Thienothiopyran-2-sulfonamides: Novel Topically Active Carbonic Anhydrase Inhibitors for the Treatment of Glaucoma

Sir:

The bulk of aqueous humor entering the eye is produced through an active secretory process by the nonpigmented epithelial cells of the ciliary process. Carbonic anhydrase is present in these cells, and following inhibition of the enzyme, bicarbonate formation is decreased, which, in turn, diminishes sodium and fluid secretion into the eye.[1-3] Maximally effective doses of systemically administered CAIs decrease aqueous humor secretion by 40–60% in both man and experimental animals[4] but not without the concomitant inhibition of the enzyme in peripheral tissues with the resultant expression of adverse experiences.[5] A strategy to circumvent these limiting side effects evolved based on the synthesis of structurally novel CAIs designed to optimize topical effectiveness and local activity. This approach culminated with the discovery of certain thienothiopyran-2-sulfonamides.

The biological profile of the thienothiopyrans was assessed through in vitro, ex vivo, and in vivo studies. In vitro determination of I_{50} and K_i values utilized HCA-II, the isozyme found in the human ciliary process.[6] I_{50} values as determined under standard conditions were highly reproducible; however, comparison with K_i values suggested that the pH stat assay may underestimate potency. When a preincubation period of 4 min at 37 °C was added prior to the pH stat titration at 3 °C, the I_{50} values obtained were in closer agreement to K_i's. This suggests that equilibrium between enzyme and inhibitor was not achieved with preincubation conditions at 3 °C. Except for compound **5**, I_{50} values for the class were in the low-nM range and comparable to standard CAIs such as acetazolamide (Table I) and methazolamide.[7] The two most potent examples in terms of inhibitory activity were **4** and **8**. In the competition assay both of these were best fit to a binding curve which assumed an enantiomeric preference; using this method of analysis, widely different K_i values were calculated for the optical antipodes. As shown in Table I, the measured affinities of **S-4** and **S-8** agreed well with the estimates obtained from the racemate. In contrast, alcohol **1** did not show an improvement in fit when it was assumed that one enantiomer was preferentially binding, and the pure enantiomers upon evaluation showed only a small separation in affinity for the enzyme.

An ex vivo assay, as described in Table II, was used to determine if the thienothiopyrans could penetrate the albino rabbit eye and reach the iris–ciliary body in sufficient concentration to induce pharmacologic effects. In order to decrease IOP, the enzyme CA must be essentially totally inhibited;[3,8] therefore, for topical activity both efficient penetration of ocular tissue and localization in the ciliary processes are required. As shown in Table II, the instillation of 0.5% solutions of **1**, **4**, and **8** blocked CA activity in a homogenate of albino rabbit iris–ciliary body at 1 h after administration by 83%, 97%, and 99%, respectively. Because a tissue homogenate is employed, the model reflects the concentration and the intrinsic inhibitory activity of the compound present in the tissue and does not necessarily imply the inhibition of CA activity under in vivo conditions.

In the α-chymotrypsin model of ocular hypertension in the albino rabbit, **1** and **8** were equally effective at 0.5% in reducing IOP while **4** appeared to be less active and **6** exhibited the greatest reduction of pressure but with a somewhat shorter duration. The IOP lowering action of both **1** and **8** at 0.5% was local because the unilateral instillation of the compound into the left eye had no effect on the elevated IOP of the right eye. Thienothiopyrans **1** and **8** were soluble to the extent of 2% in acceptable ophthalmic formulations while **6** and **7** were not. Since solubility was deemed to be important for ocular acceptability, **1** and **8** were selected for evaluation in glaucoma patients. The results obtained for **1** were disappointing relative to those observed in ocular hypertensive albino rabbits (unpublished observations). In contrast, the ocular hypotensive effect of topically administered **8** observed in rabbits has been confirmed in man. The acute instillation of **8** (MK-927), in contrast to **1**, elicits statistically significant reductions in the IOP of both normotensive, healthy volunteers[9] and glaucoma patients.[10] Compound **8** (MK-927) is the first topically applied carbonic anhydrase inhibitor to display this profile when applied in an acceptable ophthalmic formulation and expanded clinical trials are in progress. Recently, aminozolamide in a specialized gel formulation designed to prolong contact time was found to reduce IOP in glaucoma patients.[11]

The explanation for the failure of the albino rabbit to be predictive for activity in man may lie in the physiochemical difference between **1** and **8**. Compound **1** is a neutral molecule and differs in physiochemical properties from **8**, which possesses a weakly basic amine substituent. As shown in Table II, the nonbasic example **1** is essentially unbound to pigment isolated from the bovine iris–ciliary body, while **8** exhibits 12% binding under identical experimental conditions. This difference may be due to the basic substituent present in **8** and is in agreement with similar observations made with other classes of amines.[12] Although **1** and **8** possess comparable ocular hypotensive activity in albino rabbits after topical administration, the latter is more effective than the former in normotensive pigmented rabbits. The instillation of 2% **8** significantly lowered IOP at 0.5, 1, 2, and 3 h, by 2.0, 2.8, 2.7, and 1.1 mmHg, respectively. In contrast, significant reductions were present only at 1 (2.0 mmHg) and 2 h (1.6 mmHg) after 2% dosing with **1**. In this paradigm, one drop of a 2% solution was used since factors regulating aqueous humor dynamics are not the same in normotensive and hypertensive eyes.[13] The longer duration seen with **8** correlates with appreciably higher drug levels in the iris–ciliary body of the pigmented rabbit after 2% dosing. Peak

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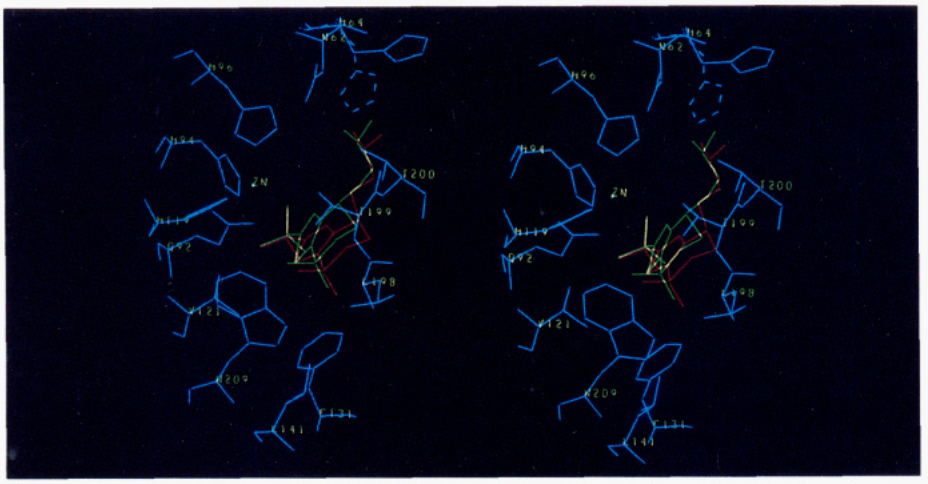
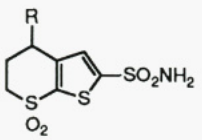


Figure 1. A stereo drawing showing a superposition of the bound conformations of **S**-8 and **R**-8 in the active site of HCA-II. Compound **S**-8 is in red, **R**-8 is in green, the amino acid residues lining the active site cavity are blue, and the position of His-64 in the native enzyme is in dashed blue. Crystals of the complexes of **S**-8 and **R**-8 with HCA-II were formed at 4 °C in 50 mM Tris-HCl at pH 8.5 with 150 mM NaCl, 3 mM NaN_3, and 1 mM CH_3HgCl in microdialysis buttons using 50–58% ammonium sulfate as the precipitant.[17] Before data collection, the crystals were backsoaked with 10 mM cysteine for 2 days to remove the mercurial. The crystals had the same space group symmetry, $P2_1$, and similar cell constants to native HCA-II. Cell constants for HCA-II/**S**-8 were a = 42.91 Å, b = 42.26 Å, c = 72.87 Å and β = 104.42° and for HCA-II/**R**-8 were a = 42.73 Å, b = 41.86 Å, c = 72.72 Å and β = 104.36°. A multiwire area detector with a rotating anode X-ray source was used for data collection. A total of 29 636 symmetry independent reflections to 1.5 Å were measured with $I > \sigma(I)$ for HCA-II/**S**-8 and 28926 for HCA-II/**R**-8. All data for each complex were collected from a single crystal. Difference electron density maps were generated with native coordinates of CA-II that had 10 waters removed from the active-site cavity[18] (also private communication: A. E. Eriksson, T. A. Jones, and A. Liljas of the University of Uppsala). Models of the inhibitors were generated[19] and fit into the difference maps.[20] Several rounds of constrained least-squares refinement[21] followed by difference map inspection and additional water molecule placement were used to define these structures of the two complexes. The present residual value for HCA-II/**S**-8 is 0.168 and for HCA-II/**R**-8 is 0.175.

Table I. In Vitro Carbonic Anhydrase Inhibitory Activity of 5,6-Dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-Dioxide Derivatives



compd	R	formula	% yield	mp, °C	I_{50}, nM; preincubation conditions: 3 °C[k]	37 °C[m]	K_i,[k] nM
1[a]	OH				5.3 ± 0.3	2.3	6.8 ± 1.3
S-1[a]	OH				4.6 ± 0.5	1.3	6.2 ± 2.2
R-1[a]	OH				6.7 ± 0.6	4.2	16 ± 2.3
2[p]	NH_2	$C_7H_{10}N_2O_4S_3$·HCl	47[b]	251–253	9.2 ± 2.3	3.2	3.7 ± 1.1[l]
3[p]	$NHCH_3$	$C_8H_{12}N_2O_4S_3$·HCl	29[c]	275–276	13.9 ± 2.9	2.5	2.3 ± 0.2[l]
4[p]	$NHCH_2CH_3$	$C_9H_{14}N_2O_4S_3$·HCl	42[d]	262–264	7.3 ± 1.4	0.8	0.69 ± 0.07[l]
S-4[n,p]	(+)$NHCH_2CH_3$	$C_9H_{14}N_2O_4S_3$·HCl	26[e]	271–273	3.6 ± 0.5	0.2	0.82 ± 0.12
R-4[n,p]	(−)$NHCH_2CH_3$	$C_9H_{14}N_2O_4S_3$·HCl	13[f]	263–267	20.6 ± 1.9	7.1	16.0 ± 0.9
5[p]	$N(CH_2CH_3)_2$	$C_{11}H_{18}N_2O_4S_3$·HCl	46[g]	236–238	70.0 ± 8.4	12.3	9.3 ± 2.8
6[p]	$NHCH_2CH_2CH_3$	$C_{10}H_{16}N_2O_4S_3$·HCl	19[h]	272–274	9.2 ± 0.6	1.5	1.1 ± 0.3[l]
7[p]	$NHCH_2CH_2CH_2CH_3$	$C_{11}H_{18}N_2O_4S_3$·HCl	35[i]	285–287	12.4 ± 0.8	2.3	1.8 ± 0.4[l]
8[p] (MK-927)	$NHCH_2CH(CH_3)_2$	$C_{11}H_{18}N_2O_4S_3$·HCl	48[j]	262–264	5.9 ± 0.3	1.2	0.7 ± 0.2[l]
S-8[o,p] (MK-417)	(*S*)(+)$NHCH_2CH(CH_3)_2$	$C_{11}H_{18}N_2O_4S_3$·HCl	7[e]	218–221	4.0 ± 0.4	0.54	0.61 ± 0.25
R-8[o,p]	(*R*)(−)$NHCH_2CH(CH_3)_2$	$C_{11}H_{18}N_2O_4S_3$·HCl	11[f]	218–222	53.1 ± 1.8	44	71 ± 4
acetazolamide					9.9 ± 0.5	3.4	22 ± 4

[a] For synthesis, see ref 15. [b] Treatment of **1** with H_2CCN in 95.5% H_2SO_4 followed by hydrolysis of the resulting amide with 12 N HCl in methanol. [c] Treatment of **1** with KCN in 1:1 F_3CCO_2H/H_2SO_4 followed by reduction with borane–dimethyl sulfide. [d] Reduction of amide described in *b* with borane–dimethyl sulfide. [e] The racemate was resolved with (+)-di-*p*-toluoyl-D-tartaric acid. [f] The racemate was resolved with (−)-di-*p*-toluoyl-L-tartaric acid. [g] Acylation of **4** with acetyl chloride/Et_3N in THF followed by reduction as in *d*. [h] Treatment of **1** with propionitrile under Ritter conditions followed reduction as in *c*. [i] Acylation of **2** with butyryl chloride followed by reduction as in *c*. [j] Acylation of **2** with isobutyryl chloride followed by reduction as in *c*. [k] In vitro determinations of the concentration of inhibitor required to inhibit 50% of the enzyme activity, I_{50}, and the equilibrium dissociation constant of the inhibitor–enzyme complex, K_i, utilized HCA-II, the isozyme found in the ciliary process. For experimental details, see ref 15. Results for both K_i and I_{50} values are expressed as mean ±SE (n = 3–5). [l] For racemic inhibitors with K_i values less than 5 nM, the data were better fitted by a model which assumes that one enantiomer is preferentially binding to the enzyme, that is, a model in which the concentration of inhibitor is one-half of the nominal concentration. [m] I_{50} values were determined as described in ref 15 after a preincubation between enzyme and inhibitor for 4 min at 37 °C. Acetazolamide was used as a control for each determination and had an I_{50} value that ranged between 3.1 nM and 3.7 nM. [n] Optical purity of these enantiomers was determined by derivatization with (−)-menthyl chloroformate and assayed by HPLC analysis. [o] Optical purity of the pure enantiomers was determined by derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) followed by HPLC assay. [p] Analysis agreed with calculated value within ±0.4%.

Table II. Biological Properties of 5,6-Dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-Dioxide Derivatives

compd	ocular pigment binding,[a] %	ex vivo CA inhibn,[b] 0.5%, %	ocular hypotensive effect,[c] 0.5%, mmHg	aqueous solubility at 2%[d]
1	1.9 ± 0.6	83	−6.9 ± 0.9 (5)	+
2	32.1 ± 4.0	85	−3.3 ± 3.0 (0)	+
3	23.7 ± 3.6	76	−3.3 ± 0.3 (3)	+
4	29.2 ± 2.9	97	−4.2 ± 0.9 (2)	+
5	9.0 ± 1.1	66	−5.3 ± 1.0 (1)	+
6	19.8 ± 1.6	92	−8.7 ± 1.3 (4)	-
7	25.6 ± 0.7	92	−5.7 ± 0.5 (5)	-
8 (MK-927)	12.3 ± 1.5	99	−6.5 ± 0.6 (5)	+
S-8 (MK-417)	12.8 ± 3.9	100	−4.3 ± 0.8 (4)	+
R-8	10.5 ± 1.6	62	−1.3 ± 0.8 (0)	+

[a] Pigment was isolated from the bovine iris–ciliary body[16] and binding was determined in a final volume of 1 ml of a sodium potassium phosphate buffer (13 mM, pH 7.2) containing 2.2 mg pigment and 10 μM of test compound. Incubation was at room temperature for 1 h. Following centrifugation, binding was calculated as the difference in UV absorbance values between control, to which no pigment was added, and the treated sample. Results are expressed as the mean ±SE (n = 3). [b] One hour after the topical administration (one drop, 50 μL) of vehicle or test compound at 0.5%, the iris–ciliary body was excised from albino rabbits and the tissue homogenized in Tris-HCl buffer (20 mM, pH 8.5). Carbonic anhydrase activity was determined by using a pH stat assay.[15] Results are expressed as mean (n = 6) of percent inhibition between vehicle- and drug-treated animals. [c] Ocular hypertension was induced in the right eye of albino rabbits by the intraocular injection of α-chymotrypsin.[13] Test compound at 0.5% was instilled (one drop, 50 μL) and the IOP of six rabbits measured just before (t_0) and at 0.5, 1, 2, 3, 4, and 5 h after treatment. Results are expressed as the maximum fall (mean ± SE) in IOP (mmHg) from the t_0 value, and values in parentheses refer to the number of time points (maximum of six) at which IOP was significantly reduced ($P \leq 0.05$). [d] Compounds were either soluble (+) or insoluble (-) in vehicle (0.5% aqueous hydroxyethylcellulose) at 2%.

levels of 8 reached 27.8 μg/g while the concentration of 1 was 8.1 μg/g. This marked difference likely reflects the ability of 8 to bind to ocular pigment, thereby providing an intraocular depot of drug.

As shown in Table I, the optical antipode of 8 which possessed the greater in vitro enzymatic inhibitory activity had the *S* absolute stereochemical configuration. This structural assignment is unambiguous and was based on X-ray crystallographic studies. In order to explain this enantiomeric preference, both **S-8** and **R-8** were cocrystallized with HCA-II. Single-crystal X-ray diffraction analysis of the two inhibitor–enzyme complexes revealed both a difference in the relative orientation of the inhibitors in the active-site cavity and a change in the tertiary structure of the protein. These differences are illustrated in stereo format by Figure 1. The only major conformational change of the protein upon binding of the inhibitors involves the side-chain movement of His-64. In both enzyme–inhibitor complexes, the center of the imidazole ring moves a total of 3.1 Å from its position in the native enzyme. This rotation is indicated by dashed bonds for the position of the histidine side chain in native CA and solid bonds for its position in the enzyme–inhibitor complex. With both enantiomers, the sulfonamide group has displaced hydroxyl ion as the fourth ligand of a distorted tetrahedron around the zinc atom. The overall geometry of the thiophenesulfonamide portion of both **S-8** and **R-8** is similar and analogous to the thiadiazolesulfonamide segment in acetazolamide.[14] However, important differences between the homologous atoms of **S-8** and **R-8** are detailed below. One of the principal differences between **S-8** and **R-8** lies in the N–S–C–S dihedral angle, being 144° in the former and 158° in the latter. Since the sulfonamide portion of both inhibitors is spatially similar, the differences in dihedral angles represents a 14° twist of the thiophene ring in **R-8** relative to **S-8**. Ab initio molecular orbital calculations indicate that the preferred N–S–C–S dihedral angle is roughly 90°; this implies that the angle in **S-8**, while not ideal, is somewhat favored over the larger angle in **R-8**. The 14° twist in the N–S–C–S dihedral angle is related to the orientation of the substituted amino side chain. In **S-8** the isobutylamino group assumes a pseudoaxial orientation with respect to the thiopyran ring, and the C–N–C–C dihedral angle in the side chain assumes a trans conformation. In this orientation, the isobutylamino side chain points directly up and into the space made available by translocation of the His-64 imidazole moiety. This pseudoaxial orientation is opposite of that predicted a priori. X-ray analysis of uncomplexed **S-8** revealed a pseudoequatorial conformation of the basic side chain. This is supported by ab initio calculations which predicted the pseudoequatorial orientation to be favored over the pseudoaxial by approximately 1 kcal. In contrast, X-ray analysis of the enzyme–inhibitor complex with **R-8** established that the alkylamino substituent was indeed in the favored pseudoequatorial orientation. However, in this case, to prevent an unfavorable steric interaction between the side chain and the wall of the enzyme's active site, geometry required the C–N–C–C dihedral to adopt a less favorable gauche conformation. In addition, the entire molecule twists relative to the sulfonamide group and as indicated above moves up and to the left as seen in Figure 1. This complex motion of **R-8** places the terminal methyl groups in essentially the same place as in **S-8**. In addition, the positioning of **R-8** relative to **S-8** in the active site leads to differences in the nonbonded interactions between the inhibitors and the enzyme. For example, in **S-8** the separation between the amide nitrogen in the side chain of residue Gln-92 and the closer sulfone oxygen in the inhibitor is 3.1 Å, while in **R-8** the separation is 2.7 Å; the separation in the latter is believed to be more conducive to hydrogen bonding. In addition, in **S-8** the distance between the para carbon in the benzene ring of residue Phe-131 and the closer sulfone oxygen in the inhibitor is 3.2 Å, while in **R-8** the separation is 2.9 Å. The latter distance may be somewhat repulsive.

The enzyme-bound conformations of **S-8** and **R-8** explain in large measure the differences in K_i and I_{50} values found for the two enantiomers. However, further analysis of the X-ray data as it relates to binding energy is continuing.

In summary, 8 (MK-927) is the first CAI to lower the intraocular pressure of glaucoma patients when applied

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in an acceptable pharmaceutical formulation. It is likely that key factors in this effect are a balance between aqueous solubility and lipophilicity along with a modest ability to bind to ocular pigment; this latter feature generates an intraocular depot. In addition, the X-ray studies of the two enantiomers, **S-8** and **R-8**, in HCA-II have provided a rare opportunity to view the effect of chirality on ligand–macromolecule interactions and to rationalize affinity for the enzyme in terms of differences in the molecular geometry of the inhibitors within the active site.

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