Supporting information

Searching for innovative quinolone-like scaffolds: synthesis and biological evaluation of 2,1-benzothiazine 2,2-dioxide derivatives.

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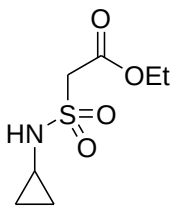
Microbiologic Procedures. MICs were determined by microdilution techniques according to CLSI guidelines.[1] The effect of combining reserpine, paroxetine or various test compounds, with scalar dilutions of freshly prepared solutions of each selected compound, on the MICs of CPX was also determined. Checkerboard combination studies using CPX, **17** and **18** were performed as described previously.[2]

EtBr Efflux inhibition assay. The loss of EtBr from *S. aureus* SA-1199B was determined fluorometrically as previously described.[3] Experiments were performed in duplicate, and the results were expressed as mean total efflux over a 5 min time course. The effect of increasing concentrations of reserpine, paroxetine, **7**, **8**, **10**, **17** and **18** on the EtBr efflux of SA-1199B was compared to that in their absence, allowing the calculation of the percentage reduction in efflux.

Synthesis. All reactions were routinely checked by thin-layer chromatography (TLC) on silica gel $60F_{254}$ (Merck) and visualized using UV illumination. Flash column chromatography was performed on Merck Silica Gel 60 (mesh 230-400) using the indicated solvents. Yields were of purified product and were not optimized. Melting points were determined in capillary tubes (Mettler PF62 apparatus) and are uncorrected. Elemental analyses were performed by a Fisons elemental analyzer (model EA1108CHN), and the data for C, H, and N are within 0.4% of the theoretical values. ^{1}H NMR spectra were recorded at 400 MHz with a Bruker Advance-DRX 400 instrument and with Me_4Si as the internal standard. The chemical shift (δ) values are reported in ppm, and the coupling constants (*J*) are given in Hz. The abbreviations used are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. The spectral data are consistent with the assigned structures. The purity of the target compounds was assessed by LC−MS according to the UV trace at 230 and 254 nm. Analytical LC−MS was run on the column Agilent Poroshell 120 EC-C18, 2.7 μm, 2.1x100mm. Gradient was from CH_3CN/H_2O (5:95) to 85% CH_3CN over 5 min. Flow was 0.3 mL/min. The LC−MS machines consisted of an HPLC Agilent 1290 Infinity System equipped with a MS detector Agilent 6540UHD Accurate Mass QTOF. Reagents and solvents were

purchased from commercial suppliers and were used as received. For routine aqueous workup, the reaction mixture was extracted with CH_2Cl_2 or EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated with a Büchi rotary evaporator at low pressure. All starting materials were commercially available unless otherwise indicated.

Ethyl 2-(*N*-cyclopropylsulfamoyl)ethanoate (3).

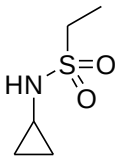


3

A solution of ethyl bromoacetate **1** (20.1 mL, 180 mmol) in EtOH (20 ml) was added dropwise at 0 °C to an aqueous solution (100 ml) of $Na_2SO_3 \cdot H_2O$ (45.3 g, 180 mmol) and the mixture was stirred at 50 °C for 3 h. After cooling, the solvent was evaporated under reduced pressure and the residue recrystallized from EtOAc/AcOH to give 11.8 g (34%) of **sodium 2-ethoxy-2-oxoethanesulfonate** as a white solid. Sodium 2-ethoxy-2-oxoethanesulfonate (19.6 g, 103 mmol) and PCl_5 (23.6 g, 113 mmol) were refluxed until the HCl development ceased, then $POCl_3$ was distilled and the residue obtained washed with benzene (3 x 20 ml) to give 11.6 g (64 mmol, 62%) of **ethyl 2-(chlorosulfonyl)ethanoate 2** as a dark brown pitch. **2** was added to a solution of cyclopropylamine (8.00 g, 140 mmol) in dry CH_2Cl_2 (75 ml) at –5 °C, and the mixture was stirred overnight at room temperature. After pouring into ice water the organic layers were separated, the solvent evaporated under reduced pressure, and the dark-brown crude obtained was purified by flash chromatography (cyclohexane:EtOAc 50/50) to give **3** (7.9 g, 59%) as a red oil: ^{1}H NMR ($CDCl_3$) δ 0.57-0.75 (4H, m, CH_2 cyclopropyl), 1.20 (3H, t, J = 7.0 Hz, CH_2CH_3), 2.42-2.57 (1H, m, CH cyclopropyl), 3.98 (2H, s, CH_2), 4.16 (2H, q, J = 7.0 Hz, CH_2CH_3), 5.23 (1H, bs, NH). Anal. ($C_7H_{13}NO_4S$) C, H, N.

Following a similar procedure, compound **12** was prepared starting from 1-ethanesulfonylchloride **11** in place of ethyl 2-(chlorosulfonyl)ethanoate **2**.

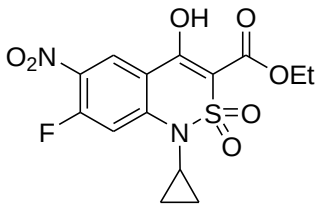
***N*-cyclopropylethanesulfonamide (12).**



12

Dark brown oil (80%): ^{1}H NMR ($CDCl_3$) δ 0.57-0.77 (4H, m, CH_2 cyclopropyl), 1.33 (3H, t, J = 7.4 Hz, CH_2CH_3), 2.48-2.62 (1H, m, CH cyclopropyl), 3.10 (2H, q, J = 7.4 Hz, CH_2CH_3), 5.00 (1H, bs, NH). Anal. ($C_5H_{11}NO_2S$) C, H, N.

Ethyl 1-cyclopropyl-7-fluoro-4-hydroxy-6-nitro-1*H*-2,1-benzothiazine-3-carboxylate 2,2-dioxide (5).

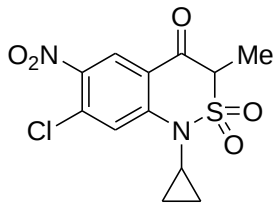


5

A solution of **3** (7.9 g, 38.2 mmol) in dry THF (50 ml) was added dropwise to a stirred suspension of NaH (60% in mineral oil, 2.82 g, 118 mmol) in dry THF (15 mL) at –10 °C. After 30 minutes, a solution of 2,4-difluoro-5-nitrobenzoyl chloride **4** (6.0 g, 29 mmol) in dry THF (15 mL) was added dropwise and the mixture reacted overnight at room temperature. The reaction mixture was poured into ice water, adjusted to pH 4 with HCl 2N and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to give a residue that was recrystallized from EtOH to give **5** (2.5 g, 23%) as a yellow solid: mp 147.2-149.0 °C; ^{1}H NMR ($CDCl_3$) δ 0.89-1.02 and 1.17-1.27 (each 2H, m, CH_2 cyclopropyl), 1.37 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.82-2.98 (1H, m, CH cyclopropyl), 4.41 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.35 (1H, d, J = 12.3 Hz, H-8), 8.75 (1H, d, J = 8.2 Hz, H-5), 13.48 (1H, s, OH). Anal. ($C_{14}H_{13}FN_2O_7S$) C, H, N.

Following a similar procedure, compound **14** was prepared starting from sulfonamide **12** and the acyl chloride **13**.

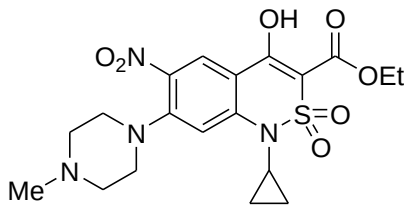
7-chloro-1-cyclopropyl-3-methyl-6-nitro-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (14).



14

Pale yellow solid (25%): mp 262.0-262.9 °C; ^{1}H NMR ($CDCl_3$) δ 0.75-1.05 and 1.22-1.37 (each 2H, m, CH_2 cyclopropyl), 1.65 (3H, d, J = 7.1 Hz, CH_3), 2.75-2.85 (1H, m, CH cyclopropyl), 4.08 (1H, q, J = 7.1 Hz, CH), 7.62 (1H, s, H-8), 8.62 (1H, s, H-5). Anal. ($C_{12}H_{11}ClN_2O_5S$) C, H, N.

Ethyl 1-cyclopropyl-4-hydroxy-7-(4-methyl-1-piperazinyl)-6-nitro-1*H*-2,1-benzothiazine-3-carboxylate 2,2-dioxide (6).

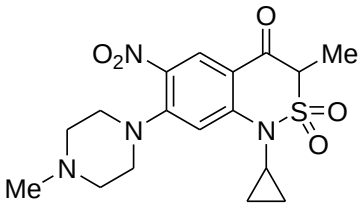


6

A solution of **5** (0.96 g, 2.6 mmol), triethylamine (1.5 mL, 10.5 mmol) and *N*-methylpiperazine (1.1 g, 10.5 mmol) in dry CH_3CN (20 mL) was stirred at 70 °C for 4 h. After cooling, the solvent was evaporated under reduced pressure and the residue purified by flash chromatography eluting with $CHCl_3$:MeOH (90/10) to give **6** (0.13 g, 83%) as a yellow-orange solid: mp 222.0-224.0 °C; ^{1}H NMR (DMSO-d_6) δ 0.64 and 1.00 (each 2H, bs, CH_2 cyclopropyl), 1.10 (3H, t, J = 7.0 Hz, $CH_2C\mathit{H}_3$), 2.13 (3H, s, CH_3 piperazine), 2.30-2.45 (4H, m, CH_2 piperazine) 2.58-2.73 (1H, m, CH cyclopropyl), 2.95-3.10 (4H, m, CH_2 piperazine), 4.03 (2H, q, J = 7.0 Hz, $C\mathit{H}_2CH_3$), 6.88 (1H, s, H-8), 8.35 (1H, s, H-5). Anal. ($C_{19}H_{24}N_4O_7S$) C, H, N.

Following a similar procedure, compound **16** was prepared starting from nitro-derivative **15**.

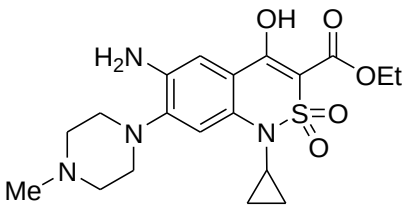
1-cyclopropyl-3-methyl-7-(4-methyl-1-piperazinyl)-6-nitro-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (16).



16

Orange solid (68%): mp 175.0-176.7 °C; ^{1}H NMR ($CDCl_3$) δ 0.70-1.02 and 1.09-1.25 (each 2H, m, CH_2 cyclopropyl), 1.55 (3H, d, J = 7.0 Hz, CH_3), 2.33 (3H, s, CH_3 piperazine), 2.50-2.64 (4H, m, CH_2 piperazine) 2.70-2.87 (1H, m, CH cyclopropyl), 3.15-3.29 (4H, m, CH_2 piperazine), 3.96 (1H, q, J = 7.0 Hz, CH), 6.86 (1H, s, H-8), 8.51 (1H, s, H-5). Anal. ($C_{17}H_{22}N_4O_5S$) C, H, N.

Ethyl 6-amino-1-cyclopropyl-4-hydroxy-7-(4-methyl-1-piperazinyl)-1*H*-2,1-benzothiazine-3-carboxylate 2,2-dioxide (7).

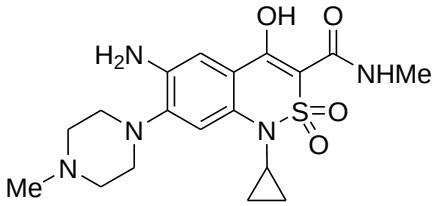


7

Pd/C 10% (0.8 g) and $HCOONH_4$ (0.6 g, 8.9 mmol) were added portion wise to a solution of **6** (0.8 g, 1.8 mmol) in dry DMF (10 mL), and the reaction mixture was stirred at room temperature for 1 h. After filtering off the catalyst over Celite, the filtrate was evaporated under reduced pressure and the residue obtained was purified by flash chromatography eluting with $CHCl_3$:MeOH:NH_4OH (67/30/3) and recrystallized from EtOH to give **7** (0.47 g, 63%) as a light brown solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 0.30-0.70 (4H, m, CH_2 cyclopropyl), 1.09 (3H, t, J = 7.0 Hz, $CH_2C\mathit{H_3}$), 2.20 (3H, s, CH_3 piperazine), 2.40-2.55 (4H, m, CH_2 piperazine) 2.55-2.88 (5H, m, CH_2 piperazine, CH cyclopropyl), 4.03 (2H, q, J = 7.0 Hz, $C\mathit{H_2}CH_3$), 4.60 (2H, bs, NH_2) 6.60 (1H, s, H-8), 7.10 (1H, s, H-5). Anal. ($C_{19}H_{26}N_4O_5S$) C, H, N.

Following a similar procedure, compound **10** and **17** were prepared starting from the nitro derivatives **9** and **16**, respectively.

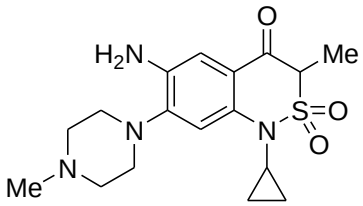
6-amino-1-cyclopropyl-4-hydroxy-*N*-methyl-7-(4-methyl-1-piperazinyl)-1*H*-2,1-benzothiazine-3-carboxamide 2,2-dioxide (10).



10

Orange solid (51%): mp>300 °C; ^{1}H NMR (DMSO-d_6) δ 0.76 (4H, bs, CH_2 cyclopropyl), 2.50 (1H, s, CH cyclopropyl), 2.58 (3H, s, CH_3 piperazine), 2.70 (3H, s, NHCH_3), 3.02 (8H, m, CH_2 piperazine) 4.82 (2H, bs, NH_2), 6.80 (1H, s, H-5), 7.12 (1H, s, H-5), 9.00 (1H, bs, NHCH$_3$). Anal. ($C_{18}H_{25}N_5O_4S$) C, H, N.

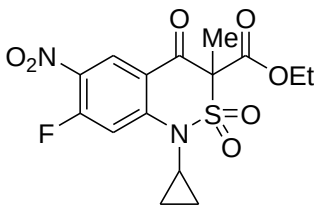
6-amino-1-cyclopropyl-3-methyl-7-(4-methyl-1-piperazinyl)-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (17).



17

Brown solid (67%): mp 240.0-240.8 °C; ^{1}H NMR ($CDCl_3$) δ 0.70-0.91 and 1.00-1.12 (each 2H, m, CH_2 cyclopropyl), 1.53 (3H, d, J = 7.1 Hz, CH_3), 2.37 (3H, s, CH_3 piperazine), 2.50-2.79 (5H, m, CH_2 piperazine and CH cyclopropyl), 2.96-3.10 (4H, m, CH_2 piperazine), 3.70 (2H, bs, NH_2), 3.88 (1H, q, J = 7.1 Hz, CH), 6.95 (1H, s, H-8), 7.30 (1H, s, H-5). Anal. ($C_{17}H_{24}N_4O_3S$) C, H, N.

Ethyl 7-fluoro-1-cyclopropyl-3-methyl-6-nitro-4-oxo-3,4-dihydro-1*H*-2,1-benzothiazine-3-carboxylate 2,2-dioxide (15).



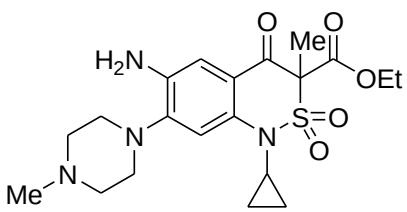
15

To a stirred solution of **14** (0.1 g, 0.3 mmol) and triethylamine (0.1 mL, 0.6 mmol) in CH_2Cl_2 (5 ml), ethyl chloroformate (0.07 mL, 0.7 mmol) was added dropwise and the mixture was stirred at room temperature for 1 h. The organic layers were washed with water (3 x 10 mL) and evaporated to dryness to give **15** (88%) as a yellow solid, used in the next step without further purification: mp 168.0-169.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.90-1.00 and 1.15-1.25 (each 2H, m,

CH_2 cyclopropyl), 1.35 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.15 (3H, s, CH_3), 2.90-3.05 (1H, m, CH cyclopropyl), 4.20 (2H, q, J = 7.1 Hz, CH_2CH_3), 7.60 (1H, s, H-8), 8.00 (1H, s, H-5). Anal. ($C_{15}H_{15}FN_2O_7S$) C, H, N.

Following a similar procedure, compound **18** was prepared starting from **17**.

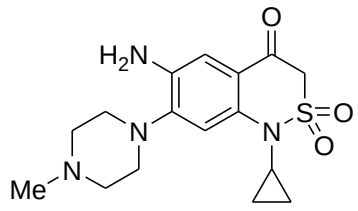
Ethyl 6-amino-1-cyclopropyl-3-methyl-7-(4-methyl-1-piperazinyl)-4-oxo-3,4-dihydro-1*H*-2,1-benzothiazine-3-carboxylate 2,2-dioxide (18).



18

Brown solid (49 %) mp 234.7-236.0 °C; ^{1}H NMR ($CDCl_3$) δ 0.75-1.00 (4H, m, CH_2 cyclopropyl), 1.30 (3H, t, J = 7.1 Hz, CH_2 CH_3), 2.20 (3H, s, CH_3), 2.32 (3H, s, CH_3 piperazine), 2.58 (4H, m, CH_2 piperazine) 2.76-2.88 (1H, m, CH cyclopropyl), 2.90-3.05 (4H, bs, CH_2 piperazine), 3.70 (2H, bs, NH_2), 4.22 (2H, q, J = 7.1 Hz, CH_2CH_3), 6.67 (1H, s, H-8), 6.99 (1H, s, H-5). Anal. ($C_{20}H_{28}N_4O_5S$) C, H, N.

6-amino-1-cyclopropyl-7-(4-methyl-1-piperazinyl)-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide (8).

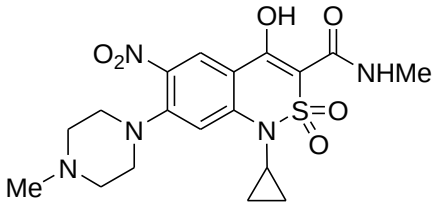


8

A solution of **7** (0.1 g, 0.25 mmol) and $LiOH{\cdot}H_2O$ (0.02 g, 0.5 mmol) in water/dioxane (1:3, 5 mL) was stirred at room temperature for 24 h. The solvent was evaporated to dryness and the residue purified by flash chromatography eluting with $CHCl_3$/MeOH/NH_4OH (89:10:1) to give **8** (0.014 g, 15%) as pale brown solid: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 0.68-0.90 and 0.98-1.09 (each 2H, m, CH_2 cyclopropyl), 2.29 (3H, s, CH_3

piperazine), 2.45-2.78 (5H, m, CH_2 piperazine, CH cyclopropyl), 3.05 (4H, bs, CH_2 piperazine), 3.72 (2H, bs, NH_2), 4.09 (2H, s, CH_2), 6.93 (1H, s, H-5), 7.29 (1H, s, H-5). Anal. ($C_{16}H_{22}N_4O_3S$) C, H, N.

1-cyclopropyl-4-hydroxy-*N*-methyl-7-(4-methyl-1-piperazinyl)-6-nitro-1*H*-2,1-benzothiazine-3-carboxamide 2,2-dioxide (9).



9

A suspension of **6** (0.4 g, 0.9 mmol) in a solution of $MeNH_2$/EtOH 33% (2 mL) was allowed to react in a sealed tube at 80 °C for 48 h. After cooling, the solvent was evaporated to dryness and the residue purified by column chromatography eluting with CH_2Cl_2/MeOH/NH_4OH (88:10:2). **9** was obtained as an orange-brown solid (0.14 g, 36%) after recrystallization from EtOH: mp 255.5-256.8 °C; ^{1}H NMR (DMSO-d_6) δ 0.40-0.75 and 1.10-1.35 (each 2H, m, CH_2 cyclopropyl), 2.68 (3H, d, J = 3.5 Hz, NHC*H*$_3$), 2.79 (3H, s, CH_3 piperazine), 3.00-3.40 (9H, m, CH cyclopropyl and CH_2 piperazine), 7.05 (1H, s, H-8), 8.52 (1H, s, H-5), 9.63 (1H, bs, N*H*CH_3). Anal. ($C_{18}H_{23}N_5O_6S$) C, H, N.

Table S1. Elemental analysis data for the synthesized compounds.

			Calculated (%)			Found (%)		
Compd.	**MW**	**Chemical Formula**	**C**	**H**	**N**	**C**	**H**	**N**
3	207.24	$C_7H_{13}NO_4S$	40.57	6.32	6.76	40.33	6.39	6.64
12	149.21	$C_5H_{11}NO_2S$	40.25	7.43	9.39	39.89	7.47	9.44
5	372.32	$C_{14}H_{13}FN_2O_7S$	45.16	3.52	7.52	45.22	3.49	7.45
14	330.74	$C_{12}H_{11}ClN_2O_5S$	43.58	3.35	8.47	43.49	3.39	8.51
6	452.48	$C_{19}H_{24}N_4O_7S$	50.43	5.35	12.38	50.54	5.31	12.29
16	394.45	$C_{17}H_{22}N_4O_5S$	51.76	5.62	14.20	51.87	5.54	13.97
7	422.50	$C_{19}H_{26}N_4O_5S$	54.01	6.20	13.26	53.79	6.32	13.05
10	407.49	$C_{18}H_{25}N_5O_4S$	53.06	6.18	17.19	52.94	6.21	17.36
17	364.46	$C_{17}H_{24}N_4O_3S$	56.02	6.64	15.37	56.33	6.52	15.18
15	386.35	$C_{15}H_{15}FN_2O_7S$	46.63	3.91	7.25	46.89	3.77	7.41
18	436.53	$C_{20}H_{28}N_4O_5S$	55.03	6.47	12.83	54.81	6.34	13.05
8	350.43	$C_{16}H_{22}N_4O_3S$	54.84	6.33	15.99	54.67	6.35	16.13
9	437.47	$C_{18}H_{23}N_5O_6S$	49.42	5.30	16.01	49.53	5.09	15.96

Table S2. LC-MS analysis data for the target compounds.

				Exact Mass	
				Calcd	**Found**
Compd.	**t_R (min)**	**Purity (%)**	**Chemical Formula**		**MS (ESI): *m/z* $(M + H)^+$**
7	2.51	> 98	$C_{19}H_{26}N_4O_5S$	422.16239	423.16975
8	1.85	> 98	$C_{16}H_{22}N_4O_3S$	350.14126	351.14909
10	2.38	> 98	$C_{18}H_{25}N_5O_4S$	407.16273	408.16969
17	2.12	> 98	$C_{17}H_{24}N_4O_3S$	364.15691	365.16459
18	2.62	> 96	$C_{20}H_{28}N_4O_5S$	436.17804	437.18578

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