of the treated mice beyond that of the control animals; untreated mice survive on average 6.2 days. Compounds were classified as active (A) when the mean survival time of the treated mice is twice that of the controls (>12.4 days), and curative (C) when one or more test animals live 60 days postinfection. Deaths from 0–2 days post-treatment were attributed to toxicity (T).

A slight modification of the Thompson test[18] was used to further quantify antimalarial activity and toxicity. Five week old CD-1 mice were inoculated on day 0 with 5×10^6 trophozoites of *P. berghei* (strain KBG 173) obtained from an infected mouse at 60% parasitemia, diluted with uninfected mouse blood, and injected intraperitonealy. On days 3–5, each group of seven mice were treated sc with the compound to be tested in eight total doses, twice a day for 3 days. A range of doses sufficient to generate a dose–response curve was used. Blood films were taken 1 day after completion of drug treatment (day 6) and weekly thereafter until day 60. Parasitemia values were determined from Giemsa-stained blood films. Drug activity was evaluated by suppression of parasitemia, extension of survival time, and curative activity. Mice living 60 days postinfection and blood film negative were considered cured. A drug was considered to be toxic if the mice died before the untreated control mice. An advantage of this method of evaluation is the ability to assess efficacy and acute toxicity at the same time in the same model.

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Thieno[2,3-*b*]furan-2-sulfonamides as Topical Carbonic Anhydrase Inhibitors

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Novel 5-[(alkylamino)methyl]thieno[2,3-*b*]furan-2-sulfonamides were prepared and evaluated in vitro for inhibition of human carbonic anhydrase II (CA II) and ex vivo for their ability to inhibit CA II in the albino rabbit eye after topical administration. Compound **11a** was found to lower intraocular pressure (IOP) in both the α-CT ocular hypertensive albino rabbit and the normal albino rabbit, but was ineffective at lowering IOP in a hypertensive, pigmented monkey model. Since **11a** was highly bound to ocular pigment, a series of less basic analogs was prepared. Examples in this series were both less extensively bound to ocular pigment and more active at reducing IOP in pigmented rabbits after topical dosing. Key examples displayed moderate reactivity toward glutathione.

Introduction

Topically effective carbonic anhydrase inhibitors that minimize systemic side effects[1] are a potentially important therapeutic advance in the treatment of open-angle glaucoma.[2] Recently, several novel classes of inhibitors, including sulfonamides of benzo[*b*]thiophene,[3] benzo[*b*]furan,[4] 4-aminothienothiopyran,[5] thieno[2,3-*b*]thiophene,[6] and 5-[(hydroxyalkyl)sulfonyl]thiophene,[7] have been reported. Leading representatives in these classes demonstrate nanomolar level potency for inhibition of human carbonic anhydrase II (CA II) in vitro, ocular hypotensive efficacy in animals after topical dosing, minimal sensitization potential, and appropriate solubility at or near physiological pH to allow for dosing as a suspension or a solution. As part of our continuing work in this area we wish to report on the biological evaluation of a series of

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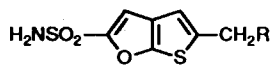
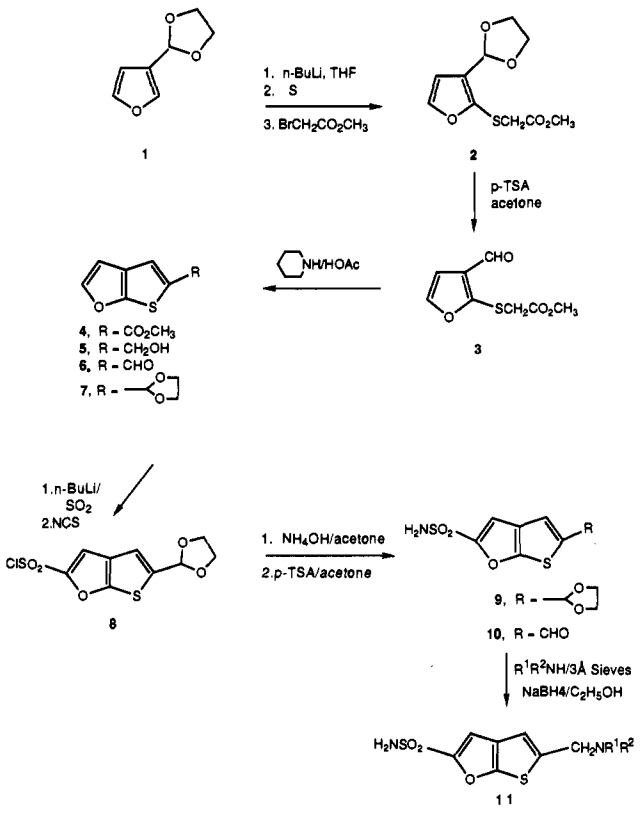
Table I. Analytical, Solubility, and Biological Data for Thieno[3,2-*b*]furan-2-sulfonamides



compd	R	formula	mp, °C	anal.	solubility[a,b] mg/mL	partition coeff[c]	% pigment binding[d]	pK_a[e]	I_{50} × 10^{-9} M[f]	K_i × 10^{-9} M[f]	ex vivo % CA inhibn[g]
11a	$NHCH_3$	$C_8H_{10}N_2O_3S_2 \cdot HCl$	221–223	C,H,N	>51[a]	0.13	77	9.25	9.0	32.2	92 (0.1)
								7.85			84 (0.02)
11b	NHC_2H_5	$C_9H_{12}N_2O_3S_2 \cdot HCl$	225–229	C,H,N	27[a]	0.26	74	9.55	11.0	19.0	93 (0.1)
								8.05			69 (0.02)
11c	NH(*n*-C_3H_7)	$C_{10}H_{14}N_2O_3S_2 \cdot HCl$	251–254	C,H,N	>22.5[a]	1.06	68	9.40	8.0		89 (0.1)
								7.85			63 (0.02)
11d	NH(*i*-C_4H_9)	$C_{11}H_{16}N_2O_3S_2 \cdot HCl$	238–243	C,H,N	15.3[a]	7.0	73	9.10	6.0	7.4	81 (0.1)
								7.55			63 (0.02)
11e		$C_{11}H_{14}N_2O_4S_2 \cdot HCl$	243–246	C,H,N	>4.7[a]	7.65	25	9.20	5.6	1.24	86 (0.1)
					0.97[b]			5.32			
11f	$NHCH_2CH_2OCH_3$	$C_{10}H_{14}N_2O_4S_2 \cdot HCl$	190–192	C,H,N	>27[a]	1.04	73	9.05	11.0		91 (0.1)
					>27[b]			7.00			47 (0.02)
11g	$N(CH_2CH_2OCH_3)_2$	$C_{13}H_{20}N_2O_5S_2 \cdot C_4H_4O_4$	96–98	C,H,N	>30[a]	19.6	27	8.95	5.1	2.6	63 (0.1)
								5.57			
11h	$NH(CH_2CH_2OC_2H_5)$	$C_{11}H_{16}N_2O_4S_2 \cdot HCl$	200–202	C,H,N	40.8[a]	3.37	75	8.90	8.0		90 (0.1)
					19.7[b]			6.85			32 (0.02)
11i	$NH(CH_2CH_2CH_2CH_2OH)$	$C_{11}H_{16}N_2O_4S_2 \cdot HCl$	218–220	C,H,N	>47[a]	0.14		9.16	11.0		70 (0.1)
								7.65			
11j	$NH(CH_2CH_2CH_2OCH_3)$	$C_{11}H_{10}N_2O_4S_2 \cdot HCl$	203–205	C,H,N	>34[a]	0.42	78	9.00	12.0		82 (0.1)
								7.25			69 (0.02)
11k	$NH(CH_2)_3O(CH_2)_2OCH_3$	$C_{13}H_{20}N_2O_5S_2 \cdot HCl$	182–184	C,H,N	>17.5[a]	0.30	73	9.00	14.0		77 (0.1)
								7.42			
11l	$N[(CH_2)_2OCH_3][(CH_2)_2O(CH_2)_2OCH_3)]$	$C_{16}H_{28}N_2O_{10}S_2$	74–76	C,H,N			21	8.85	6.49	3.6	75 (0.5)
								5.55			
11m		$C_{13}H_{13}N_3O_3S_2 \cdot C_4H_4O_4$	149–151	C,H,N	20.4[a]	2.76	69	9.08	8.2	8.2	77 (0.1)
					16.9[b]			7.02			59 (0.02)
11n	$NHCH_2CH_2F$	$C_6H_{11}FN_2O_3S_2 \cdot HCl$	202–204	C,H,N	>32[a]	2.2	60	9.00	12.0	3.4	88 (0.1)
								6.55			35 (0.02)

[a] Solubility measured in water at 25 °C. [b] Solubility measured in pH 7.4 buffer at 25 °C. [c] Partition coefficients were determined by equilibrating each test compound between 1-octanol and 0.1 ionic strength pH 7.4 buffer. [d] The binding of each test compound to bovine iris and ciliary body was determined as described in ref 18. [e] pK_a was determined in 30% ethanol/H_2O. [f] In vitro inhibition of human carbonic anhydrase II (see Experimental Section for details). [g] The ability of each test compound to inhibit albino rabbit iris and ciliary body carbonic anhydrase after topically installation was assessed ex vivo as described in ref 13. [h] In this assay the known carbonic anhydrase inhibitors acetazolamide and methazolamide displayed inhibition levels of 26% and 12%, respectively, when dosed as 0.1% suspensions in 0.5% aqueous (hydroxyethyl)cellulose.

Scheme I



thieno[2,3-*b*]furan-2-sulfonamides as topical ocular hypotensive agents. Specifically, expanding on an earlier series,[6] we were interested in appraising the effects on enzyme inhibition and ocular transport as heteroatoms were varied in a fused [5.5] aromatic system.

Chemistry

The synthesis[8,9] of the compounds listed in Table I was carried out as shown in Scheme I. Metalation[10] of acetal **1** followed by thiolation and alkylation gave **2**. Acetal deprotection and subsequent treatment of **3** under Knoevenagel conditions with piperidinium acetate in benzene[11] afforded the desired ester **4** in 61% yield. Reduction of **4** with lithium aluminum hydride gave alcohol **5**, which was converted to aldehyde **6** with PCC and protected as its acetal **7** under standard conditions. Deprotonation of **7** was effected with *n*-BuLi in THF at −78 °C and subsequent conversion to the sulfonyl chloride was carried out by sequential treatment with sulfur dioxide and *N*-chlorosuccinimide. Treatment of **8** with concentrated NH_4OH in acetone provided sulfonamide **9**, which was deprotected and subjected to reductive amination to provide compounds in the aminomethyl sulfonamide series **11**.

Results and Discussion

Compounds were evaluated initially in vitro for their ability to functionally inhibit CO_2 hydration catalyzed by human erythrocyte CA II (I_{50})[12] and to inhibit the binding of dansylamide (K_i). Among the more potent compounds in these in vitro assays were the morpholino analog **11e** and the bis(2-methoxyethyl)amine **11g**; however, several other compounds displayed I_{50} values of less than 10 nM. Although the functional (I_{50}) assay and the binding (K_i) assay provided inhibition potencies that were in general comparable, deviation was seen for the *N*-methyl (**11a**) and *N*-ethyl (**11b**) analogs which were 2–3-fold more potent in the functional assay than in binding. Overall, potency was neither dramatically increased nor decreased by N-substitution on the 5-aminomethyl group.

An ex vivo assay[13] (Table I) was used to determine the ability of compounds to penetrate the albino rabbit eye and to inhibit the target enzyme in iris–ciliary body homogenate 1 h after topical dosing. Test compounds were evaluated at 0.1% (1 drop, 50 μL), while lower concentrations were used to evaluate the more potent compounds. In this assay **11a**, **11b**, **11c**, **11d**, and **11j** showed good activity at both 0.1% and 0.02% dosing.

On the basis of these results, **11a** was selected for expanded study in a series of physical and biological models. The solubility of **11a** was >51 mg/mL in pH 5.2 buffer, allowing ready access to concentrated solutions of this compound. The octanol/pH 7.4 buffer partition coefficient of **11a** was 0.13 and the level of binding to bovine ocular pigment[14] was determined to be 77%. Further in vivo evaluation of **11a** was conducted in the α-chymotrypsinized (α-CT) albino rabbit, a model of chronic ocular hypertension.[15] In this assay **11a** maximally lowered intraocular pressure (IOP) by 7.2 mmHg and 5 mmHg at 0.5% and 0.1% dosing (1 drop, 50 μL), respectively. This level of activity compares favorably with the documented potency of the more active members of the benzo[*b*]furan-[4] and thieno[2,3-*b*]thiopyransulfonamide classes.[5] Subsequent evaluation showed that **11a** was effective at reducing IOP in normotensive albino rabbits after topical dosing [2.5 mmHg decrease with 1 drop (50 μL) of a 2% solution]. However, despite these initial favorable findings and much to our initial surprise, topical administration of a 2% solution of **11a** to laser-treated, ocular hypertensive, pigmented monkeys[16] effected no significant depression of IOP. Considering the contrasting results in albino rabbits and pigmented monkeys, we reasoned that the lack of activity of **11a** in the monkey was probably due to its high degree of binding to ocular pigment, thereby critically diminishing the free concentration of drug available for interaction with CA II. The noncovalent, ocular pigment binding of planar aromatic structures containing appended basic functionality has earlier been recognized to be an

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Table II. Pigment-Binding, IOP-Lowering, and GSH-Reactivity Data

compd	% bovine ocular pigment binding	IOP lowering[a] (2%, 3 drops), mmHg	GSH reactivity[b]
11b	76.1	0	11%
11e	24.8	2.5	10%
11g	26.9	3.9	18%

[a] Compounds were bilaterally instilled in three 50-μL drops at 2% concentration (w/v) in 0.5% aqueous (hydroxyethyl)cellulose vehicle. Results are expressed as percent change from the control. [b] Reaction with 5 equiv of GSH, pH 7.4, 37 °C, 16–22 h as described in ref 20.

important feature of ocular pharmacology.[17-19]

In an attempt to modulate the extent of ocular pigment binding of thieno[2,3-*b*]furansulfonamides, we decided to diminish the basicity of the appended amino groups. As shown in Table I, substitution on the nitrogen of the parent aminomethyl compound with 2-methoxyethyl provided **11f**, which displayed a 1 pK_a unit lowering (8.05 to 7.00). Analogous bis-substitution gave **11g**, which was 1.4 pK_a units less basic than **11f** and 2.4 pK_a units less basic than **11b**. Similarly, the 2-(2-pyridylmethyl) (**11m**) and 2-fluoroethyl (**11n**) substituted analogs displayed a reduction in basicity of 1.0–1.5 pK_a units, while the morpholino analog **11e** was 2.7 pK_a units less basic then **11b**. Determination of pigment binding in this series of less basic amines demonstrated that association with ocular pigment was comparable to **11b** for the monosubstituted examples such as **11f**, **11h**, and **11n**, but was significantly reduced for tertiary amines **11e**, **11g**, and **11l**.[20]

To evaluate the possible relationship between ocular pigment binding and IOP lowering in ocular normotensive pigmented rabbits, a series of compounds (Table II) was studied for their ability to reduce IOP after topical dosing (3 drops of a 2% solution) in pigmented rabbits. Under conditions where the highly pigment-bound **11b** was inactive, the less efficiently pigment-bound compounds **11e** and **11g** displayed significant IOP-lowering activity. This result, coupled with the lack of activity of **11a** in pigmented monkeys, strongly suggests that optimum topical carbonic anhydrase inhibitors for the treatment of elevated IOP in humans should display no more than moderate levels of pigment binding.

Since some aryl sulfonamides have been found to be causative agents in ocular or dermal sensitization,[3,4] several of the compounds studied above were evaluated for their ability to covalently react with reduced glutathione (5 equiv of GSH, pH 7.4, 37 °C, 16–22 h)[21] (Table II). Under these conditions **11b**, **11e**, and **11g** all displayed significant reactivity. The extent of reaction of **11b**, **11e**, and **11g** with glutathione was much greater than that of the corresponding thieno[2,3-*b*]thiophene analogs,[6] and indicates that in-depth evaluation of members of this structural class for their sensitization potential is warranted. In this regard **11a** (2% reaction with GSH) was tested in the Magnusson–Kligman guinea pig maximum-sensitization protocol[22] and was found not to cause sensitization.

Conclusion

A novel series of 5-substituted thieno[2,3-*b*]furan-2-sulfonamides was prepared and evaluated as topical agents for reduction of intraocular pressure. Compound **11a** displayed nanomolar potency in vitro for CA II inhibition, high water solubility in pH 5.2 buffer, and extensive binding to ocular pigment. Although **11a** showed topical activity in both the ocular normotensive and hypertensive albino rabbit, no IOP-lowering activity was found in a pigmented, ocular hypertensive monkey model. Analogs were prepared that displayed lower basicity, diminished pigment binding, and enhanced activity in pigmented rabbits following topical dosing. Optimum clinical candidates will very likely possess only moderate levels of binding to ocular pigment. The reactivity of key sulfonamides toward reduced glutathione was found to be moderate, indicating the need for in-depth evaluation of sensitization potential.

Experimental Section

3-[2-(1,3-Dioxolanyl)]furan (1). To a solution of 100 g (1.04 mol) of 3-furancarboxaldehyde in 400 mL of benzene was added 225 g (3.63 mol) of ethylene glycol and 100 mg of pyridinium *p*-toluenesulfonate (PPTS) and the resulting mixture was stirred and heated at reflux utilizing a Dean–Stark trap. After the theoretical amount of water was collected (usually 3 h), the cooled reaction mixture was diluted with 100 mL of ether and 200 mL of water. The organic phase was separated and the aqueous phase was reextracted with 100 mL of ether. The organic phases were combined, washed with 2 × 100-mL portions of water and brine, and then dried over anhydrous sodium sulfate. The drying agent was removed by filtration, and after solvent removal on the rotary evaporator, the residue was distilled to provide 125 g (86%) of pure **2**. Bp: 78–82 °C/20 mm. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.03 (2 H, m), 4.11 (2 H, m), 5.88 (1 H, s), 6.50 (1 H, d, J = 2 Hz), 7.44 (1 H, dd, J = 2.2 Hz), 7.58 (1 H, d, J = 4 Hz).

Methyl [[3-[2-(1,3-Dioxolanyl)]furan-2-yl]thio]acetate (2). To a mechanically-stirred solution of 69.0 g (0.49 mol) of 3-[2-(1,3-dioxolanyl)]furan (**1**) in 250 mL of THF under N_2 and cooled to −78 °C was added 0.49 mol of *n*-butyllithium (in hexane) dropwise at <−70 °C. The resulting reaction mixture was stirred at −78 °C for 45 min to provide a white suspension. Then, 16.64 g (0.52 mol) of sulfur was added portionwise via Gooch tubing over 5 min (the internal temperature rose to −65 °C) to provide an orange suspension. The suspension was stirred at −78 °C for 30 min and then at −50 °C for 30 min to afford a deep purple reaction mixture. Then, 91.8 g (0.60 mol) of methyl bromoacetate in 50 mL of THF was added dropwise over 15 min. After stirring at −78 °C for 30 min, the reaction mixture was allowed to gradually warm to −10 °C over 1.5 h.

The reaction mixture was quenched with 250 mL of brine/250 mL of ether and the aqueous phase was separated and re-extracted with ether. The organic phases were combined, washed with brine, and dried over sodium sulfate, and the solvent was removed in vacuo. The resulting oil was taken up in ether and passed through a silica gel pad to provide a clear filtrate. The solvent was removed in vacuo to give crude **3** as a clear oil (R_f 0.4 on silica gel eluting with 20% EtOAc/hexane). This oil was purified by flash chromatography on silica gel eluting with 25% EtOAc/hexane to give 90.8 g (76%) of **2** as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.50 (2 H, s), 3.69 (3 H, s), 4.0 (2 H, m), 4.12 (2 H, m), 5.87 (1 H,

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s), 6.51 (1 H, d, J = 2 Hz), 7.49 (1 M, d, J = 2 Hz). MS: m/z 244.

Methyl [(3-Formylfuran-2-yl)thio]acetate (3). To a solution of 6.8 g (0.028 mol) of **2** dissolved in 50 mL of acetone was added 100 mg of p-toluenesulfonic acid monohydrate, and the resulting solution was kept at room temperature for 3 h. To this was added 15 mL of saturated sodium bicarbonate solution and the resulting suspension was stripped on a rotary evaporator to remove acetone. The resulting slurry was extracted with 2 × 40-mL portions of ether, and the combined organic extracts were washed with brine and dried. The solvent was removed in vacuo to give 4.65 g (83%) of **3** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.71 (5 H, s), 6.79 (1 H, d, J = 2 Hz), 7.51 (1 H, d, J = 2 Hz), 10.0 (1 H, s). MS: m/z 200.

Methyl Thieno[2,3-b]furan-5-carboxylate (4). Piperidine (6.97 mL, 0.0704 mol) was added to a solution of acetic acid (4.40 mL, 0.0705 mol) in benzene (200 mL). The slightly warm solution was stirred for 15 min and then 11.4 g (0.057 mol) of **3** in benzene (150 mL) was added. An additional amount of benzene (150 mL) was used to rinse out the flask. The vessel was purged with N_2, and the contents were heated to reflux with water removal via a Dean–Stark trap. The originally bright yellow solution became dark red upon heating. After 2.5 h, 85% of the theoretical amount of water was collected.

The reaction mixture was cooled to 20 °C, poured into a 2-L separatory funnel, and diluted with 1.2 L of ether. This solution was extracted with 2 × 200 mL of 5% HCl, 1 × 200 mL of 50% saturated $NaHCO_3$, 1 × 200 mL of H_2O, and 1 × 50 mL of saturated NaCl. The washed organic phase was dried over $MgSO_4$ and filtered, and solvents were removed in vacuo to give 19 g of a black-brown solid. This was taken up in a minimum of EtOAc and passed through a silica gel column (150 g silica gel) using 20% ethyl acetate/hexane as eluant, to give 6.57 g (61%) of **4** as a yellowish solid. Recrystallization reduced the bad smell, and was effected by dissolving the product in ether (100 mL) and cooling to −35 °C. The white crystals were collected and dried at 20 °C (house vacuum) for 72 h to yield 4.40 g of product **4**. Mp: 95–96 °C. HPLC (at 210 nm): 99.4% pure. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (3 H, s), 6.74 (1 H, d, J = 2.2 Hz), 7.66 (9 H, d, J = 2.2 Hz), 7.72 (1 H, s). Anal. Calcd for $C_8H_6O_3S$: C, 52.53; H, 3.31. Found: C, 52.83; H, 3.37.

Thieno[2,3-b]furan-5-carbinol (5). To a suspension of 3.80 g (0.1 mol) of lithium aluminum hydride in 650 mL of ether cooled to 0–10 °C under N_2 was added a solution of 9.19 g (0.05 mol) of **4** in ether (150 mL) dropwise over 20 min. This suspension was stirred at room temperature for 4 h, at which time all the starting material was consumed. The reaction mixture was cooled and quenched with 30 mL of saturated sodium/potassium tartrate solution added dropwise.

The resulting mixture was then stirred at room temperature for 30 min. The ether phase was decanted away from the gummy solid and this solid was thoroughly triturated with ether. The organic extracts were combined, washed with brine, and dried over sodium sulfate, and the solvent was removed in vacuo. The resulting amber oil was purified by flash chromatography on silica gel eluting with 20% EtOAc/hexane to give 7.3 g (94%) of **5** as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.80 (2 H, s), 6.65 (1 H, d, J = 2 Hz), 6.87 (1 H, s), 7.59 (1 H, d, J = 2 Hz). MS: m/z 154. Anal. Calcd for $C_7H_6O_2S$: C, 54.53; H, 3.92. Found: C, 54.29; H, 3.88.

Thieno[2,3-b]furan-5-carboxaldehyde (6). To a mechanically-stirred suspension of 15.22 g (0.071 mol) pyridinium chlorochromate in 150 mL of methylene chloride at room temperature was added a solution of 7.26 g (0.047 mol) of **5** in 150 mL of methylene chloride in one portion. After stirring for 2 h at room temperature, all starting material was consumed. The reaction mixture was diluted with 200 mL of ether and was filtered through a silica gel pad. The residue in the flask and on the pad was washed thoroughly with methylene chloride. The organic extracts were combined, and the solvent was removed in vacuo to provide 5.42 g (76%) of **6** as an oil that partially crystallized on standing. It was anticipated that this material would have only moderate stability, so it was generally used directly in the next step. ^{1}H NMR (300 MHz, $CDCl_3$: δ 6.80 (1 H, d, J = 2 Hz), 7.69 (1 H, s), 7.71 (1 H, d, J = 2 Hz), 9.90 (1 H, s). MS: m/z 152. Anal. Calcd for $C_7H_4O_2S$: C, 55.25; H, 2.65. Found: C, 55.46; H, 2.30.

5-[2-(1,3-Dioxolanyl)]thieno[2,3-b]furan (7). To a solution of 5.91 g (0.039 mol) of **6** and 4.96 (0.08 mol) of ethylene glycol in 75 mL of benzene was added 100 mg of pyridinium p-toluenesulfonate and the resulting mixture was heated at reflux utilizing a Dean–Stark trap. After approximately 8 h the theoretical amount of water was evolved and TLC showed that all the starting material was consumed. The cooled reaction mixture was diluted with 75 mL of water/100 mL of ether and the organic phase was separated, washed with water and brine, and dried. This was passed through a silica gel pad and the solvent was removed in vacuo to give crude **7**. This was purified by flash chromatography on silica gel eluting with 12% EtOAc/hexane to give 6.2 g (81%) of pure **7** (R_f 0.6 on silica gel eluting with 20% EtOAc/hexane), which crystallized on standing. Mp: 60–63 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.03 (2 H, m), 4.13 (2 H, m), 6.09 (1 H, s), 6.67 (1 H, d, J = 2 Hz), 7.02 (1 H, s), 7.58 (1 H, d, J = 2 Hz). MS: m/z 196. Anal. Calcd for $C_9H_8O_3S$: C, 59.98; H, 4.47. Found: C, 60.16; H, 4.55.

5-[2-(1,3-Dioxolanyl)]thieno[2,3-b]furan-2-sulfonamide (9). To a solution of 1.96 g (0.01 mol) of **7** in 20 mL of THF cooled to −78 °C under N_2 was added 0.01 mol of n-butyllithium dropwise at <−70 °C and the resulting solution was stirred for 45 min at −78 °C. Then, SO_2 gas was bubbled close to the surface of the reaction mixture as the temperature rose to ca. −60 °C. This suspension was stirred at −65 °C for 30 min while SO_2 gas was continuously admitted and the mixture was allowed to gradually warm to 0 °C over 1 h. The solvent was removed at <35 °C under reduced pressure (water aspirator) to afford the desired sulfinic acid as a tan solid. This solid was taken up in 20 mL of saturated $NaHCO_3$ solution, and with cooling to 0–10 °C, 2.0 g (0.015 mol) of N-chlorosuccinimide was added portionwise over 5 min with vigorous stirring. The resulting suspension was stirred vigorously at 0–10 °C for 1.5 h. This mixture was extracted with 3 × 50-mL portions of $CHCl_3$, and the combined organic extracts were washed with brine and dried. The solvent was removed in vacuo to give the intermediate sulfonyl chloride **8** as a tan solid. This solid was dissolved in 15 mL of acetone, and with cooling to 5 °C, 15 mL of concentrated NH_4OH solution was added in one portion. This was stirred at 5 °C for 1.5 h and then extracted with 5 × 50-mL portions of EtOAc. The combined organic extracts were washed with brine and dried, and the solvent was removed in vacuo to give 1.66 g (60%) **9** as a tan solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.97 (2 H, m), 4.03 (2 H, m), 6.09 (1 H, s), 7.29 (1 H, s), 7.36 (1 H, s), 7.90 (2 H, bs). MS: m/z 275. Anal. Calcd for $C_9H_9NO_5S_2$: C, 39.27; H, 3.30; N, 5.09. Found: C, 39.16; H, 3.60; N, 5.28.

5-Formylthieno[2,3-b]furan-2-sulfonamide (10). To a solution of 1.66 g (0.006 mol) of **9** in 35 mL of acetone was added 25 mg of p-toluenesulfonic acid monohydrate and the resulting solution was kept at room temperature for 2 h. Then, 25 mL of saturated sodium bicarbonate solution was added and the acetone was removed in vacuo. The residue was extracted with 3 × 50-mL portions of ethyl acetate, and the combined organic extracts were washed with brine, dried, and passed through a silica gel pad. The solvent was removed from the filtrate to give 1.34 g (95%) of **10** as a tan solid. Mp: 165–167 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.56 (1 H, d, J = 2 Hz), 8.09 (2 H, bs), 8.17 (1 H, d, J = 2 Hz), 10.0 (1 H, s). MS: m/z 231. Anal. Calcd for $C_7H_5NO_4S_2$: C, 36.36; H, 2.18; N, 6.06. Found: C, 36.64; H, 2.22; N, 6.30.

5-[(N-Methylamino)methyl]thieno[2,3-b]furan-2-sulfonamide (11a). A solution of methylamine (excess) in 10 mL of ethanol was treated at 0–10 °C with 3-Å molecular sieves (1 g) and a solution of **10** (0.46 g, 0.002 mol) in ethanol (5 mL). This solution was stirred for 3 h at room temperature and the solvent was removed at low pressure. Another 10 mL of EtOH was then added, followed by sodium borohydride (0.30 g, 0.008 mol) and the resulting solution was stirred at room temperature for 16 h.

The reaction mixture was then poured into 75 mL of H_2O, acidified at 0–10 °C with 6 N HCl, and stirred for 0.5 h. This solution was then extracted with ethyl acetate (50 mL), basified to pH 8–9 with concentrated NH_4OH, and extracted with 4 × 50-mL portions of ethyl acetate. The organic phase was washed with brine and dried (Na_2SO_4) and the solvent was removed. The resulting residue was purified by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (85/15) to give pure **11a** (0.32 g) as a white solid. Mp: 150–155 °C. This was dissolved in 1 mL of ethanolic HCl (1 M) at 0–10 °C, stirred for 10 min, and then

gradually diluted with 30 mL of ether. The resulting hydrochloride salt (0.33 g) was dried at high vacuum. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.55 (3 H, bs), 4.40 (2 H, m), 7.33 (1 H, s), 7.95 (1 H, s), 9.17 (2 H, m).

The following compounds were prepared in a similar manner.

5-[(*N*-Ethylamino)methyl]thieno[2,3-*b*]furan-2-sulfonamide (11b). Purification of crude free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (9/1) followed by hydrochloride formation provided pure **11b**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.03 (3 H, t), 2.59 (2 H, q), 3.92 (2 H, s), 6.96 (1 H, s), 7.28 (1 H, s), 7.80 (2 H, b).

5-[(*N*-Isopropylamino)methyl]thieno[2,3-*b*]furan-2-sulfonamide (11c). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (9/1) followed by hydrochloride formation gave pure **11c**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.79 (3 H, t), 1.35 (2 H, m), 2.59 (2 H, s), 3.87 (2 H, bs), 6.89 (1 H, s), 7.18 (1 H, s), 7.76 (2 H, b).

5-[(*N*-Isobutylamino)methyl]thieno[2,3-*b*]furan-2-sulfonamide (11d). Purification of the crude base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (95/5) followed by hydrochloride formation gave pure **11d**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.94 (2 H, d), 1.99 (1 H, m), 2.73 (2 H, m), 4.42 (2 H, m), 7.38 (1 H, s), 7.42 (1 H, d), 7.96 (1 H, bs), 9.20 (2 H, m).

5-[*N*-(Morpholinomethyl)amino]thieno[2,3-*b*]furan-2-sulfonamide (11e). Purification of the crude free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (97/3) followed by hydrochloride formation provided pure **11e**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.34 (4 H, m), 3.50 (4 H, m), 3.63 (2 H, s), 6.90 (1 H, s), 7.18 (1 H, s), 7.75 (2 H, s).

5-[*N*-(2-Methoxyethyl)amino]thieno[2,3-*b*]furan-2-sulfonamide (11f). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (92/8) followed by hydrochloride formation gave pure **11f**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.60 (2 H, t), 3.06 (3 H, s), 3.30 (2 H, t), 3.82 (2 H, s), 6.85 (1 H, s), 7.18 (1 H, s), 7.73 (2 H, bs).

5-[[*N*,*N*-Bis(2-methoxyethyl)amino]methyl]thieno[2,3-*b*]furan-2-sulfonamide (11g). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (97/3) followed by maleate formation gave pure **11g**. Free base ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.80 (4 H, t), 3.32 (6 H, s), 3.50 (4 H, t), 3.95 (2 H, s), 6.78 (1 H, s), 7.22 (1 H, s), 7.26 (2 H, s).

5-[[*N*-(2-Ethoxyethyl)amino]methyl]thieno[2,3-*b*]furan-2-sulfonamide (11h). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (94/6) followed by hydrochloride formation gave pure **11h**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.11 (3 H, t), 2.59 (2 H, t), 3.43 (4 H, m), 3.95 (2 H, s), 6.96 (1 H, s), 7.27 (1 H, s), 7.83 (2 H, bs).

5-[[*N*-(4-Hydroxybutyl)amino]methyl]thieno[2,3-*b*]furan-2-sulfonamide (11i). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (85/15) followed by hydrochloride formation provided pure **11i**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.48 (4 H, m), 3.39 (4 H, m), 3.93 (2 H, s), 6.97 (1 H, s), 7.28 (1 H, s), 7.85 (2 H, b).

5-[[*N*-(3-Methoxypropyl)amino]methyl]thieno[2,3-*b*]furan-2-sulfonamide (11j). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (9/1) followed by hydrochloride formation afforded pure **11j**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.88 (2 H, m), 2.95 (2 H, m), 3.24 (3 H, s), 3.38 (2 H, t), 4.43 (2 H, s), 7.36 (1 H, s), 7.43 (1 H, s), 7.96 (2 H, bs).

5-[[*N*-[3-(2-Methoxyethoxy)propyl]amino]methyl]thieno[2,3-*b*]furan-2-sulfonamide (11k). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (97/3) followed by hydrochloride formation gave pure **11k**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.75 (2 H, m), 2.60 (2 H, t), 3.21 (3 H, s), 3.33 (2 H, s), 3.42 (4 H, m), 3.91 (2 H, s), 6.95 (1 H, s), 7.26 (1 H, s), 7.82 (2 H, b).

5-[[*N*-(2-Methoxyethyl)-*N*-[2-(2-Methoxyethoxy)ethyl]amino]methyl]thieno[2,3-*b*]furan-2-sulfonamide (11l). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (9/1) followed by maleate formation have pure **11l**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.00 (4 H, m), 3.25 (3 H, s), 3.28 (3 H, s), 3.48 (2 H, m), 3.65 (2 H, m), 4.32 (2 H, m), 6.14 (2 H, s), 7.20 (1 H, bs), 7.35 (1 H, s), 7.90 (2 H, s).

5-[[*N*-(2-Pyridylmethyl)amino]methyl]thieno[2,3-*b*]furan-2-sulfonamide (11m). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (9/1) followed by maleate formation gave pure **11m**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (2 H, s), 3.97 (2 H, s), 6.96 (1 H, s), 7.25 (2 H, m), 7.45 (1 H, d), 7.72–7.90 (4 H, m), 8.50 (1 H, d).

5-[[*N*-(2-Fluoroethyl)amino]methyl]thieno[2,3-*b*]furan-2-sulfonamide (11n). Purification of the free base by flash chromatography on silica gel eluting with $CHCl_3$/MeOH (95/5) followed by hydrochloride formation gave pure **11n**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.48 (2 H, s), 4.65 (2 H, t), 4.81 (2 H, t), 7.36 (1 H, s), 7.42 (1 H, s), 7.97 (2 H, s).

H_2O Solubility. A standard solution was prepared by dissolving 1 mg of sample in 10 mL of CH_3OH. The standard solution was scanned by UV (Acta M VI Beckman spectrophotometer) to determine the wavelength of maximum absorbance, diluting as necessary. A saturated solution was prepared by stirring magnetically a small volume of pH 7.4 phosphate buffer 0.039 M (~500 μL) in the presence of excess compound. The saturated solution was checked every 30 min and additional compound added if necessary to maintain saturation. After 4 h, the solution was filtered to remove excess compound, using HA 0.45-μm Millipore filters. The saturated solution was diluted to at least 3 mL and then scanned by UV at the wavelength of maximum absorbance. Total solubility was then determined by the relationship $C' = AC/A$ where C = concentration of saturated solution in milligrams/milliliter, A = absorbance of the standard solution (correcting for any dilutions), A' = absorbance of the saturated solution (correcting for any dilutions), and C' = concentration of saturated solution in milligrams/milliliter.

pK_a. The half-neutralization point was measured by titrating the organic acids and bases with 0.5 N NaOH and 0.5 N HCl in H_2O and mixed solvents, using a glass-columned electrode system. All of the compounds were run in 30% EtOH/H_2O.

Partition Coefficients. Partition coefficients were obtained by equilibrating the test compound between octanol and 0.1 ionic strength pH 7.4 phosphate buffer. The concentration in each phase was determined by UV spectrophotometry.

In Vitro Inhibition of Human Carbonic Anhydrase II. Human erythrocyte CA II was isolated from lysed red blood cells by the following affinity-chromatography procedure. Citrated human blood (500 mL) was centrifuged at 5000*g* for 10 min at 4 °C and the resultant plasma decanted. Red blood cells were washed with cold 0.9% NaCl solution and then centrifuged. The supernatant was discarded and the process of washing and centrifugation repeated. Cell lysis was achieved at 4 °C by adding an equal volume of cold water and cellular debris was removed by centrifugation. Lysed human red blood cells (80 mL) were diluted 5-fold with 0.05 M Tris-SO_4 buffer, pH 8.8, and poured onto a 0.9 × 8 cm [4-(aminomethyl)benzenesulfonamide-CM agarose] affinity chromatography gel column. Chromatography was carried out at 4 °C, and fractions were monitored by determining optical density at 280 nm with an LKB Uvicard III.

The column was eluted with 0.2 M sodium sulfate in 0.1 M Tris-SO_4 buffered at pH 8.8 to remove all hemoglobin and other proteins not specifically bound. Low-activity carbonic anhydrase I was eluted as a single peak with 0.6 M potassium chloride in 0.1 M potassium phosphate buffer (pH 7.2). Elution was continued until the optical density at 280 nm was less than 0.1. Highly purified carbonic anhydrase II was eluted with 0.6 M potassium chloride in 0.1 M potassium phosphate buffer (pH 5.2). Carbonic anhydrase II purity was assessed by disk gel and starch gel electrophoresis. The gels were stained for protein with Coomassie Blue, and carbonic anhydrase II bands were visualized by fluorescein diacetate staining. The enzyme solution was desalted and concentrated to 1 mg of protein/mL of 0.1 M phosphate, pH 7.2, on an Amicon UM-10 Ultrafiltration membrane and stored at 2–5 °C.

Inhibition of the purified human erythrocyte carbonic anhydrase II was assessed by using a pH stat assay. This assay measures the rate of hydration of CO_2[17] by determining the rate at which a standard solution of NaOH has to be added to a lightly buffered solution to maintain a constant pH as CO_2 is bubbled into the buffer. Enzymatic activity is proportional to the volume of a standard NaOH solution that is required to maintain the pH at a given value, e.g., 8.3. To 4 mL of 0.02 M Tris-Cl buffer, pH

8.6, in a 5-mL Radiometer V531 jacketed assay vessel equilibrated at 2 °C was added buffer-diluted enzyme (25 mL). CO_2/air (5/95) was bubbled into the assay vessel at a rate of 150 mL/min. The pH stat end point was set at pH 8.3, and the volume of 0.025 N NaOH added over a 3-min period in order to maintain pH 8.3 was measured. Enzyme inhibition was measured by the addition of an inhibitor in 0.1–3.9 mL of buffer followed by the addition of enzyme and titration with NaOH. Results were expressed as the I_{50} values, which were obtained from semilog plots of percent inhibition against log concentration.

In Vitro Binding for Human Carbonic Anhydrase II. The binding of test compounds to purified human erythrocyte carbonic anhydrase II was determined by a fluorescence competition assay employing the fluorescent CA inhibitor dansylamide. This compound has been shown to produce a large increase in fluorescence upon binding to the active site of carbonic anhydrase. A fluorescence cuvette containing 1×10^{-7} M human CA II (HCA II) and 2×10^{-6} M dansylamide in pH 7.4, 0.1 ionic strength phosphate buffer was placed in the thermostated cell holder of a Perkin-Elmer MPF-44B fluorescence spectrophotometer. The temperature was maintained at 37 °C by using a constant-temperature water circulator. The excitation and emission wavelengths were set at 280 and 460 nm, respectively. Fluorescence intensities were recorded following addition, with stirring, of small, measured aliquots of a solution of the test compounds in pH 7.4 buffer. The resulting data were converted to fluorescence intensity vs compound concentration, corrected for dilution by the titrant, and fitted by nonlinear least squares to a model in which the compound and dansylamide compete for a single binding site on HCA II. The dissociation constant of the dansylamide–HCA II complex, which is needed for these calculations, was found to be 1.98×10^{-6} M under these conditions. It was found in all cases that the data fitted well to a single-site model. There was no evidence for additional, lower-affinity binding sites. All binding determinations were done a minimum of three times.

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PgH_2 Analogs as Potential Antiplatelet Derivatives

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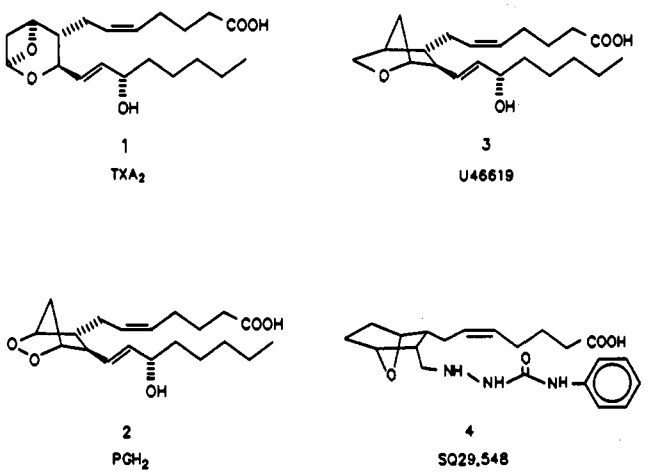
Previous observations implicating PgH_2 as a direct activator of platelets suggested that derivatives of U46619, a well-characterized TxA_2 receptor agonist having structural homology with PgH_2, might possess antiplatelet activity. The present work describes the synthesis of [1*S*-(1α,2β,3α,4α)]-3-[(tetrahydropyranyloxy)methyl]-2-[2-[(triphenylmethyl)oxy]ethyl]-5-oxabicyclo[2.2.1]heptane (**14**) a potentially useful intermediate for the synthesis of various epoxymethano derivatives. The latter was converted to [1*S*-(1α,2β(*Z*),3α,4α)]-7-[3-[[2-[(phenylamino)carbonyl]-hydrazino]methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (**23**), an epoxymethano derivative of PgH_2 containing a hydrazide lower side chain as previously used in the TxA_2 antagonist, SQ 29,548. The intermediate **14** was also converted to [1*S*-(1α,2β(*Z*),3α,4α)]-7-[3-[(hexylamino)methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (**25**) which contained a simple aza side chain as used in earlier antagonists. Derivatives **23** and **25** appeared to be specific antagonists of the human platelet TxA_2 receptor as evidenced by their inhibition of U46619 (1.5 μM) induced aggregation of human platelet rich plasma (IC_{50} = 22 and 7 μM, respectively), while having little effect on ADP (2 μM) induced aggregation at much higher concentrations. In addition, one of these derivatives, the bicycloamine **25**, was shown to compete for [^{3}H]U46619 binding to washed human platelets with an IC_{50} value of 25 μM, supporting the notion that these derivatives were acting at the thromboxane receptor. However, the potency of these derivatives was less than for previously reported TxA_2 antagonists, suggesting that simple linear combinations of functionality from molecules active at the human platelet thromboxane receptor will be of limited predictive value.

Considerable effort has been expended in an attempt to find specific TxA_2 (**1**) synthase inhibitors and TxA_2 receptor antagonists as potential antithrombotic agents. The latter work has resulted in a number of diverse structures which are reported to be specific TxA_2 antagonists.[1-9] The emphasis on TxA_2 logically stems from the original notion that TxA_2 production in the platelet was prerequisite to platelet activation by arachidonic acid. Yet evidence has indicated that PgH_2 (**2**) itself is directly capable of stimulating platelet functional change,[10] and interaction of PgH_2 with the platelet receptor appears to be coupled to calcium mobilization and thus presumably platelet functional change.[11] In addition, at least one of the best characterized TxA_2 receptor agonists, U46619 (**3**),[12,13] is closely related to PgH_2 in its structure.

On the basis of such observations, it seemed reasonable to explore compounds directly related to the structure of PgH_2 as a possible new class of antiplatelet derivatives. In the present work we describe a general synthetic scheme for preparation of side chain modified derivatives of PgH_2 which also incorporate the stable epoxymethano functionality of U46619 while retaining the natural PgH_2 chirality. It is hoped that the study of such derivatives



may ultimately lead to effective antiplatelet compounds and to a better understanding of structure–activity rela-

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