by the reported method;[1-2] They have identical analysis data with the literature. **3c** were obtained by the cleavage of corresponding ether **2c**,[3] *N,N*-Dialkylaminophenols **3f-h** were obtained with the same manner with **3a**.[2,4] **4c**, **4e** were prepared by the same way of **4a**; **5c**, **5e**, **5g-s** were obtained by the same way of **5a.**[2]

4-(3-Methoxyphenyl)thiomorpholine (**2e**). A Schlenk flask was charged with 1-bromo-3-methoxybenzene (1.26 mL, 10 mmol), thiomorpholine (1.2 mL, 12 mmol), sodium tert-butoxide (1.35g, 1.4 mmol), tris(dibenzylideneacetone)dipalladium(0) (45.8 mg, 0.05 mmol), BINAP (93.4 mg, 0.15 mmol), and toluene (20 mL) under argon. The flask was immersed in an 80 °C oil bath with stirring for 1 day. The solution was then allowed to cool to room temperature, taken up in ether (100 mL), filtered, and concentrated. The crude product was then purified further by flash chromatography on silica gel eluted by petroleum ether: ethyl acetate= 10:1 (v/v). Slightly yellow oil, yield 94%, IR ν (neat, cm^{-1}): 2952, 2907 (alkyl-CH), 1604, 1496 (C_6H_4), 1161 (C-O-C); ^{1}H NMR (270 MHz, $CDCl_3$) δ_{ppm}: 2.71-2.75 (m, 4H, 2×CH_2), 3.53-3.57 (m, 4H, 2×CH_2), 3.79 (s, 3H, CH_3), 6.39-6.52, 7.14-7.20 (m, 4H, 4×Ar-*H*); ^{13}C NMR (68 MHz, $CDCl_3$) δ_{ppm}: 26.6 (2×CH$_2$), 52.0 (2×CH$_2$), 55.2 (CH$_3$), 103.4 (Ar CH), 104.3 (Ar CH), 109.6 (Ar CH), 129.9 (Ar CH), 152.4 (Ar C), 160.7 (Ar C). Anal. Calcd. For $C_{11}H_{15}NOS$: C, 63.12; H, 7.22; N, 6.69; Found: C, 63.41; H, 6.98; N, 6.64.

3-(Piperidin-1-yl)phenol (**3c**). 1-(3-methoxyphenyl)piperidine **2c** (3.1 g, 13.6 mmol) was refluxed in a solution of 47% HI (22 mL) and 37% HCl (22 mL) for two days. The solution was cooled in ice-water bath and neutralized to pH 6.0 by Na_2CO_3 and then phosphate buffer (6.9 g $NaH_2PO{\cdot}H_2O$ and 1.4 g Na_2HPO_4 in 100 mL water). The water solution was extracted with Et_2O (3×50 mL), the organic layer was washed by brine and dried over Na_2SO_4. After removing the solvent, the product was pure enough for next step. White solid, yield 84%, ^{1}H NMR (270 MHz, $CDCl_3$) δ_{ppm}: 1.51-1.60 (m, 2H, CH_2), 1.65-1.76 (m, 4H, 2×CH_2), 3.05-3.09 (m, 4H, 2×CH_2), 6.27-6.30, 6.40-6.48, 6.70-7.05 (m, 4H, 4×Ar-*H*); ^{13}C NMR (68 MHz, $CDCl_3$) δ_{ppm}: 25.6 (*C*H_2), 26.8 (2×*C*H_2), 52.4 (2×*C*H_2), 105.3 (Ar *C*H), 108.1 (Ar *C*H), 109.9 (Ar *C*H), 130.6 (Ar *C*H), 155.0 (Ar *C*), 159.1 (Ar *C*);

General procedure of synthesis 3-(dialkylamino)phenols (**3f**-**h**): A mixture of 3-aminophenol **5** (30 mmol), N_2CO_3 (42 mmol), i-PrOH (15 mL), H_2O (15 mL) and 1-bromoalkane was refluxed overnight. The organic layer was separated and purified by flash chromatography, eluted by petroleum ether: AcOEt= 10: 1 (v/v), to obtain the product.

3-(Dibutylamino)phenol (**3f**). Colorless oil, yield 83%, ^{1}H NMR (270 MHz, $CDCl_3$) δ_{ppm}: 0.94 (t, 6H, *J*=7.2 Hz, 2×CH_3), 1.27-1.40 (m, 4H, 2×CH_2), 1.50-1.61 (m, 4H, 2×CH_2), 3.19-3.25 (m, 4H, 2×CH_2), 4.63 (br, 1H, O*H*), 6.07-6.13, 6.21-6.25, 7.00-7.06 (m, 4H, 4×Ar-*H*); ^{13}C NMR (68 MHz, $CDCl_3$) δ_{ppm}: 14.0 (2×*C*H_3), 20.3 (2×*C*H_2), 29.4 (2×*C*H_2), 50.8 (2×*C*H_2), 98.5 (Ar *C*H), 102.0 (Ar *C*H), 104.6 (Ar *C*H), 130.0 (Ar *C*H), 149.8 (Ar *C*), 156.7 (Ar *C*);

3-(Dipentylamino)phenol (**3g**). Colorless oil, yield 76%, ^{1}H NMR (270 MHz, $CDCl_3$) δ_{ppm}: 0.91 (t, 6H, *J*=6.9 Hz, 2×CH_3), 1.22-1.42 (m, 8H, 4×CH_2), 1.51-1.62 (m, 4H, 2×CH_2), 3.18-3.24 (m, 4H, 2×CH_2), 4.63 (br, 1H, O*H*), 6.06-6.12, 6.21-6.24, 7.00-7.06 (m, 4H, 4×Ar-*H*); ^{13}C NMR (68 MHz, $CDCl_3$) δ_{ppm}: 14.1 (2×*C*H_3), 22.6 (2×*C*H_2), 26.9 (2×*C*H_2), 29.3 (2×*C*H_2), 51.0 (2×*C*H_2), 98.5 (Ar *C*H), 102.0 (Ar *C*H), 104.6 (Ar *C*H), 130.0 (Ar *C*H), 149.8 (Ar *C*), 156.7 (Ar *C*);

3-(Dihexylamino)phenol (**3h**). Colorless oil, yield 77%, ^{1}H NMR (270 MHz, $CDCl_3$) δ_{ppm}: 0.89 (t, 6H, *J*=6.6 Hz, 2×CH_3), 1.30 (br, 12H, 6×CH_2), 1.50-1.61 (m, 4H, 2×CH_2), 3.18-3.23 (m, 4H, 2×CH_2), 4.66 (br, 1H, O*H*), 6.06-6.12, 6.20-6.23, 7.00-7.06 (m, 4H, 4×Ar-*H*); ^{13}C NMR (68 MHz, $CDCl_3$) δ_{ppm}: 14.1 (2×*C*H_3), 22.7 (2×*C*H_2), 26.8 (2×*C*H_2), 27.2 (2×*C*H_2), 31.7 (2×*C*H_2), 51.1 (2×*C*H_2), 98.5 (Ar *C*H), 102.0 (Ar *C*H), 104.6 (Ar *C*H), 130.0 (Ar *C*H), 149.8 (Ar *C*), 156.6 (Ar *C*);

General method of preparation of N,N-dialkyl-3-methoxy-4-nitrosoanilines (**4c, 4e**):

A mixture of *N,N*-dialkyl-3-methoxyaniline (10 mmol) and HCl (40 mmol) in H_2O (30 mL) was stirred in an ice bath. $NaNO_2$ (14 mmol) was slowly added to the mixture during 60 min, and then the resulting mixture was stirred in ice bath for another 1 hr. After adding K_2CO_3 powder to basify the above solution until pH ~9, the mixture was filtrated and the green mass was washed with water. The mass was ultrasonicated in Et_2O and filtrated to give nitroso compounds. Due to the quaternary carbon of the free base was difficult to detected, ^{13}C-NMR was tested by corresponding hydrochloric salts. To a stirred solution of free base (1 mmol) in EtOH (~8 mL), hydrochloric acid (3 mmol) was

added, and then Et_2O (-20 mL) was added. The hydrochloric salt was obtained after filtration.

1-(3-Methoxy-4-nitrosophenyl)piperidine (**4c**). Green powder, yield 94%, mp 159-160 °C; IR ν (neat, cm^{-1}): 2936, 2854 (alkyl-CH), 1603, 1517 (C_6H_3), 1221 (C-O-C); ^1H NMR (400 MHz, *$CDCl_3$*) δ_{ppm}: 1.68-1.77 (m, 6H, 3×C*H_2*), 3.53-3.56 (m, 4H, 2×C*H_2*), 4.16 (s, 3H, C*H_3*), 6.24-6.28, 6.65-6.67 (m, *3*H, 3×Ar-*H*); ^{13}C NMR of corresponding HCl salt (68 MHz, *CD_3OD*) δ_{ppm}: 24.6 (*C*H_2), 28.6 (2×*C*H_2), 53.8 (2×*C*H_2), 58.1 (*C*H_3), 96.4 (Ar *C*H), 120.2 (Ar *C*H), 125.6 (Ar *C*H), 146.0 (Ar *C*), 163.1 (Ar *C*), 167.2 (Ar *C*); MS (ESI^+): *m/z*: 221.2 [M + H]$^+$. Anal. Calcd. For $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72; Found: C, 65.57; H, 7.45; N, 12.57.

4-(3-Methoxy-4-nitrosophenyl)thiomorpholine (**4e**), yield 95%, mp 125-126 °C; IR ν (neat, cm^{-1}): 2904 (alkyl-CH), 1601, 1512 (C_6H_3), 1197 (C-O-C); ^1H NMR (400 MHz, *$CDCl_3$*) δ_{ppm}: 2.73-2.76 (m, 6H, 3×C*H_2*), 3.93-3.95 (m, 4H, 2×C*H_2*), 4.17 (s, 3H, C*H_3*), 6.22 (dd, *J* = 9.4, 2.5 Hz, 1H, Ar-*H*), 6.30 (d, *J* = 2.5 Hz, 1H, Ar-*H*), 6.60 (d, *J* = 9.4 Hz, 1H, Ar-*H*); ^{13}C NMR of corresponding HCl salt (68 MHz, *CD_3OD-D_2O*) δ_{ppm}: 29.9 (2×*C*H_2), 55.5 (2×*C*H_2), 58.4 (*C*H_3), 96.2 (Ar *C*H), 119.8 (Ar *C*H), 125.7 (Ar *C*H), 145.2 (Ar *C*), 163.7 (Ar *C*), 167.1 (Ar *C*); MS (ESI^+): *m/z*: 239.2 [M + H]$^+$. Anal. Calcd. For $C_{11}H_{14}N_2O_2S$: C, 55.44; H, 5.92; N, 11.76; Found: C, 55.15; H, 6.02; N, 11.55.

Combustion data of new compounds: Elemental analyses were performed on Yanagimoto MT-3, and the results (C, H, N) were within ±0.4% of theoretical values. Because of deliquescence and hygroscopicity, correct elemental analyses for most of the compounds could only be obtained by factoring in partial hydration of these organic salts.

	Calcd. For				Found		
		C	H	N	C	H	N
2e	$C_{11}H_{15}NOS$	63.12	7.22	6.69	63.41	6.98	6.64
4c	$C_{12}H_{16}N_2O_2$	65.43	7.32	12.72	65.57	7.45	12.57
4e	$C_{11}H_{14}N_2O_2S$	55.44	5.92	11.76	55.15	6.02	11.55
5c	$C_{22}H_{26}ClN_3O{\cdot}2.25H_2O$	62.25	7.24	9.90	62.08	7.21	9.83
5e	$C_{18}H_{20}ClN_3O{\cdot}1.25H_2O$	61.36	6.44	11.93	61.50	6.26	11.68
5g	$C_{22}H_{28}ClN_3O{\cdot}2.25H_2O$	61.96	7.68	9.85	61.83	7.45	9.68
5h	$C_{18}H_{20}ClN_3O_2{\cdot}2.75H_2O$	54.68	6.50	10.63	54.62	6.37	10.48
5i	$C_{20}H_{24}ClN_3O_2{\cdot}1.25H_2O$	60.60	6.74	10.60	60.33	6.63	10.49
5j	$C_{22}H_{28}ClN_3O_2{\cdot}1.25H_2O$	62.25	7.24	9.90	62.09	7.41	9.64
5k	$C_{18}H_{20}ClN_3OS{\cdot}3H_2O$	51.98	6.30	10.10	51.70	6.12	9.97
5l	$C_{20}H_{24}ClN_3OS{\cdot}H_2O$	58.88	6.42	10.30	58.62	6.67	10.15
5m	$C_{22}H_{28}ClN_3OS\ 2.25H_2O$	57.63	7.14	9.16	57.59	7.06	9.01
5n	$C_{19}H_{22}ClN_3O{\cdot}1.5H_2O$	61.53	6.79	11.33	61.85	6.61	11.14
3h	$C_{21}H_{26}ClN_3O{\cdot}H_2O$	64.69	7.24	10.78	64.94	7.34	10.76
5p	$C_{23}H_{30}ClN_3O{\cdot}2H_2O$	63.36	7.86	9.64	63.39	7.62	9.42
5q	$C_{25}H_{34}ClN_3O{\cdot}0.75H_2O$	68.01	8.10	9.52	67.99	8.28	9.36
5r	$C_{27}H_{38}ClN_3O{\cdot}1.75H_2O$	66.51	8.58	8.62	66.23	8.50	8.55
5s	$C_{29}H_{42}ClN_3O{\cdot}2.75H_2O$	65.27	8.97	7.87	65.05	8.88	7.90

Reference:

(1) Skowronska-Ptasinska, M.; Verboom, W.; Reinhoudt, D. N. Effect of Different Dialkylamino Groups on the Regioselectivity of Lithiation of O-protected 3-(Dialkylamino)phenols. *J. Org. Chem.* **1985**, *50*, 2690-2698.

(2) Ge, J.; Arai, C.; Ihara, M. The convenient Synthesis of Zinc Chloride-Free 3,7-Bis(dialkylamino)phenoxazinium Salts. *Dyes Pigme.* **2008**, doi:10.1016/j.dyepig.2008.01.001.

(3) Gompel, J. V.; Schuster G. B. Chemiluminescence of Organic Peroxides: Intramolecular Electron-Exchange Luminescence from a Secondary Perester. *J. Org. Chem.* **1987**, *52*, 1465-1468.

(4) Dong, X. Z.; Cai, C. Synthesis of 3-Methyl-7-dipropylamino-1,4-benzoxazin- 2-one. *Jingxi Huagong* **2003**, *20*, 761.