Topically Active Ocular Carbonic Anhydrase Inhibitors: Novel Biscarbonylamidothiadiazole Sulfonamides as Ocular Hypotensive Agents (43612)

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Abstract. **A novel homologous series of bis(carbonyl)amidothiadiazole sulfonamides has been synthesized for structure-activity relationship studies, and initial characterization has been performed. The goal was synthesis of thiadiazole derivatives with appropriate lipid and water solubilities for utility as topically (corneal application) active carbonic anhydrase (CA) inhibitors. This series has solubility properties and pK_a which bracket those of acetazolamide—the prototypical CA inhibitor. All of these compounds are active as *in vitro* CA inhibitors, and are 10–25% as potent as acetazolamide as *in vitro* enzyme inhibitors. Two of these compounds act as ocular hypotensive agents after topical application of a single dose to the corneas of normotensive New Zealand albino rabbits. The efficacy of the lead compound of this series (in this one model) is approximately equivalent to that of topical CA inhibitors that are presently in clinical trial.**

None of these novel compounds reacts to an appreciable extent with free sulfhydryl groups (a predictor of toxicity). This family of compounds will be useful for future studies of ocular pharmacokinetics, as well as ocular and systemic effects of topical administration of CA inhibitors. These and future studies may lead to development of thiadiazole sulfonamides useful in the management of glaucoma. [P.S.E.B.M. 1993, Vol 203]

Carbonic anhydrase (CA) (carbonate hydrolyase; E.C. 4.2.1.1) is a zinc metalloenzyme found in many tissues that secrete acidic or alkaline (bicarbonate or carbonate rich) fluids, including the ocular ciliary epithelium (1, 2). Carbonic anhydrase inhibitors decrease aqueous humor formation and intraocular pressure. Orally administered carbonic anhydrase inhibitors are effective ocular hypotensive agents, and can be useful for the management of glaucoma. When used systemically, however, the classical heterocyclic sulfonamide CA inhibitors lead to a constellation of severe, nuisance side effects which compromise patient compliance (3, 4). The obvious solution to the problem, topical corneal application of CA inhibitors, has been discussed for a generation and actively sought for a decade. It is presumed that the low total dose to be used topically would preclude systemic side effects, with the therapeutic effect confined to the eye.

The pharmacology of acetazolamide, the prototypical heterocyclic sulfonamide CA inhibitor, has been studied extensively, and acetazolamide has solubility properties that do not allow adequate penetration of the cornea or demonstration of any ocular hypotensive effect after topical administration, except under extreme conditions (5, 6). Many compounds that are substantially different in structure and in physicochem-

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ical properties have been evaluated as candidate topical carbonic anhydrase inhibitors. These strategies have included evaluation of methazolamide analogs (7), benzothiazole derivatives (8–11), prodrug strategies (12, 13), and development of entirely novel heterocyclic sulfonamides (14–19). The most novel of these have shown promise in clinical trials as ocular hypotensive agents. These compounds are efficacious, although their ultimate clinical utility is not certain.

Compounds that are modifications of thiadiazole CA inhibitors (e.g., acetazolamide) and that might traverse the cornea and lower intraocular pressure (IOP) have been studied (20), but no useful candidate from this class has been found. This paper is a report of the synthesis, physicochemical characterization and a structure-activity relationship study of a series of novel bis(carbonyl)amidothiadiazole sulfonamides as ocular hypotensive agents following topical administration to the corneas of conscious, normotensive New Zealand White rabbits.

Materials and Methods

Synthesis. Novel thiadiazole derivatives were produced from a common precursor, 2-amino-1,3,4-thiadiazole-5-sulfonamide (2-ATS). 2-ATS was produced by hydrolysis of its commercially available acetamide, acetazolamide. Unless otherwise noted, all reagents were purchased from Aldrich Chemical Co. Acetazolamide (0.2 mole) was slurried with constant stirring in 600 ml of methanol (Fisher) to which was added 60 ml of 12 *N* aqueous HCl (Fisher). This mixture was heated to reflux and maintained for 6 hr as the slurry became a true solution. At this point, the reaction was approximately 90% completely, as judged by high-performance liquid chromatography (HPLC described below). Addition of another 30 ml of 12 *N* HCl, followed by heating for an additional 2 hr, was sufficient to drive the reaction to completion. Product was recovered by raising the pH to 7.0 by slow addition of 10 *M* aqueous NaOH, maintaining the temperature at less than 10°C, followed by recrystallization from methanol, filtration, and drying under reduced pressure.

Synthesis of biscarbonylamides of 2-ATS was accomplished by condensation of 2-ATS with the appropriate one of three ethyl ester-hemiacylchlorides: ethyl oxaloylchloride, ethyl succinoylchloride, or ethyl adipoylchloride. 2-ATS (0.09 mole) was slurried in dry tetrahydrofuran along with 0.11 mole of dry pyridine. The appropriate ethyl ester-hemiacylchloride (0.09 mole in 100 ml of dry diethylether) was added at room temperature with stirring over 30 min. The resulting solution was maintained with stirring at room temperature for 18 hr. The reaction was terminated by addition of ice-cold water. Product was recovered by filtration.

Physicochemical Characterization. *High Performance Liquid Chromatography.* For qualitative and quantitative drug analyses, HPLC was used. A 25-cm × 4.6-mm reverse-phase column, packed with LC18-DB (Supelco) was used with one of two different mobile phases. Isocratic analysis was conducted using 50 m*M* sodium phosphate buffer (pH = 2.0) with 15% CH_3OH at a flow rate of 2.0 ml/min. Alternatively, gradient elution analysis was performed using an initial mobile phase composition of 50 m*M* sodium phosphate buffer (pH = 2.0):CH_3OH in the proportion 95:5, v:v. This was varied in a linear fashion over 12 min to a final composition of 10:90, (phosphate buffer:CH_3OH). For detection, a photodiode array detector was used (model 1040A; Hewlett-Packard).

Determination of pK_a. For each compound, pK_a were estimated by monitoring changes in the UV-visible absorption spectrum (400 nm–210 nm) as a function of pH in the range from 2.0 to 10.0. Molar extinction coefficients were determined using UV-visible absorbance spectra and the Beer-Lambert law. Melting points were determined using a standard Fisher-Johns apparatus and a heating rate of 2–3 K minute^{-1}.

Maximum solubility of candidate compounds in water was determined by constructing saturated solutions in aqueous 50 m*M* phosphate buffer, pH = 7.4, followed by HPLC analysis. Partition coefficients were determined for partitioning between a buffered aqueous system and diethyl ether or chloroform. Solutions of test compounds were prepared in aqueous sodium phosphate buffer (pH = 7.4) and saturated with organic solvent and in buffer-saturated organic solvent. Equal volumes of these two solutions were mixed with their drug-free counterparts by gentle inversion for 18 to 24 hr at room temperature. Solutions were recovered and analyzed using a modification of a standard enzymatic method or by HPLC either directly (for aqueous samples) or after evaporation at 50°C under a stream of dry N_2 and reconstitution with aqueous HPLC mobile phase. The enzymatic method was a modification of the classical method of Maren (21), which is an end point analysis of CO_2 hydratase activity using a phenol red indicator. Assays were performed using pre-equilibration (60 sec) of CA-II (purified from bovine erythrocytes) with a range of concentrations of inhibitor. Essentially, a pre-equilibrated enzyme-inhibitor mixture was added to CO_2 saturated bicarbonate/carbonate buffer at 0°C in a reaction volume of 0.8 ml, and the time required to cause a color change was the dependent variable monitored. Drug concentrations were assessed by use of the standard enzymatic method. Preincubation of the enzyme-inhibitor mixtures results in a time-dependent, exponential decrease in IC_{50}, which is stable after 60 sec. Acetazolamide was used as the internal standard for quantitative analysis in the HPLC analysis.

The partition coefficients reported are the ratio of drug concentrations found in organic:aqueous phases.

Sulfhydryl reactivity was determined *in vitro*. Test compound (1 m*M*) was incubated at room temperature in a buffered (pH = 7.4) solution that was also 20 m*M* with respect to thiol. The thiols used were either reduced glutathione (Sigma) or cysteine. The loss of CA inhibitor was determined using purified CA-II (Sigma) and the enzymatic method.

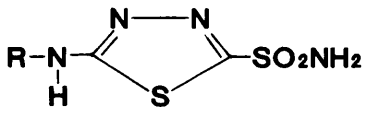
Ocular Hypotensive Effect. Adult normotensive New Zealand albino rabbits, each weighing approximately 2–3 kg, were used (six in each set) for IOP measurements after topical anesthesia as necessary (tetracaine HCl). Intraocular pressure was determined using pneumotonometry (Digilab model 30D; BioRad). All experiments were started at the same morning hour and at least 90 min after traveling from the animal facility, to minimize anxiety and diurnal variation in the IOP.

Experiments were performed in a double-masked fashion using paired design and randomized assignment of treated and control eyes. On days of experimentation, fresh preparations of test compounds were made, using the greater of 90% of maximal solubility at pH = 7.4 or 1% suspension, and drugs were applied topically in 100 μl volume. The investigator who prepared the drugs provided coded drug preparations to a different investigator who measured IOP. The drug vehicle was phosphate (10 m*M*, pH = 7.8)-buffered saline that was isotonic with respect to tears and contained 1% hydroxypropylmethylcellulose. To each rabbit, drug plus vehicle was administered to one eye; vehicle alone was administered to the contralateral eye. Time 0 was defined as the time of drug administration, and IOP was determined at 30, 60, 90, 120, 180, 240, 300, and 360 min after drug administration, with examination of the anterior segment for signs of toxicity. At the end of the experiments, IOP differences (treated-control) were calculated and Student's *t* test for paired data was used to test the null hypothesis that the difference between each treatment/control pair was zero. A value for $P < 0.05$ was selected as criterion for statistically supported significance.

Results

The structures of the test compounds, 2-ATS, and acetazolamide are shown in Figure 1. Some of the physicochemical properties of these compounds are listed in Table I.

Ethyloxaloylazolamide (2-oxalamido-1,3,4-thiadiazole-5-sulfonamide ethyl ester; EtOxAz) is approximately 10–30 times as water soluble as acetazolamide, while ethylsuccinoylazolamide (2-succinylamido-1,3,4-thiadiazole-5-sulfonamide ethyl ester; EtSuxAz) is as soluble and ethyladipoylazolamide (2-adipamido-1,3,4-thiadiazole-5-sulfonamide ethyl ester; EtAdipAz) is



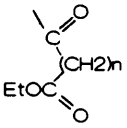
R-	*n*	*Name*	*Abbreviation*
H –	–	aminothiadiazole sulfonamide	2-ATS
acetyl –	–	acetazolamide	Actz
	0	ethyloxaloylazolamide	EtOxAz
	2	ethylsuccinylazolamide	EtSuxAz
	4	ethyladipoylazolamide	EtAdipAz

Figure 1. Structures of test compounds and their precursors.

much less water soluble than the prototype acetazolamide. Partition coefficients were determined as well (chloroform:buffer and ether:buffer) at pH = 7.4. All three novel compounds, EtOxAz, EtSuxAz, and EtAdipAz, have partition coefficients greater than or equal to that of acetazolamide, with EtAdipAz being most lipophilic. Predictably, melting points for the novel compounds decrease as chain length increases. The UV-visible absorption maximum (266 nm) is the same for all 2-amidothiadiazole sulfonamides. HPLC capacity factor (k′) shows the same rank order of lipid solubility as do the organic:buffer partition coefficients.

Thiol reactivity, a potential indicator of toxicity (22), was negligible for all the compounds studied. IC_{50} values indicate that each of the novel compounds is a potent inhibitor of carbonic anhydrase. Values are also listed for pK_as and, with one exception, are similar among the compounds. This exception (the first pK_a of EtOxAz) explains this compound's striking water solubility at pH = 7.4.

The results from *in vivo* topical administration experiments are given in Figure 2, and demonstrate the efficacy of EtOxAz as a topically active ocular hypotensive agent. For EtOxAz, measurements at all times after drug administration showed a significant ocular hypotensive effect. A decrease of 3.0 mm Hg in the IOP was observed 1 hr after drug application, and this effect persisted for at least 5 hr. EtSuxAz, given in 1% suspension, led to a modest hypotensive effect (maximal, −2.2 mm Hg) of short duration; measurements at t = 0.5, 0.75, and 1 hr showed a significant IOP lowering. EtAdipAz had no ocular hypotensive effect when given topically in the form of a 1% suspension. Saturated solutions of EtSuxAz or EtAdipAz (approximately 5 m*M* and 0.5 m*M*, respectively) had no effect on IOP.

Table I. Physicochemical Properties

	EtOxAz	EtSuxAz	EtAdipAz	Acetazolamide	2-ATS
Solubility[a] (m*M*)	90.9	5.8	0.5	3–8	
Partition coefficient[b]					
$CHCl_3$:buffer	0.0014	0.024	0.0292	0.001	
Ether:buffer	0.099	0.495	1.394	0.14	
Melting range (°C)	215–216	191–194	147–150	258–259	208–211
UV absorbance maximum (nm)	266	266	266	266	280
ϵ[c] ($M^{-1} \times cm^{-1}$)	2440	7503	6820	8740	6940
k′[d] (HPLC)	3.04	3.65	4.52	2.13	0.38
Thiol reactivity[e] (%)	<1	<1	<1	<1	<1
IC_{50} (n*M*)	1.3	2.3	2.5	0.6	4.1
pK_{a1}	4.8	7.4	7.4	7.4	6.8
pK_{a2}	9.5	9.3	8.8	8.8	8.2

[a] Maximum solubility at 18°C in 50 m*M* phosphate buffer (pH = 7.4).
[b] Organic: buffer partition coefficient using 50 m*M* phosphate buffer (pH = 7.4).
[c] Molar extinction coefficient.
[d] k′ = capacity factor = (*Ve* − *Vo*)/*Vo*; where *Vo* = void volume and *Ve* = elution volume.
[e] Percentage loss of CA inhibitor activity over 6 hr at room temperature in the presence of 20 m*M* glutathione.

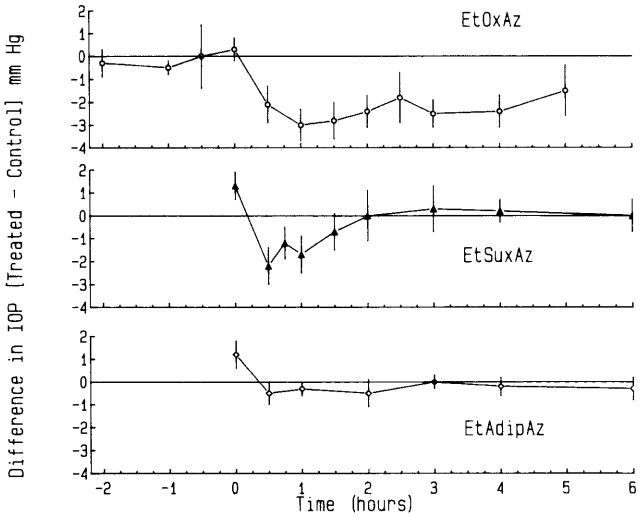


Figure 2. Time course of ocular hypotensive effects. Data presented are differences (mean ± SEM) in IOP ($IOP_{treated} - IOP_{control}$). After a single topical administration of candidate drug to the treated eye (and vehicle to the contralateral control eye), IOP measurements were obtained. Six animals were used for each group. Student's *t* test for paired data was used to test the null hypothesis that the mean difference was equal to zero, and a value of $P < 0.05$ was selected as criterion for statistically supported significance. For EtOxAz, measurements at all times showed a significant ocular hypotensive effect. For EtSuxAz, measurements at $t = 0.5$, 0.75, and 1 hr showed a significant IOP lowering. No significant effects of EtAdipAz were noted.

Discussion

The purpose of this study was synthesis of a homologous series of amidothiadiazole sulfonamides and study of the pharmacology of these agents as topically active ocular hypotensive agents. In the quest for topically useful ocular carbonic anhydrase inhibitors, the goal for years has been achievement of the proper balance of hydrophilicity and lipophilicity, to allow for the simultaneous (i) construction of solutions of sufficient drug concentrations, (ii) rapid absorption across the (relatively lipophilic) corneal epithelium, and (iii) ready passage through the (relatively hydrophilic) corneal stroma.

This series is useful for such ocular pharmacokinetic studies because its members have water and lipid solubilities that bracket those of acetazolamide—the prototypical thiadiazole CA inhibitor. These compounds also differ from some of the benzothiazole CA inhibitors in that they are not subject to displacement of the required sulfonamide group after attack by a free thiol. CA inhibitors that react readily with sulfhydryl groups are not useful, as they are rapidly inactivated and may serve as haptens, later provoking an immunologic response.

When tested as ocular hypotensive agents after application to the cornea, only EtOxAz has the ability to lower intraocular pressure to a significant degree. The maximum effect observed was somewhat variable (Table II). We tentatively attribute this to pharmacokinetic variables, but also note that EtOxAz has never failed to lower IOP in this model. While the decrease produced by EtSuxAz was statistically significant, this distinction is of dubious value. Unless a drug is capable of lowering IOP by 3–4 mm Hg in the normotensive New Zealand White rabbit, it will most likely not be pharmacologically useful. Application of EtAdipAz topically has no effect on IOP.

Due to its high degree of water solubility, a substantial amount of EtOxAz can be dissolved in aqueous solution at pH values that do not deviate extremely from the physiologic range. When mixed with the tear film (pH approximately 7.6), EtOxAz, with a first pK_a of 4.8, is far more water soluble than either EtSuxAz or EtAdipAz. The ethyloxaloyl side chain increases

Table II. Integrated Time-Effect Data

Compound[a]	Maximal decrease in IOP[b] (mm Hg)	Duration (hr)	Area under curve (mm Hg × hr)
Florida (Maren)			
Trifluoromethazolamide (7)	−3.1 ± 0.3	5.7	5.9
"Propionylazolamide" (7)	−2.2 ± 0.5	8.0	7.5
Iowa (Schoenwald & Barfknecht)			
6-OH ethoxzolamide susp. (10)	−1.9 ± 0.5	4.0	2.6
6-OH ethoxzolamide gel (10)	−2.6 ± 0.6	5.0	6.7
Aminozolamide gel (7)	−2.2 ± 0.3	5.0	6.2
N-Methyl Actz (13)	−2.0 ± 0.4	3.5	4.2
Merck			
L-645,151 (12)	−2.6 ± 0.3	>5.0	>8.0
MK-927 (15)	−2.9 ± 0.4	>6.0	>11.2
MK-507 (16)	−3.4 ± 0.4	>4.0	>10.3
This report			
EtOxAz	−3.0 ± 1.7	5.0	9.5
EtSuxAz	−2.2 ± 0.8	2.0	1.5
EtAdipAz	−1.0 (approx.)	2.0	<1.0

[a] Numbers in parentheses after compound are reference numbers.
[b] Decrease = treated eye IOP − control eye IOP. Data are expressed as mean ± SE.

water solubility of EtOxAz with respect to the prototype acetazolamide without significantly altering lipid solubility.

All three of the members of this class have approximately the same potency *in vitro* as CA inhibitors; thus, the differences in efficacy among these three compounds is most likely due to pharmacokinetic properties, i.e., the ability to traverse the cornea before being washed away in the tears. In these short-term, single-dose experiments, no effect was observed on IOP in the contralateral eye.

A useful method for comparing the efficacy of ocular hypotensive compounds is the use of the area under curve (or area under the line [7]), the integrated time-effect curves. This is a more representative index that takes into account both the magnitude and duration of effect.

Table II shows a comparison of results of this study with data obtained from other laboratories and other compounds using the same model. This comparison demonstrates that EtOxAz effect is comparable to the values reported by other research groups. This series is the first of a group of modified thiadiazole CA inhibitors from this laboratory. Future studies of the pharmacology of these compounds will address questions of ocular and systemic pharmacokinetics, and further modifications of structure will be attempted to increase the magnitude and the duration of the ocular hypotensive effect.

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