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Endothelin Receptor Antagonists: Synthesis and Structure–Activity Relationships of Substituted Benzothiazine-1,1-dioxides

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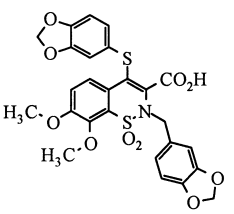
Abstract—The development of benzothiazine-1,1-dioxide derivatives as a new structural class of potent endothelin receptor antagonists is described. Structure–activity relationships (SAR) revealed that PD164800 (**1**) is a potent antagonist of the ET_A receptor subtype.

Introduction

Endothelin-1 (ET-1) is an endogenous peptide that has been implicated in a number of human diseases including hypertension, congestive heart failure, renal failure, pulmonary hypertension, ischemia and cerebral vasospasm.[1–9] In humans, ET-1 interacts with least two receptor subtypes (ET_A and ET_B).[10–13] A third receptor subtype (ET_C) has been identified in the frog, Xenopus laevis; however, this receptor subtype has not been described in humans.[14,15]

A number of nonpeptide ET_A/ET_B nonselective antagonists have been reported including Ro 46-2005,[9] Ro 47-0203 (bosentan),[16] CGS 27830,[17] and particularly from these laboratories a series of 1,3-diaryl-2-carboxyindoles.[18] In addition, nonpeptide ET_A selective antagonists that have been reported include PD 156707,[19] BMS 182874,[20] L-749,329,[21] SKF 209670,[22] and from these laboratories a series of 1,3-disubstituted-2-carboxyindoles.[18,23]



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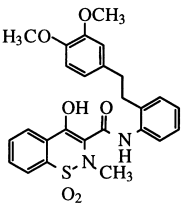
In our continuing efforts to design novel endothelin antagonists we have optimized a weakly active lead, **2** (ET_A $IC_{50} = 16\,\mu M$), obtained by screening the Parke-Davis library of compounds.



2

Results and Discussion

Chemistry

The endothelin antagonists in this study were prepared via the intermediacy of a 4-trifluoromethanesulphonyl-oxy-benzothiazine dioxide derivative which underwent: (i) displacement with a suitably substituted sodium thiophenoxide, followed by hydrolysis, to afford the compounds of Table 1, or alternatively, (ii) a palladium mediated aryl cross coupling reaction, followed by hydrolysis, to afford the compounds of Table 2.

Both procedures proved to be particularly general and were tolerant of a number of thiophenols and boronic

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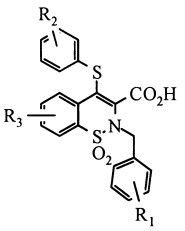
acids. The requisite thiophenols were readily prepared by treatment of aryl lithiums with elemental sulphur[24] or from the reaction of the corresponding aryl iodides with thiourea and a nickel catalyst.[25] The boronic acid derivatives were generated by trapping the aryl lithium, or Grignard, with trimethylborate.[26]

Scheme 1 depicts an illustrative example for the synthesis of the compounds without substitution on the parent benzo-ring. The reaction sequence was initiated by the alkylation of saccharin, **3**, with methyl bromoacetate followed by ring expansion with sodium methoxide in dimethylformamide (DMF) to give the benzothiazine dioxide **4**.[27] Fortunately, benzylation with 3,4-methylenedioxybenzyl chloride proceeded selectively to afford the derivative **5**. In fact, the difference in pK_a of the sulfonamide and the enol ester was sufficient to allow benzylation with a variety of substituted benzyl halides. With a readily accessible route to compounds of type **5** we treated the 2-benzyl benzothiazine dioxide with trifluoromethanesulphonic anhydride and pyridine in dichloromethane to afford the crucial 4-trifluoromethane sulphonate intermediate **6** which could be isolated, but was generally used directly. Addition of the sodium salt of a thiophenol to a solution of the trifluoromethane sulphonate **6** in DMF readily afforded the addition–elimination adduct which was hydrolyzed with aqueous base to afford the final target compounds of Table 1. Alternatively, the trifluoromethane sulphonate **6** was treated with 3,4-methylenedioxyphenyl boronic acid and catalytic palladium(0) tetrakistriphenylphosphine in toluene/DMF, which upon refluxing afforded the 4-aryl adduct in good yield. Hydrolysis with aqueous base afforded the target compounds of Table 2.

Table 1. SAR of pharmacophore **18**



18

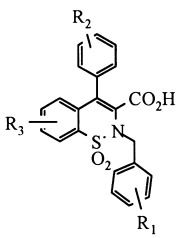
Compd	R_1	R_2	R_3	ET_A IC_{50} (nM)	ET_B IC_{50} (nM)
7	3,4-methylenedioxy	3,4-methylenedioxy	H	210	6100
19	3,4-methylenedioxy	3,4-dimethoxy	H	230	8200
20	3,4-methylenedioxy	3,4,5-trimethoxy	H	230	22000
21	3,4-methylenedioxy	3,4-ethylenedioxy	H	420	5600
22	3,4-methylenedioxy	4-methoxy	H	440	21000
23	3,4-methylenedioxy	4-methyl	H	450	20000
24	3,4-methylenedioxy	3-methoxy	H	460	11000
25	3,4-methylenedioxy	H	H	540	22000
26	3,4-methylenedioxy	4-chloro	H	790	20000
27	3,4-methylenedioxy	3,4-dichloro	H	800	20000
28	3,4-methylenedioxy 5-methoxy	3,4-methylenedioxy	H	580	3900
29	H	3,4-methylenedioxy	H	230	16000
30	4-methyl	3,4-methylenedioxy	H	370	5300
31	3,4,5-trimethoxy	3,4-methylenedioxy	H	460	19000
32	4-chloro	3,4-methylenedioxy	H	530	14000
33	3,4-methylenedioxy 6-chloro	3,4-methylenedioxy	H	590	13000
34	3,4-dichloro	3,4-methylenedioxy	H	520	19000
35	4-methoxy	3,4-methylenedioxy	H	800	3900
36	3,4-methylenedioxy	3,4-methylenedioxy	8-methoxy	280	11000
1	3,4-methylenedioxy	3,4-methylenedioxy	7,8-dimethoxy	100	4000
37	3,4-methylenedioxy	3,4-methylenedioxy	7-methoxy	240	5300
38	3,4-methylenedioxy	3,4-methylenedioxy	6,7-methylenedioxy	290	2100
39	3,4-methylenedioxy	3,4-methylenedioxy	7-chloro	530	4500
40	3,4-methylenedioxy	3,4-methylenedioxy	6-chloro	670	17000
41	3,4-methylenedioxy	3,4-methylenedioxy	6,7-dimethoxy	800	5200
42	3,4-methylenedioxy	3,4-methylenedioxy	6-methoxy	2700	20000

The synthesis of compounds with substitution on the parent benzo-ring required significantly more synthetic steps. The 5-methoxysaccharin derivative that was required to prepare compounds **42** and **64** was synthesized as described by Shkulev.[28] Schemes 2 and 3 illustrate the synthetic routes employed to prepare the other compounds with substitution on the parent benzo-ring.

The 8-methoxy-benzothiazine dioxide **12**, which was used to prepare compounds **36** and **58**, was synthesized as outlined in Scheme 2. Esterification of **9** with hydrogen chloride gas in methanol followed by Newman rearrangement[29] of the corresponding thiocarbamate yielded **10**. Oxidation with sulfuryl chloride and potassium nitrate[30] and addition of glycine methyl ester produced the saccharin derivative **11**. Ring expansion with sodium methoxide in DMF afforded the benzothiazine dioxide **12**. Compounds **37** and **59** were prepared from 2-hydroxy-4-methoxy-benzoic acid by the same reaction sequence that was used to prepare compounds **36** and **58**.

The 7,8-dimethoxy-benzothiazine dioxide **17**, which was used to prepare compound **1**, was synthesized as outlined in Scheme 3. Starting with 3,4-dimethoxy-2-nitrobenzoic acid **13**, prepared as described by Hess,[31] the substituted methyl anthranilate **14** was isolated after esterification of the carboxylic acid and reduction of the nitro group. Diazotization with sodium nitrite and cupric chloride in acetic acid followed by treatment with sulfur dioxide surprisingly gave the disulfide **15**, not the expected sulfonyl chloride.[32] Fortunately, the disulfide was easily oxidized to the sulfonyl chloride in a manner similar to that described for the oxidation of **10**.[30] The saccharin derivative **16** was isolated after treating the sulfonyl chloride with glycine methyl ester, triethylamine, and 4-dimethylaminopyridine (DMAP) in dichloromethane. Ring expansion occurred upon exposure to sodium methoxide in DMF to afford the benzothiazine dioxide **17**.

Table 2. SAR of pharmacophore **43**



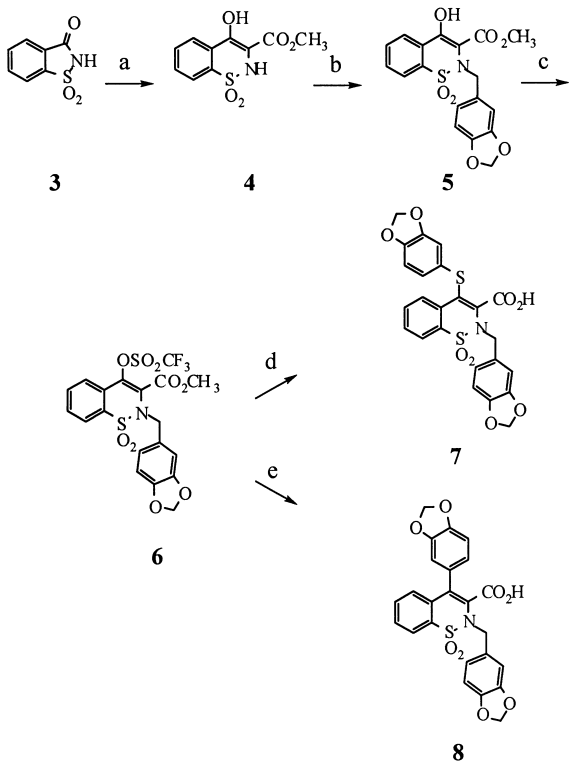
43

Compd	R_1	R_2	R_3	ET_A IC_{50} (nM)	ET_B IC_{50} (nM)
8	3,4-methylenedioxy	3,4-methylenedioxy	H	380	2500
44	3,4-methylenedioxy	3,4-dimethoxy	H	2000	4400
45	3,4-methylenedioxy	3,4,5-trimethoxy	H	15000	25000
46	3,4-methylenedioxy	3,4-ethylenedioxy	H	630	6500
47	3,4-methylenedioxy	4-methoxy	H	5700	21000
48	3,4-methylenedioxy	3-methoxy	H	1400	13000
49	3,4-methylenedioxy	4-chloro	H	11000	33000
50	3,4-methylenedioxy 5-methoxy	3,4-methylenedioxy	H	540	2900
51	H	3,4-methylenedioxy	H	410	9300
52	4-methyl	3,4-methylenedioxy	H	900	32000
53	3,4,5-trimethoxy	3,4-methylenedioxy	H	530	18000
54	4-chloro	3,4-methylenedioxy	H	700	13000
55	3,4-methylenedioxy 6-chloro	3,4-methylenedioxy	H	980	2700
56	3,4-dichloro	3,4-methylenedioxy	H	2300	15000
57	4-methoxy	3,4-methylenedioxy	H	450	5100
58	3,4-methylenedioxy	3,4-methylenedioxy	8-methoxy	1400	7400
59	3,4-methylenedioxy	3,4-methylenedioxy	7-methoxy	12000	8300
60	3,4-methylenedioxy	3,4-methylenedioxy	6,7-methylenedioxy	2800	1300
61	3,4-methylenedioxy	3,4-methylenedioxy	7-chloro	3900	8900
62	3,4-methylenedioxy	3,4-methylenedioxy	6-chloro	390	3800
63	3,4-methylenedioxy	3,4-methylenedioxy	6,7-dimethoxy	830	1700
64	3,4-methylenedioxy	3,4-methylenedioxy	6-methoxy	1800	1700

Similarly, compounds **38** and **60** were prepared from 2-nitro-4,5-methylenedioxy benzoic acid,[33] compounds **41** and **63** were prepared from methyl 2-amino-4,5-dimethoxybenzoate, and compounds **39**, **40**, **61**, and **62** were prepared from the appropriately substituted disulfide.[34]

Structure–activity relationships

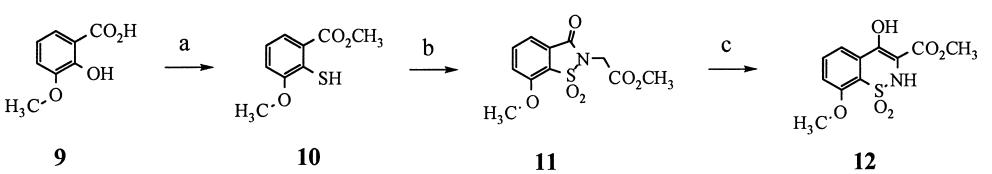
Structure–activity relationships were investigated using IC_{50} values obtained from receptor binding in Ltk-cells expressing recombinant human receptors (ET_A), and CHO-K1 cells expressing recombinant human receptors (ET_B).[19,35]



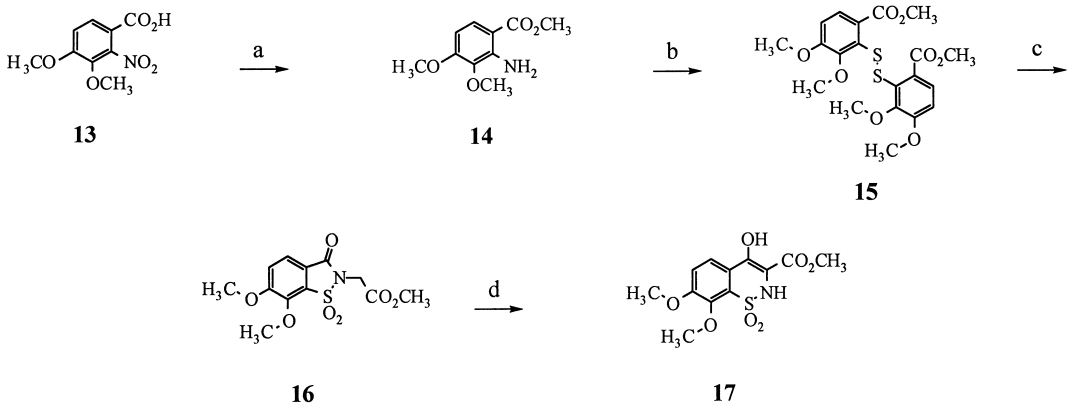
Scheme 1. (a) i.$BrCH_2CO_2CH_3$, NaH, DMF, rt, 18 h (65%). ii. $NaOCH_3$, rt, 0.5 h (68%); (b) 3,4-methylenedioxybenzyl chloride, NaH, DMF, rt, 5 h (69%); (c) trifluoromethanesulfonic anhydride, pyr, CH_2Cl_2, rt, 0.5 h (95%); (d) i. 3,4-methylenedioxythiophenol, NaH, DMF, 2 h, rt, (79%); ii. LiOH, THF, H_2O, CH_3OH, rt, 18 h (90%); (e) i. 3,4-methylenedioxyphenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, toluene, DMF, reflux, 2 h (75%); ii. LiOH, THF, H_2O, CH_3OH, rt, 18 h (90%).

Previously we have demonstrated that 1-benzyl-3-thioaryl-2-carboxyindoles and 1-benzyl-3-aryl-2-carboxyindoles are potent endothelin antagonists.[18,23] Therefore, we decided that in the optimization of the weakly active chemical lead compound **2** we would incorporate pharmacophores evident from those series of compounds. This strategy proved to be particularly successful as judged by the activity of the compounds in Tables 1 and 2. Typically we investigated the effect of electron donating substituents on ET_A receptor binding affinity by comparing binding affinity to the appropriate unsubstituted parent. In Table 1, and in particular compounds **7**, and **19**–**27**, it was apparent that while the effect of the substituent R2 was not particularly striking, electron donating substituents were preferred. Furthermore multiple electron donating groups appeared to favorably effect binding affinity of the compounds. Thus for further SAR investigations the 3,4-methylenedioxy substituent was selected as the preferred substituent at R2. Investigation of the R1 substituent with compounds **7**, and **28**–**35** indicated, in analogy with previous SAR from the 1,3-disubstituted 2-carboxy indoles, that the 3,4-methylenedioxybenzyl substituent was optimal. Thus at this point compound **7** appeared most optimal in terms of binding affinity as it relates to substitution pattern at R1 and R2. Upon investigation of the R3 substituent with compounds **36**–**42** we observed a modest increase in binding affinity occurred with compound **1**. This result stood in stark contrast to 1-benzyl-3-thioaryl-2-carboxy indoles[23] where bis methoxy substitution on the benzo-ring dramatically improved ET_A receptor binding affinity. Clearly there are some subtle differences between the binding mode and associated protein interaction of the benzothiazine dioxides and the indole derivatives.

Since **1** represented the most active compound from Table 1, we advanced this compound to some secondary assays. The first of these assessed the ability of the compound to inhibit the ET-1 induced release of arachidonic acid in cultured rabbit renal vascular smooth



Scheme 2. (a) i. HCl/CH_3OH, reflux, 2 h (97%); ii. *N*,*N*-dimethylthiocarbamoyl chloride, DABCO, DMF, rt, 18 h (72%); iii. neat, 180 °C, 3 h (85%); iv. $NaOCH_3$, CH_3OH, relux, 18 h (95%); (b) i. KNO_3, SO_2Cl_2, CH_3CN, rt. 18 h (65%); ii. glycine methyl ester, Et_3N, DMAP, CH_2Cl_2, 18 h (47%); (c) $NaOCH_3$, DMF, 0.5 h (83%).



Scheme 3. (a) i. CH_3I, Cs_2CO_3, DMF, rt, 18 h (96%); ii. H_2 Pd/C, rt. 18 h (quantitative); (b) $NaNO_2$, SO_2, CH_3CO_2H, 0 °C to rt, 18 h (52%); (c) i. KNO_3, SO_2Cl_2, CH_3CN, 5 h (78%); ii. glycine methyl ester, Et_3N, DMAP, CH_2Cl_2, 0 °C, 1 h (69%); (d) $NaOCH_3$, DMF, 0.5 h (35%).

muscle cells which express ET_A receptors coupled to arachidonic acid.[35] The IC_{50} of 150 nM indicated that **1** displayed reasonable antagonistic activity. The second functional assay then assessed the ability of the test compound to inhibit the contraction of rabbit femoral artery rings upon stimulation with ET-1, a functional response known to be mediated by the agonistic activity of ET-1 upon ET_A receptors.[35] A pA2 of 5.8 confirmed that **1** was a functional ET_A antagonist.

Interestingly, Table 2 shows that pharmacophore **43** afforded less potent compounds. We explored SAR in a similar fashion to pharmacophore **18** by observing the effects of substituents at R1, R2, and R3. While similar trends in activity were seen for these substituents to pharmacophore **18** none of these compounds were as potent as compound **1**.

Conclusion

A new series of endothelin antagonists was designed and synthesized by incorporating structure–activity relationships afforded by a previous series of indole based endothelin antagonists, in combination with a chemical lead compound uncovered by screening the historical library collection of Parke-Davis compounds. The most potent compound synthesized, **1**, was demonstrated to be a functional antagonists of the ET_A receptor. Further in vivo studies are currently underway to aid in the elucidation of the physiological and pathophysiological role of endothelin.

Experimental

Proton NMR spectra were recorded on a Varian Gemini 2000 (300 MHz) or a Varian Unity plus (400 MHz) instruments; shifts are reported in δ units relative to internal tetramethylsilane. Mass spectra were obtained on a Finnigan 4500 or a VG analytical 7070 E/HF spectrometer. High Resolution mass spectra were obtained on a Finnigan MAT 900Q spectrometer. Elemental analyses were performed on a CEC Model 240 elemental analyzer. Column chromatography was performed using E. Merck silica gel 60 (230–400 mesh). Thin-layer chromatography was performed with glass-backed silica gel ($60F_{254}$) plates employing UV light, iodine vapor, or aqueous potassium permanganate/potassium carbonate stain.

The binding assay is explained in detail in reference 35. Briefly, cultured Ltk-cells expressing human cloned ET_A receptors and CHO-K1 cells expressing human cloned ET_B receptors were used. Binding of [^{125}I]ET-1 (ET_A) or [^{125}I]ET-3 (ET_B) to membranes prepared from the cells above was performed by adding radiolabeled ET-1 or ET-3 to membranes in the absence or presence of increasing concentrations of the tested compound. At the end of the incubation (37 °C, 2 h), free and bound ligands were separated by filtration and counted with a gamma counter. Nonspecific binding was defined as the binding in the presence of 100 nM unlabeled ET-1 or ET-3. Specific binding was computer-analyzed by non-linear least squares curve fitting giving the best fit for a one-site model. IC_{50} values were derived from the average of two to ten competition experiments in which data points were measured in triplicate.

1,2-Benzisothiazole-2(3*H*)-acetic acid, 3-oxo-, methyl ester, 1,1-dioxide. To a solution of saccharin, **3** (40 g, 0.218 mol) in DMF (100 mL) at 0 °C was added sodium hydride (8.73 g, 60% in mineral oil, 0.218 mol). After 15 min methyl bromoacetate (20.7 mL, 0.218 mol) was added and the mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with

dichloromethane (250 mL) and washed with saturated sodium bicarbonate (2×180 mL), water (100 mL), and brine (2×150 mL). The organic phase was dried with magnesium sulfate, evaporated in vacuo, and the product crystallized from hot ethanol to give the title compound (36.3 g, 65%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.81 (s, 3H), 4.47 (s, 2H), 7.84–7.94 (m, 2H), 7.96 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H). Anal. calcd for $C_{10}H_9N_1O_5S_1$: C, 47.06; H, 3.55; N, 5.49; found C, 47.02; H, 3.68; N; 5.37. MS (CI) 256 (M + 1).

4-Hydroxy-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid methyl ester (4). To methanol (100 mL) was added sodium (5.4 g, 0.23 mol) portionwise. Once all the sodium had dissolved the solution was concentrated in vacuo and the final traces of methanol were removed under high vacuum. The sodium methoxide was suspended in dry DMF (65 mL) and then added to a solution of 1,2-benzisothiazole-2(3*H*)-acetic acid, 3-oxo-, methyl ester, 1,1-dioxide (20 g, 0.078 mol) in DMF (30 mL) at 0 °C over 7 min. Upon complete addition of the sodium methoxide the reaction mixture was allowed to stir for 30 min more. The product was precipitated from the reaction mixture with the dropwise addition of 1 N hydrochloric acid (430 mL), washed with water (200 mL), and dried at 52 °C under vacuum overnight to give **4** (13.6 g, 68%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.95 (s, 3H), 6.27 (bs, 1H), 7.68–7.77 (m, 2H), 7.90 (d, J = 7.5 Hz, 1H), 8.08 (d, J = 8.7 Hz, 1H). Anal. calcd for $C_{10}H_9N_1O_5S_1$: C, 47.06; H, 3.55; N, 5.49; found C, 47.11; H, 3.67; N, 5.16. MS (CI) 256 (M + 1).

2-Benzo[1,3]dioxol-5-ylmethyl-4-hydroxy-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid methyl ester (5). To **4** (1.48 g, 5.84 mmol) in DMF (10 mL) was added sodium hydride (0.257 g, 60% in mineral oil, 6.4 mmol). After 5 min 3,4-methylenedioxybenzyl chloride (2.2 g, 50 wt.% in dichloromethane, 6.4 mmol) was added and the mixture stirred at room temperature. After 18 h the solution was diluted with ethyl acetate (100 mL), washed with water (2×80 mL), brine (80 mL), and dried over magnesium sulfate. The organic phase was evaporated in vacuo, and the product crystallized from ethyl acetate/heptane to give **5** (1.63 g, 72%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.97 (s, 3H), 4.60 (bs, 2H), 5.76 (s, 2H), 6.33–6.42 (m, 3H), 7.52–7.62 (m, 2H), 7.71–7.77 (m, 2H), 12.06 (s, 1H). Anal. calcd for $C_{18}H_{15}N_1O_7S_1$: C, 55.52; H, 3.88; N, 3.60; found C, 55.45; H, 3.72; N, 3.49. MS (CI) 389 (M + 1).

2-Benzo[1,3]dioxol-5-ylmethyl-6,7-dimethoxy-1,1-dioxo-4-(trifluoro-methanesulfonyloxy)-1,2-dihydro-1λ^6-benzo[*e*]-[1,2]thiazine-3-carboxylic acid methyl ester (6). To a solution of **5** (3.0 g, 7.71 mmol) in dichloromethane (15 mL) and pyridine (3.1 mL, 38.6 mmol) at 0 °C was added trifluoromethanesulfonic anhydride (1.56 mL, 9.25 mmol). After 2 h the reaction was diluted with ethyl acetate (100 mL), washed with 1 N hydrochloric acid (2×50 mL), brine (50 mL), dried with magnesium sulfate, and then the organic phase was evaporated in vacuo to give **6** (4.0 g, quantitative). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.94 (s, 3H), 4.85 (s, 2H), 5.82 (s, 2H), 6.28 (s, 1H), 6.36 (d, J = 7.8 Hz, 1H), 6.49 (d, J = 7.8 Hz, 1H), 7.66–7.73 (m, 3H), 7.88–7.90 (m, 1H). MS (CI) 521 (M + 1).

2-Benzo[1,3]dioxol-5-ylmethyl-4-(benzo[1,3]dioxol-5-yl-sulfanyl)-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid methyl ester. A solution of **6** in DMF (3 mL) was added to sodium 1,3-benzodioxole-5-thiolate (0.31 g, 2.0 mmol)—prepared by dissolving 1,3-benzodioxole-5-thiol (0.31 g, 2.0 mmol) in DMF (3 mL) and stirring with sodium hydride (0.081 g, 2.0 mmol) for 5 min—in DMF (3 mL). After stirring at room temperature for 2 h the mixture was diluted with ethyl acetate (100 mL), washed with 1 N sodium hydroxide (2×50 mL), brine (50 mL), dried with magnesium sulfate, and evaporated in vacuo. Silica gel column chromatography eluting with 25% ethyl acetate in hexane afforded the title compound as a foam (0.64 g, 79%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.86 (s, 3H), 4.76 (s, 2H), 5.85 (s, 2H), 5.86 (s, 2H), 6.31–6.63 (m, 4H), 6.69–6.73 (m, 2H), 7.43–7.50 (m, 2H), 7.79–7.86 (m, 2H). Anal. calcd for $C_{25}H_{19}N_1O_8S_2$: C, 57.14; H, 3.64; N, 2.67; found C, 56.93; H, 3.93; N; 2.51. MS (CI) 525 (M + 1).

2-Benzo[1,3]dioxol-5-ylmethyl-4-(benzo[1,3]dioxol-5-yl-sulfanyl)-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid (7). To 2-benzo[1,3]dioxol-5-ylmethyl-4-(benzo[1,3]dioxol-5-ylsu)-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid methyl ester (0.467 g, 0.89 mmol) in THF (5 mL), methanol (2 mL), water (2 mL) was added lithium hydroxide (0.46 g, 19 mmol) and the reaction mixture was stirred at room temperature. After 5 h the reaction mixture was diluted with water (80 mL) and 50% hydrochloric acid (10 mL) and extracted with chloroform (3×80 mL). The organic phase was dried with magnesium sulfate and evaporated in vacuo to afford **7** as a foam (0.4 g, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.78 (s, 2H), 5.77 (s, 2H), 5.83 (s, 2H), 6.41 (s, 1H), 6.44–6.50 (m, 2H), 6.57–6.68 (d, J = 11.6 Hz, 1H), 6.69–6.73 (m, 2H), 7.38–7.40 (t, 1H), 7.42–7.46 (t, 1H), 7.74–7.79 (m, 2H) (CO_2H absent). Anal. calcd for $C_{24}H_{17}N_1O_8S_2$: C, 56.35; H, 3.35; N, 2.74 found C, 56.13; H, 3.49; N, 2.62. MS (CI) 511 (M +).

4-Benzo[1,3]dioxol-5-yl-2-benzo[1,3]dioxol-5-ylmethyl-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid methyl ester. To **6** (0.66 g, 1.27 mmol) in toluene (10 mL) and DMF (2 mL) was added 3,4-methylene-

dioxy-phenylboronic acid (0.35 g, 2.13 mmol), potassium carbonate (0.29 g, 2.13 mmol), tetrakis(triphenylphosphine)palladium(0) (0.16 g, 0.14 mmol) and the mixture heated at reflux. After 2 h the reaction mixture was cooled to room temperature, diluted with ethyl acetate (100 mL) washed with sodium bicarbonate (80 mL aq satd), brine (80 mL), dried with magnesium sulfate, and evaporated in vacuo. The residue was purified with a silica gel column eluted with 50% ethyl acetate in hexane to give the title compound (0.47 g, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.59 (s, 3H), 4.83 (s, 2H), 5.80 (s, 2H), 6.00 (s, 2H), 6.52–60 (m, 4H) 6.64 (s, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 8.0 Hz, 1H), 7.39–7.45 (t, 1H), 7.47–7.53 (t, 1H), 7.84 (d, J = 8.0 Hz, 1H) Anal. calcd for $C_{25}H_{19}N_1O_8S_1$: C, 60.85; H, 3.88; N, 2.84: found C, 61.02; H, 3.96; N, 2.71. MS (CI) 494 (M + 1).

4-Benzo[1,3]dioxol-5-yl-2-benzo[1,3]dioxol-5-ylmethyl-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid (8). To 4-benzo[1,3]dioxol-5-yl-2-benzo[1,3]dioxol-5-ylmethyl-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid methyl ester (0.47 g, 0.95 mmol) in THF (10 mL), methanol (3 mL), water (3 mL) was added lithium hydroxide (0.6 g, 25.1 mmol) and the reaction mixture was stirred at room temperature. After 18 h 1 N hydrochloric acid (100 mL) was added and the solution extracted with chloroform (3×80 mL). The combined organic layers were washed with brine (80 mL), dried with magnesium sulfate, and evaporated in vacuo to give **8** (0.42 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.85 (s, 2H), 5.79 (s, 2H), 6.03 (s, 2H), 6.48–6.52 (m, 3H), 6.58–6.61 (m, 2H), 6.82 (d, J = 7.8Hz, 1H), 6.92 (d, J = 7.8Hz, 1H), 7.38–7.42 (t, 1H), 7.52–7.56 (t, 1H) 7.87 (d, J = 7.6 Hz, 1H), (CO_2H absent). Anal. calcd for $C_{24}H_{17}N_1O_8S_1$: C, 60.12; H, 3.57; N, 2.92: found C, 59.80; H, 3.73; N, 2.86. MS (CI) 479 (M + 1).

2-Hydroxy-3-methoxy-benzoic acid methyl ester. To 3-methoxysalicylic acid, **9** (1.0 g, 5.94 mmol) in methanol (25 mL) was bubbled hydrogen chloride (g) for 10 min. The reaction mixture was then heated at reflux. After 2 h the solvent was evaporated in vacuo, the residue was dissolved in chloroform (25 mL), passed through a pad of silica gel, and evaporated filtrate to give the title compound (1.06 g, 98%) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.86 (s, 3H), 3.91 (s, 3H), 6.77–6.80 (t, 1H), 7.01 (d, J = 8.1 Hz, 1H), 7.38 (d, J = 8.1 Hz, 1H), 10.97 (s, 1H).

2-Dimethylthiocarbamoyloxy-3-methoxy-benzoic acid methyl ester. To 2-hydroxy-3-methoxy-benzoic acid methyl ester (1.05 g, 5.77 mmol) in DMF (5 mL) was added DABCO (2.59 g, 23.1 mmol) and *N*,*N*-dimethylthiocarbamoyl chloride (2.85 g, 23.1 mmol), the reaction mixture was stirred at room temperature. After 18 h the reaction mixture was diluted with ethyl acetate (100 mL), washed with 1 N hydrochloric acid (80 mL), brine (80 mL). The organic phase was dried with magnesium sulfate, evaporated in vacuo, and purified with silica gel column eluted with 50% ethyl acetate in hexane to give the title compound (1.11 g, 72%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.36 (s, 3H), 3.43 (s, 3H), 3.80 (s, 3H), 3.82 (s, 3H), 7.12 (d, J = 8.2 Hz, 1H), 7.22 (t, 1H), 7.53 (d, J = 8.2 Hz, 1H). Anal. calcd for $C_{12}H_{15}N_1O_4S_1$: C, 53.52; H, 5.61; N, 5.20; found C, 53.60; H, 5.63; N, 5.14. CI (MS) 269 (M + 1).

2-Dimethylcarbamoylsulfanyl-3-methoxy-benzoic acid methyl ester. Heated 2-dimethylthiocarbamoyloxy-3-methoxy-benzoic acid methyl ester (15.0 g, 55.8 mmol) neat at 180 °C for 3 h. The residue was purified with a silica gel column eluted with 65% ethyl acetate in hexane to give the title compound (12.78 g, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.95 (bs, 3H), 3.10 (bs, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 7.05 (d, J = 8.3 Hz, 1H), 7.29–7.40 (m, 2H). MS (CI) 270 (M + 1).

2-Mercapto-3-methoxy-benzoic acid methyl ester (10). To 2-dimethylcarbamoylsulfanyl-3-methoxy-benzoic acid methyl ester: (12.0 g, 44.6 mmol) in methanol (120 mL) was added sodium hydride (5.4 g of 60%, 133.8 mmol) and the reaction mixture was heated at reflux. After 1.5 h the solvent was evaporated in vacuo. Then 1 N hydrochloric acid (300 mL) was added and the solution was extracted with ether (3×150 mL). The combined organic phases were washed with brine (100 mL), dried with magnesium sulfate, and evaporated in vacuo to give **10** (8.36 g, 95%), which was used without further purification. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.79 (s, 3H), 3.85 (s, 3H), 5.30 (s, 1H), 7.12–7.31 (m, 2H), 7.53 (d, J = 7.7, 1H).

2-Chlorosulfonyl-3-methoxy-benzoic acid methyl ester. To **10** (8.28 g, 41.8 mmol) in acetonitrile (100 mL) was added potassium nitrate (10.6 g, 105 mmol). The reaction was cooled to 0 °C and sulfuryl chloride (8.3 mL, 105.0 mmol) was added over 5 min. The reaction mixture was warmed to room temperature and stirred for 18 h. The solvent volume was reduced to 25 mL in vaccuo, and sodium bicarbonate (120 mL, aq satd) was added. The resulting solution was extracted with ethyl acetate (3×100 mL) and the combined organic phases were washed with brine (100 mL), dried with magnesium sulfate, and evaporated in vacuo to give the title compound (7.0 g, 64%), which was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.89 (s, 3H), 4.04 (s, 3H), 7.04 (d, J = 7.9 Hz, 1H). 7.15 (d, J = 7.9 Hz, 1H), 7.64–7.68 (t, 1H). MS (CI) 264 (M +).

(7-Methoxy-1,1,3-trioxo-1,3-dihydro-1λ^6-benzo[d]isothiazol-2-yl)-acetic acid methyl ester (11). To 2-chlorosulfonyl-3-methoxy-benzoic acid methyl ester (6.5 g, 24.5 mmol) in dichloromethane (75 mL) at 0 °C was

added triethylamine (7.5 mL, 54.0 mmol), glycine methyl ester hydrochloride (3.4 g, 27.0 mmol), 4-dimethylaminopyridine (0.1 g, 0.8 mmol) and the reaction mixture stirred at room temperature. After 18 h the solvent volume was reduced to 30 mL in vacuo and ethyl acetate (120 mL) was added. The organic phase was washed with 1 N hydrochloric acid (80 mL), brine (80 mL), dried with magnesium sulfate, and evaporated in vacuo to give an oil. The oil was dissolved in ethyl acetate and the product was precipitated with the addition of hexane to give **12** (3.3 g, 47%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.76 (s, 3H), 4.02 (s, 3H), 4.40 (s, 2H), 7.28 (d, J = 7.9, 1H), 7.58 (d, J = 7.9 Hz, 1H), 7.72–7.59 (t, 1H). MS (CI) 286 (M + 1).

4-Hydroxy-8-methoxy-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*]-[1,2]thiazine-3-carboxylic acid methyl ester (12). To **11** (3.0 g, 9.46 mmol) in DMF (10 mL) was added sodium methoxide (2.0 g, 37.9 mmol). The reaction mixture was stirred for 30 min at room temperature then quenched with 1 N hydrochloric acid (100 mL) and **12** (2.24 g, 83%) was collected as a light yellow precipitate. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.82 (s, 3H), 3.87 (s, 3H), 7.42 (d, J = 8.2 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.69–7.73 (t, 1H), 9.70 (s, 1H), 11.35 (s, 1H). MS (CI) 286 (M + 1).

3,4-Dimethoxy-2-nitro-benzoic acid methyl ester. To **13** (25 g, 110 mmol) in DMF (70 mL) was added cesium carbonate (71.8 g, 220 mmol) and methyl iodide (10.3 mL, 165 mmol) and the reaction mixture stirred at room temperature. After 18 h water (1500 mL) was added and crystals of the title compound (25.4 g, 96%) were collected. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.87 (s, 3H), 3.92 (s, 3H), 3.98 (s, 3H), 7.02 (d, J = 8.8 Hz, 1H), 7.81 (d, J = 8.8 Hz, 1H). Anal. calcd for $C_{10}H_{11}N_1O_6$: C, 49.80; H, 4.60; N, 5.81; found C, 49.76; H, 4.57; N, 5.75. MS (CI) 242 (M + 1).

2-Amino-3,4-dimethoxy-benzoic acid methyl ester (14). To 3,4-dimethoxy-2-nitro-benzoic acid methyl ester (25.3 g, 0.1 mol) in THF (500 mL) and methanol (500 mL) was added 5%Pd/C (3.0 g). The reaction mixture was then hydrogenated at 50 psi overnight. The reaction solution was filtered through celite and the solvent volume reduced by 50% in vacuo. Hydrochloric acid (gas) was bubbled through the solution for 10 min, ether (400 mL) was added, and **14** (25 g, quantitative) was collected as a light-brown precipitate. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.66 (s, 3H), 3.76 (s, 3H), 3.82 (s, 3H), 6.38 (d, J = 9.2 Hz, 1H), 7.52 (d, J = 9.2 Hz, 1H), (NH_2 absent). MS (CI) 242 (M + 1).

Methyl-2,2′-dithiobis[3,4]-dimethoxybenzoate (15). To **14** (16.94 g, 68.44 mmol) in acetic acid (150 mL) and concentrated hydrochloric acid (150 mL) at 0 °C was slowly added a solution of sodium nitrite (4.73 g, 68.4 mmol) in water (30 mL). After 30 min sulfur dioxide (g) was bubbled through the solution for 15 min, followed by the addition of cupric chloride dihydrate (5.85 g, 34.3 mmol) in water (20 mL), and the reaction mixture was warmed to room temperature. After 8 h, sulfur dioxide (g) was bubbled through solution for 10 min and the reaction mixture stirred for 18 h. The reaction mixture was diluted with water (1000 mL) and extracted with ethyl acetate (3×600 mL). The combined organic phases were washed with sodium bicarbonate (2×400 mL, aq satd), brine (400 mL), dried with magnesium sulfate, evaporated in vacuo, and crystallized from dichloromethane/diisopropyl ether to give **15** (8.1 g, 52%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.62 (s, 6H), 3.80 (s, 6H), 3.87 (s, 6H), 6.85 (d, J = 8.6 Hz, 2H), 7.81 (d, J = 8.6 Hz, 2H). Anal. calcd for $C_{20}H_{22}O_8S_2$: C, 52.85; H, 4.88; found C, 52.55; H, 4.74; MS (CI) 455 (M + 1).

2-Chlorosulfonyl-3,4-dimethoxy-benzoic acid methyl ester. To **15** (6.55 g, 14.4 mmol) and potassium nitrate (4.37 g, 43.0 mmol) in acetonitrile (45 mL) was added sulfuryl chloride (3.45 mL, 43.0 mmol) dropwise over 7 min and the reaction mixture was stirred at room temperature. After 5 h sodium bicarbonate (500 mL, aq satd) was added and the solution extracted with ethyl acetate (600 mL). The organic phase was washed with brine (400 mL), dried with magnesium sulfate, and evaporated in vacuo to give the title compound (6.6 g, 78%) as an oil. Used without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.90 (s, 3H), 3.96 (s, 3H), 4.08 (s, 3H), 7.21 (s, 2H).

(6,7-Dimethoxy-1,1,3-trioxo-1,3-dihydro-1λ^6-benzo[d]isothiazol-2-yl)-acetic acid methyl ester (16). To 2-chlorosulfonyl-3,4-dimethoxy-benzoic acid methyl ester (3.4 g, 11.5 mmol) in dichloromethane (130 mL) at room temperature was added triethylamine (6.4 mL, 46.2 mmol) and glycine methyl ester hydrochloride (1.45 g, 11.5 mmol) and the solution stirred at room temperature. After 2 h the solution was diluted with dichloromethane (300 mL), washed with 1 N hydrochloric acid (3×300 mL), brine (300 mL), dried with magnesium sulfate, and the solvent evporated in vacuo. The residue was purified with a silica gel column eluted with 25–35% ethyl acetate in hexane. The product was then crystallized from dichloromethane/diisopropyl ether to give **16** (2.5 g, 69%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.80 (s, 3H), 4.00 (s, 3H), 4.16 (s, 3H), 4.42 (s, 2H), 7.25 (d, J = 8.2 Hz, 1H), 7.73 (d, J = 8.2 Hz, 1H). Anal. calcd for $C_{12}H_{13}N_1O_7S_1$: C, 45.71; H, 4.16; N, 4.44 found C, 45.73; H, 4.12; N, 4.38 MS (CI) 316 (M + 1).

4-Hydroxy-7,8-dimethoxy-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid methyl ester (17). To **17** (0.23 g, 0.73 mmol) in DMF (1.0 mL) at 0 °C was

added sodium methoxide (0.12 g, 2.22 mmol) and the reaction mixture stirred for 4 min. The reaction solution was added to concentrated hydrochloric acid (2 mL)/ice (2.0 g) and **17** (0.105 g, 46%) was collected as a white precipitate. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.79 (s, 3H), 3.80 (s, 3H), 3.90 (s, 3H), 7.45 (d, J = 8.8 Hz, 1H), 7.70 (d, J = 9.0 Hz, 1H), 9.65 (s, 1H), 11.48 (s, 1H). Supplementary material. Those interested in additional compound information may contact the author.

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