and reconstituted as described for **5d**. Extraction efficiency was greater than 80%.

Separation Procedures. Three HPLC procedures were developed, one for the isolation and identification of **5d** and two for **5m**.

Procedure I: HPLC Separation of 5d. Stationary phase: Ultrasphere ODS precolumn (4.6 mm × 45 mm) with a 5-μm Ultrasphere ODS analytical column (4.6 mm × 250 mm). Mobile phase: 60% CH_3CN, 40% sodium phosphate buffer (0.025 M), pH 7.2. Flow rate: 1 mL/min. Temperature: ambient. Either detection was spectrophotometric using a fixed-wavelength detector with a 229-nm filter (cadmium lamp) or fractions were collected for subsequent scintillation counting. The retention time for **5d** was 10.4 min.

Procedure II: HPLC Separation of 5m Using Reversed-Phase Ion-Pair Chromatography. Stationary phase: Ultrasphere ion-pair precolumn (4.6 mm × 45 mm) with a 5-μm Ultrasphere ion-pair analytical column (4.6 mm × 250 mm). Mobile phase: 55% MeOH, 22.5% tetrabutylammonium hydroxide (0.025 M), 22.5% potassium phosphate, pH 8.0, buffer (0.02 M). Flow rate: 1.2 mL/min. Temperature: 35 °C. Either detection was spectrophotometric using a fixed-wavelength detector with a 229-nm filter (cadmium lamp) or fractions were collected for subsequent scintillation counting. The retention time for **5m** was 28 min.

Procedure III: HPLC Separation of 5m Using Reversed-Phase Chromatography. Stationary phase: Ultrasphere ODS precolumn (4.6 mm × 45 mm) with a 5-μm ultrasphere ODS analytical column (4.6 mm × 250 mm). Mobile phase: 35% MeOH, 65% potassium phosphate, pH 5.3, buffer (20 mM). Flow rate: 1 mL/min. Temperature: ambient. Detection was as described above. The retention time for **5m** was 24.5 min.

Mass Spectral Analysis of M_1. A sample of metabolite M_1 (50 mg) was purified under HPLC conditions III by collecting fractions eluting from the HPLC column that coeluted with the radioactive peak for M_1. The material collected was evaporated to dryness under N_2. Mass spectra were obtained by using a Finnigan 4000 series quadrupole mass spectrometer. Solid-probe electron-impact ionization spectra of both **5m** and M_1 were obtained.

β-Glucuronidase Deconjugation and Mass Spectral Analysis of M_2. The purified metabolite (0.1 mg) M_2 was incubated in a volume of 2 mL in potassium phosphate, pH 5.0, buffer (0.2 M) containing 1000 units of β-glucuronidase derived from *Helix pomatia*. The incubation was maintained at 37 °C for 6 h prior to extraction and chromatography under the conditions previously described. Under the conditions of the incubation any sulfatase activity present in the β-glucuronidase would have been inhibited by the high concentration of phosphate present. The deconjugated product was subjected to mass spectral analysis as above.

Acknowledgment. We thank R. L. Dyer and M. D. Gray for the synthesis of [^{14}C]-**5d** and R. Brownsill and J. Firth for mass spectral analysis. We also express our appreciation to J. Brand for the preparation of the manuscript.

Registry No. **3a**, 108836-41-5; **3b**, 108836-42-6; **4a**, 108836-43-7; **4b**, 108836-44-8; **5a**, 108836-45-9; **5b**, 108836-45-9; **5c**, 108836-46-0; **5d**, 108836-47-1; **5e**, 108836-48-2; **5f**, 108836-49-3; **5g**, 108836-50-6; **5h**, 108836-51-7; **5i**, 108836-52-8; **5j**, 108836-53-9; **5k**, 108836-54-0; **5l**, 108836-55-1; **5m**, 108836-56-2; **5n**, 108836-57-3; **5o**, 108836-58-4; **5p**, 108836-59-5; **5q**, 108836-60-8; **5r**, 108836-61-9; **5s**, 108836-62-0; **5t**, 108836-63-1; **5u**, 108836-64-2; **5v**, 108836-65-3; 4-methoxybenzyl alcohol, 105-13-5; epibromohydrin, 3132-64-7; ethyl 4-(hydroxymethyl)benzoate, 15852-63-8; imidazole, 288-32-4; ethyl (4-(bromomethyl)benzoate, 26496-94-6; 4-(methoxybenzyl chloride, 824-94-2; 4-(methylthio)benzyl chloride, 874-87-3; methyl 4-(bromomethyl)benzoate, 2417-72-3; ethyl 3-(bromomethyl)-benzoate, 62290-17-9; 5-(bromomethyl)-1,3-dihydroisobenzofuran-1-one, 63113-10-0.

Novel Inhibitors of Rat Lens Aldose Reductase: *N*-[[(Substituted amino)phenyl]sulfonyl]glycines

Charles A. Mayfield and Jack DeRuiter*

Division of Medicinal Chemistry, Department of Pharmacal Sciences, School of Pharmacy, Auburn University, Auburn University, Alabama 36849. *Received February 9, 1987*

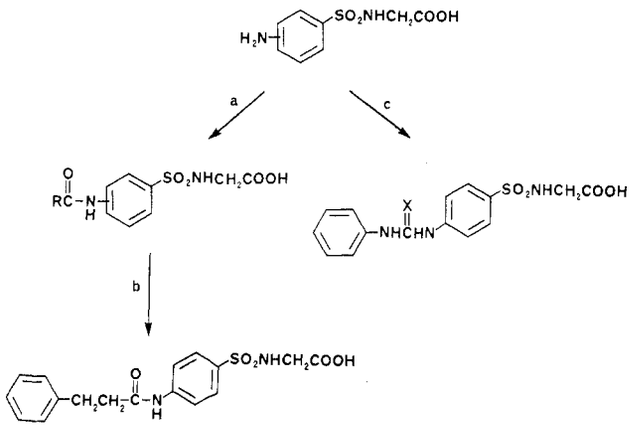
A number of *N*-[[(substituted amino)phenyl]sulfonyl]glycines **3a–n** were synthesized as analogues of the simple (phenylsulfonyl)glycines **1a–c** with increased lipophilic character and therefore greater aldose reductase inhibitory potential. The 2-benzoylamino derivative **3c** was found to be less potent than the corresponding amine **1c** as an inhibitor of rat lens aldose reductase, but both the 3- and 4-benzoylamino analogues, **3b** and **3a**, are substantially more potent than their amines **1b** and **1a**; compound **3a** is the most effective inhibitor of this series, with an IC_{50} of 0.41 μM. The 4-benzoylamino derivative **3a** is also significantly more active than the 4-acetylamino analogue **3d** and the 4-benzylamino (**3e**) and 4-dimethylamino (**3f**) derivatives, suggesting that both the additional carbonyl moiety and aromatic ring present in this compound may bind to complementary sites present on the enzyme. Furthermore, structure–activity studies reveal that increasing the number of atoms between the carbonyl and aromatic moieties of **3a** results in a decrease in inhibitory activity. Kinetic studies demonstrate that **3a**, like other known inhibitors of aldose reductase, functions as an uncompetitive inhibitor with respect to the substrate and therefore may interact at the proposed common inhibitor binding site of this enzyme.

As described earlier in considerable detail,[1-3] there is mounting evidence linking the conversion of glucose to sorbitol by aldose reductase in ocular and nerve tissues to the development of several chronic diabetic pathologies including cataracts, retinopathy, and peripheral neuropathy.[1-3] Thus, one approach currently investigated to prevent or at least delay the onset of diabetic pathologies has involved the development of inhibitors of the enzyme aldose reductase.[3-7] In a previous paper, we described the

(1) Dvornik, D.; Simard-Duquesne, N.; Krami, M.; Sestanj, K.; Gabbay, K. H.; Varma, S. D.; Merola, L. O. *Science (Washington, D.C.)* **1973**, *182*, 1146.
(2) Lipinski, C. A.; Hutson, N. J. *Annu. Rep. Med. Chem.* **1984**, *19*, 169.
(3) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. *J. Med. Chem.* **1985**, *28*, 841.
(4) DeRuiter, J.; Brubaker, A. N.; Millen, J.; Riley, T. N. *J. Med. Chem.* **1986**, *29*, 627.
(5) Brubaker, A. N.; DeRuiter, J.; Whitmer, W. L. *J. Med. Chem.* **1986**, *29*, 1094.
(6) DeRuiter, J.; Brubaker, A. N.; Whitmer, W. L.; Stein, J. L. *J. Med. Chem.* **1986**, *29*, 2024.
(7) DeRuiter, J.; Brubaker, A. N.; Garner, M. A.; Barksdale, J. M.; Mayfield, C. A. *J. Pharm. Sci.* **1987**, *76*, 149.

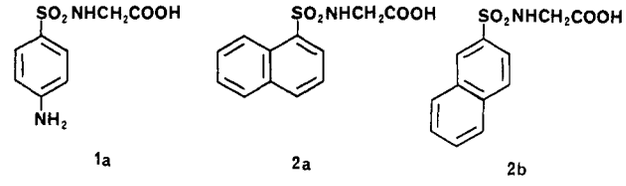
0022-2623/87/1830-1595$01.50/0

Scheme I[a]



[a] a, RCOCl, $NaHCO_3$, THF, H_2O or NaOH, H_2O; b, H_2, Pd/C, EtOH; c, $C_6H_5N{=}C{=}X$, $CHCl_3$ or EtOH.

synthesis and in vitro aldose reductase inhibitory activity of a number of *N*-(arylsulfonyl)amino acids as compounds that may contain the minimum structural requirements for enzyme inhibition.[7] Of these compounds, the *N*-(phenylsulfonyl)glycines were found to display modest inhibitory activity against enzyme obtained from rat lens, with the 4-amino derivative **1a** being among the most



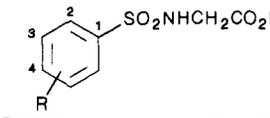
1a **2a** **2b**

potent monosubstituted analogues, with an IC_{50} of 16 μM. Also, the α- and β-naphthalene analogues **2a** and **2b**, whihch represent derivatives of **1a** in which the benzene ring has been replaced with a moiety of greater lipophilic character, were found to be the most potent compounds of this series, with IC_{50}'s of 1.3 and 0.4 μM, respectively.[7] These observations prompted us to prepare a number of derivatives of **1** that contain an additional lipophilic moiety linked to the amino moiety. The (phenylsulfonyl)glycine derivative **1a** was selected as the parent compound for these studies since it is one of the most potent monosubstituted glycine derivatives, and it can be easily functionalized by using standard synthetic procedures.

Chemistry

[(4-Aminophenyl)sulfonyl]glycine (**1a**), which served as a key intermediate for these syntheses, and the corresponding 2- and 3-amino isomers **1c** and **1b** were prepared by reaction of commercially available nitrobenzenesulfonyl chlorides with glycine in aqueous sodium hydroxide, followed by catalytic hydrogenation.[9,10] Treatment of amines **1a–c** with acid chlorides in the presence of aqueous base provided the acylamino derivatives **3a–c,g,i,m,n** (Scheme I and Table I). Also, catalytic reduction of the cinnamic acid amide **3i** prepared by this method gave the phenylpropionamide **3h**. The acetylamino derivative **3d** was obtained upon chlorosulfonation of acetanilide followed by treatment of the intermediate with glycine in aqueous sodium hydroxide. The urea and thiourea derivatives **3j** and **3k** were prepared by reaction of the 4-amino intermediate **1a** with the appropriate isocyanate or isothiocyanate (Scheme I and Table I). *N*-Alkyl analogues **3e** and **3f** were prepared by reductive alkylation. Treatment of the intermediate amine **1a** with benzaldehyde in the presence of sodium cyanoborohydride afforded the *N*-benzylamine analogue **3e**. The corresponding reaction with formaldehyde gave the *N*,*N*-dimethyl product **3f** (Table I).

Table I. *N*-[[(Substituted amino)phenyl]sulfonyl]glycines



no.	R	mp,[a] °C	yield,[b] %	formula[c]
1a	4-NH_2	169–170 (170)[d]	81	$C_8H_{10}N_2O_4S$
1b	3-NH_2	149–150 (148)[e]	82	$C_8H_{10}N_2O_4S$
1c	2-NH_2	145–147 (155)[e]	89	$C_8H_{10}N_2O_4S$
3a	4-(C_6H_5CONH)	232–235	74	$C_{15}H_{14}N_2O_5S\cdot {}^1/_3H_2O$
3b	3-(C_6H_5CONH)	209–212	90	$C_{15}H_{14}N_2O_5S$
3c	2-(C_6H_5CONH)	170–174	18	$C_{15}H_{14}N_2O_5S$
3d	4-(CH_3CONH)	230–234 dec	74	$C_{10}H_{12}N_2O_5S$
3e	4-($C_6H_5CH_2NH$)	132–138	22	$C_{15}H_{16}N_2O_4S\cdot {}^1/_4H_2O$
3f	4-$(CH_3)_2N$	165–173	14	$C_{10}H_{14}N_2O_4S$
3g	4-($C_6H_5CH_2CONH$)	213–217	96	$C_{16}H_{16}N_2O_5S$
3h	4-($C_6H_5CH_2CH_2CONH$)	188–190	63	$C_{17}H_{18}N_2O_5S$
3i	4-($C_6H_5CH{=}CHCONH$)	218–220	54	$C_{17}H_{16}N_2O_5S$
3j	4-($C_6H_5NHCONH$)	185	27	$C_{15}H_{15}N_3O_5S\cdot {}^1/_3H_2O$
3k	4-($C_6H_5NHCSNH$)	135–137	34	$C_{15}H_{15}N_3O_4S_2\cdot H_2O$
3l	4-($C_6H_5CH_2OCONH$)	143–148	22	$C_{16}H_{16}N_2O_6S$
3m	4-($C_6H_5OCH_2CONH$)	173–174	80	$C_{16}H_{16}N_2O_6S\cdot H_2O$
3n	4-($C_6H_5CH_2OCH_2CONH$)	156–158	56	$C_{17}H_{18}N_2O_6S$

[a] Compounds **1–c** were recrystallized from watter, and compounds **3a–c,g,i,k,n** were recrystallized from ethanol. Compounds **3d–f,h,j,l,m** were recrystallized from mixtures of ethanol and water. [b] Yields given for **1a–c** represent the yields obtained from reduction of the corresponding nitro compounds. Yields for **3a–n** represent yields of final acylation or alkylation reaction. [c] All products exhibited IR and ^1H NMR spectra consistent with the assigned structures and gave satisfactory C, H, N, and S combustion analyses. [d] Reference 9. [e] Reference 10.

Results and Discussion

All of the *N*-[[(acylamino)phenyl]sulfonyl]- and *N*-[[(alkylamino)phenyl]sulfonyl]glycines **3a–n**, as well as the [(aminophenyl)sulfonyl]glycines **1a–c**, were tested for their ability to inhibit aldose reductase as described previously.[6] Those compounds found to produce greater than 50% enzyme inhibition at 100 μM were tested at additional concentrations to generate log dose–response curves. Inhibitor IC_{50} values were then calculated from the linear portion of the log dose–response curves using the LINEFIT linear regression program of Barlow (Table II).[8]

In the rat lens assay, the simple [(aminophenyl)-sulfonyl]glycines **1a–c** were found to be relatively weak inhibitors of aldose reductase, with IC_{50} values ranging from 16 to 38 μM (Table II). In this series the 4-amino derivative **1a** is more potent than the 3-amino derivative **1b**, which is slightly more active than the 2-amino analogue **1c**. The 2-benzoylamino derivative **3c**, with an IC_{50} of 78 μM, displays only $^1/_2$ the inhibitory activity of the corresponding 2-amino derivative **1c**. Both the 3- (**3b**) and 4-benzoylamino (**3a**) analogues, however, are substantially

(8) Barlow, R. B. *Biodata Handling with Microcomputers*; Elsevier Science: Amsterdam, 1983; p 76.
(9) Ghosh, N. N.; Majunmder, M. N. *J. Indian Chem. Soc.* **1967**, *44*, 115.
(10) Ghosh, N. N.; Dasgupta, M. *J. Indian Chem. Soc.* **1966**, *43*, 545.
(11) Kador, P. F.; Sharpless, N. E. *Mol. Pharmacol.* **1983**, *24*, 521.

Table II. Aldose Reductase Inhibitory Activity

compd	R	% inhibn at 100 μM (SEM)[a]	IC_{50},[b] μM
1a	4-NH_2	76 (2)	16
1b	3-NH_2	73 (2)	29
1c	2-NH_2	74 (4)	38
3a	4-(C_6H_5CONH)	81 (2)	0.41
3b	3-(C_6H_5CONH)	79 (3)	7.12
3c	2-(C_6H_5CONH)	52 (3)	78
3d	4-(CH_3CONH)	88 (5)	4.22
3e	4-($C_6H_5CH_2NH$)	71 (1)	7.04
3f	4-$(CH_3)_2N$	80 (3)	10.9
3g	4-($C_6H_5CH_2CONH$)	100 (1)	2.17
3h	4-($C_6H_5CH_2CH_2CONH$)	86 (1)	11.5
3i	4-(C_6H_5CH=$CHCONH$)	72 (2)	0.69
3j	4-($C_6H_5NHCONH$)	83 (2)	2.75
3k	4-($C_6H_5NHCSNH$)	65 (1)	8.42
3l	4-($C_6H_5CH_2OCONH$)	82 (1)	6.03
3m	4-($C_6H_5OCH_2CONH$)	87 (3)	2.64
3n	4-($C_6H_5CH_2OCH_2CONH$)	79 (2)	3.50
sorbinil		ND[c]	0.073[d]

[a] Percent inhibition at an inhibitor concentration of 100 μM followed by the standard error of the mean (SEM). [b] IC_{50} values represent the concentration required to produce 50% enzyme inhibition. The significance level for the least-squares fit of the log dose–response curves was <0.01. [c] Not determined. [d] The literature IC_{50} value for sorbinil is 0.07 μM.[11]

more potent than their corresponding amino derivatives **1b** and **1a**. For example, the 4-benzoylamino derivative **3a** is 40 times as potent as **1a**, while the 3-benzoylamino analogue **3b** is ca. 4 times as active as **1b**. Also, the 4-acetylamino compound **3d**, with an IC_{50} of 4.22 μM, is nearly 4 times as potent as the 4-amino derivative **1a**.

The increased inhibitory activity observed for compounds **3a**, **3b**, and **3d** suggests that addition of a carbonyl moiety to the amino group of **1a** or **1b** may provide an additional site for interaction with the enzyme. Also, since the 4-benzoylamino derivative **3a** is 10 times more active than the 4-acetylamino derivative **3d**, it appears that the additional lipophilic ring of **3a** may also contribute toward inhibitory potency, perhaps through direct interactions with complementary binding sites prsent on aldose reductase. Additional support for these hypotheses is derived from consideration of the inhibitory data obtained with both the 4-benzylamino derivative **3e** and the 4-dimethylamino compound **3f**. These compounds, with IC_{50} values of about 7 and 11 μM, respectively, are somewhat more potent than the parent 4-amino compound **1a**, but since they lack the amide carbonyl moiety, they are not as potent as the corresponding benzoylamino derivative **3a**. Therefore both the additional carbonyl moiety and, to a lesser extent, the added aromatic ring present in **3a** appear to contribute toward the enhanced inhibitory activity of this compound. Furthermore, the significant differences in potency observed for **3a–c** demonstrate the importance of the position of these moieties relative to the (phenylsulfonyl)glycine fragment for activity.

The high level of inhibitory activity observed for the 4-benzoylamino derivative **3a** prompted an investigation of the influence of varying the number and nature of atoms between the amide carbonyl and the additional aromatic ring present in this compound. The importance of the number of carbon atoms between these functional groups is demonstrated by comparison of the inhibitory data obtained with the 4-benzoylamino **3a**, 4-phenylacetylamino **3g**, and 4-phenylpropionylamino **3h** derivatives where as the number of atoms is increased, inhibitory activity steadily decreases from 0.41 to 11.5 μM (Table II). One exception to this trend is the cinnamoylamino analogue **3i**, which displays inhibitory potency comparable to that

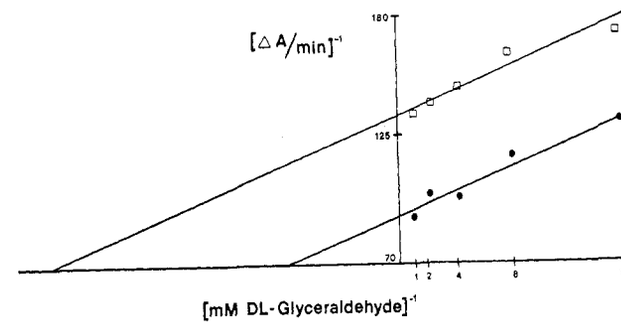


Figure 1. Double-reciprocal plot of initial enzyme velocity vs. concentration of substrate (DL-glyceraldehyde) with and without added inhibitor **3a**: no inhibitor (●); 0.5 μM inhibitor (□).

of the 4-benzoylamino derivative **3a**. The greater inhibitory activity of this compound compared to the 4-phenylpropionyl derivative **3h** may be a result of the ability of its vinylic moiety to partially mimic the aromatic ring of **3a** in the inhibitor–enzyme interaction.

Examination of the inhibitory data obtained for compounds **3j–n** reveals that varying the nature of the atoms in the 4-aroylamino side chain does not dramatically alter activity (Table II). For example, the phenylurea derivative **3j** is essentially equipotent with the isoelectronic 4-phenylacetylamino compound **3g**, while the thiourea analogue **3k** is 4 times less active. The oxygen isosteres of the phenylpropionyl compound, **3l** and **3m**, are ca. 2 and 5 times more potent, respectively, than **3h**. All of these derivatives (**3j–n**), however, are significantly less active as inhibitors of aldose reductase than the 4-benzoylamino derivative **3a**.

Therefore, modification of [(4-aminophenyl)sulfonyl]-glycine (**1a**) and, to a lesser extent, the 3-amino derivative **1b** by N-benzoylation, as in **3a** and **3b**, results in a significant increase in aldose reductase inhibitory activity. Also, while **3a** is as potent as the (β-naphthalenylsulfonyl)glycine derivative **2b** (IC_{50} = 0.40 μM), its enhanced inhibitory potency appears to be a result of the presence of both the additional carbonyl and lipophilic moieties.

Other known inhibitors of aldose reductase have been shown to produce uncompetitive inhibition with respect to the substrte (glyceraldehyde), and this observation, along with data obtained from competition studies, has led to the postulte that these compounds bind to a common site on the enzyme which is distinct from the substrate binding site; this common site of interaction has been designated as the "inhibitor binding site".[3] Enzyme kinetic studies with **3a** reveal that it produces uncompetitive inhibition relative to the substrate (Figure 1), suggesting that this compound may also interact at the distinct inhibitor binding site. Therefore **3a** may serve as a valuable lead structure for the design of additional analogues to probe the inhibitor binding site of aldose reductase and further determine the steric and electronic requirements for optimal inhibitory activity.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian T-60A NMR spectrometer in $CDCl_3$ or Me_2SO-d_6 solutions, with Me_4Si as an internal standard. IR spectra were recorded on a Beckman 4230 infrared spectrophotometer as Nujol mulls. UV spectra and enzyme reaction rates were measured with a Shimadzu UV-160 spectrophotometer. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are within 0.4 of theoretical percentages. Common reagent grade chemicals were purchased from commercial sources and were used as received. DL-

Glyceraldehyde and NADPH (type 1) were purchased from Sigma Chemical Co.

[(Aminophenyl)sulfonyl]glycines 1a–c. Nitrobenzenesulfonyl chloride (5.2 g, 20 mmol) was added portionwise over a period of 0.5 h to a warm (60–70 °C), stirred solution of glycine (1.5 g, 20 mmol) and NaOH (1.6 g, 40 mmol) in H_2O (20 mL). After addition was complete, the reaction mixture was stirred for an additional 0.5 h at 60–70 °C and then cooled to room temperature followed by further cooling in an ice bath. Upon acidification (pH 1) of the reaction mixture with concentrated HCl, the crude products formed as thick precipitates. The products were isolated by filtration and recrystallized from aqueous ethanol to give the [(nitrophenyl)sulfonyl]amino acids **5a–c** (31–58%). Solutions of the intermediate nitro compounds (10 mmol) in ethanol (100 mL) containing 5% Pd/C (200 mg) were shaken under an H_2 atmosphere (initially at 50 psi) on a Parr apparatus until consumption of H_2 ceased (within 2 h). The reaction mixture was then filtered and the filtrate evaporated under vacuum to yield a yellow solid. Recrystallization from aqueous ethanol gave the [(aminophenyl)sulfonyl]glycines **1a–c** (Table I).

***N*-[[(Acylamino)phenyl]sulfonyl]glycines 3a–c,g,i,m,n.** A solution of the acid chloride (5.5 mmol) in THF (10 mL) was added dropwise over a period of 0.5 h to a stirred solution of **1a–c** (10 mmol) in THF (10 mL) and H_2O (20 mL) containing $NaHCO_3$ (0.84 g). After addition was complete, the reaction mixture was stirred at reflux overnight and then allowed to cool to room temperature. The mixture was evaporated in vacuo to yield a solid or oil, which was suspended in H_2O (35 mL). The resultant solution was cooled (ice bath) and acidified (to pH 1) with concentrated HCl to yield the crude product as a thick precipitate. Recrystallization gave the *N*-[[(acylamino)phenyl]sulfonyl]glycines (Table I).

***N*-[[4-[(Benzyloxy)carbonyl]amino]phenyl]sulfonyl]glycine (3l).** Benzyl chloroformate (0.62 mL, 4.3 mmol) was added dropwise to a warm (60–70 °C), stirred solution of **1a** (1.0 g, 4.3 mmol) and NaOH (0.35 g, 8.7 mmol) in H_2O (20 mL) and the reaction mixture stirred at 60–70 °C for 0.5 h. Upon cooling (ice bath) and acidification (pH 1) with concentrated HCl, the crude product formed as a thick white precipitate. Recrystallization from aqueous ethanol gave **3l** (0.35 g, 22%) as white crystals (Table I).

***N*-[[4-(Acetylamino)phenyl]sulfonyl]glycine (3d).** Chlorosulfonic acid (50 mL, 772 mmol) was added dropwise with stirring to dry acetanilide (20 g, 0.145 mmol). After addition was complete, the reaction mixture was heated at reflux for 1 h, then allowed to cool to room temperature, and poured into a stirred slurry of ice water (300 g). The resulting suspension of white solid was stirred for several minutes to obtain an even suspension, and then filtered to yield the intermediate 4-acetamidobenzenesulfonyl chloride, which was added portionwise (1.13 g, 4.8 mmol) with stirring to a warm (80–90 °C) solution of glycine (0.36 g, 4.8 mmol) and NaOH (0.40 g) in H_2O (20 mL). After addition was complete, the reaction mixture was stirred for 0.5 h at 80–90 °C and then cooled to room temperature followed by further cooling (ice bath). Upon acidification (pH 1) with concentrted HCl, the crude product formed as a thick white precipitate. Isolation by vacuum filtration followed by recrystallization from aqueous ethanol provided the product **3d** as feathery white crystals (0.97 g, 74%) (Table I).

***N*-[[4-[(Phenylpropionyl)amino]phenyl]sulfonyl]glycine (3h).** A solution of **3i** (1.0 g, 2.8 mmol) in EtOH (100 mL) containing 5% Pd/C (500 mg) was shaken under an H_2 atmosphere (initially at 50 psi) on a Parr apparatus for 16 h. The reaction mixture was filtered and the filtrate evaporated under vacuum to yield an off-white solid. Recrystallization from aqueous ethanol gave **3h** (0.64 g, 63%) as shiny silver crystals (Table I).

***N*-Phenyl-*N'*-[4-(*N*-glycinylsulfonyl)phenyl]urea (3j).** A solution of **1a** (1.73 g, 7.5 mmol) and phenyl isocyanate (1.1 mL, 10 mmol) in $CHCl_3$ (25 mL) was stirred at room temperature for 2 h. Water (10 mL) and concentrated HCl (3 drops) were then added, and the mixture was stirred for 0.5 h followed by evaporation under vacuum to yield a peach colored solid. The solid was dissolved in H_2O (30 mL) and the pH adjusted to 1–2 with concentrated HCl (30 mL). $CHCl_3$ was added, and the resulting suspension was isolated by vacuum filtration. The solid was dissolved in saturated $NaHCO_3$ (75 mL) and the solution filtered. The filtrate volume was reduced, then cooled (ice bath), and acidified to pH 1 with concentrated HCl to yield the crude product as a yellow precipitate. Recrystallization from aqueous ethanol gave **3j** (0.70 g, 26.7%) as tan crystals (Table I).

***N*-Phenyl-*N'*-[4-(*N*-glycinylsulfonyl)phenyl]thiourea (3k).** A solution of phenyl isothiocyanate (1.2 mL, 10 mmol) and **1a** (2.30 g, 10 mmol) in EtOH (20 mL) was stirred at reflux for 0.5 h. Water was then added dropwise to the hot solution until a permanent cloudiness resulted. The mixture was allowed to cool to room temperature followed by further cooling in an ice bath. The resulting white solid was collected by filtration and washed with hexane. Recrystallization from ethanol gave **3k** (1.25 g, 34%) as granular yellow crystals (Table I).

***N*-[[4-(Alkylamino)phenyl]sulfonyl]glycines 3e and 3f.** A mixture of aldehyde (7.0 mmol), [(4-aminophenyl)sulfonyl]-glycine (**1a**) (1.61 g, 7.0 mmol), and sodium cyanoborohydride (1.33 g, 21 mmol) in acetonitrile (50 mL) was stirred at room temperature for 4.5 h. The reaction mixture was then evaporated to dryness in vacuo and the residue dissolved in H_2O (35 mL) and then acidified to pH 5 with glacial acetic acid. The acidified solution was extracted with $CHCl_3$ (2 × 75 mL), and the combined $CHCl_3$ extracts were washed with H_2O (100 mL) and evaporated in vacuo to yield the crude products. Recrystallization from aqueous ethanol gave **3e** and **3f** (Table I).

Enzyme Assay. Frozen rat eyes were purchased from Charles River Breeding Labs., Inc., and the lenses were dissected from the partially thawed eyes and were then kept at −6 °C until used for the assay. Crude enzyme supernatant was prepared by homogenizing 50 lenses in distilled water (10 mL) and then centrifuging the crude homogenate at 10000 rpm for 10 min while an ambient temperature of 0–5 °C was maintained.

Aldose reductase activity of the freshly prepared supernatant was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm with a Shimadzu UV-160 double-beam spectrophotometer. The reaction mixture contained 0.1 M phosphate buffer, pH 6.2; 0.104 mM NaDPH; 10 mM DL-glyceraldehyde; and 0.2 mL of the enzyme supernatant in a total volume of 2.0 mL. The reference blank contained all of the above reagents except glyceraldehyde, to correct for nonspecific reduction by NADPH. The reaction was initiated by the addition of glyceraldehyde, and it was monitored spectrophotometrically for 3.0 min. Enzyme activity was adjusted by diluting the supernatant with distilled water so that 0.2 mL of enzyme supernatant gave an average reaction rate for the control sample of 0.010 ± 0.002 absorbance units/min. The inhibitory activity of compounds **1a–c** and **3a–n** was determined by including 0.2 mL of each inhibitor solution at the desired concentration in the enzyme reaction mixture. The percent inhibition for each compound at each concentration (tested at four concentrations with three determinations at each concentration) was calculated by comparing the reaction rate of the solution containing both substrate and inhibitor with that of control solutions containing only the substrate. Inhibitor IC_{50} values were then calculated from the inhibitory data from least-squares analysis of the log dose–response curves using the LINEFIT program of Barlow.[8] Enzyme kinetic studies were performed with **3a** by using five concentrations of substrate (1.0, 0.5, 0.25, 0.125, and 0.0625 mM) and approximately the IC_{50} concentration of **3a** (0.5 μM). The type of inhibition produced by **3a** was determined by analysis of the plots of reciprocal initial velocities vs. reciprocal substrate concentrations with and without inhibitor **3a** present (Figure 1). These plots were generated by least-squares fit of the data, and the effect of **3a** on K_m and V_{max} was verified by using the nonlinear least-squares HYPMIC program of Barlow.[8]

Registry No. **1a**, 5616-30-8; **1b**, 109065-68-1; **1c**, 13514-59-5; **3a**, 109065-69-2; **3b**, 109065-70-5; **3c**, 109086-31-9; **3d**, 23776-98-9; **3e**, 109065-71-6; **3f**, 109065-72-7; **3g**, 109065-73-8; **3h**, 109065-74-9; **3i**, 109065-75-0; **3j**, 109065-76-1; **3k**, 109065-77-2; **3l**, 58960-90-0; **3m**, 109065-78-3; **3n**, 109065-79-4; **5a**, 15054-44-1; **5b**, 1215-64-1; **5c**, 15054-42-9; 4-$O_2NC_6H_4SO_2Cl$, 98-74-8; 3-$O_2NC_6H_4SO_2Cl$, 121-51-7; 2-$O_2NC_6H_4SO_2Cl$, 1694-92-4; H-Gly-OH, 56-40-6; PhCOCl, 98-88-4; $PhCH_2COCl$, 103-80-0; PhCH=CHCOCl, 102-92-1; $PhOCH_2COCl$, 701-99-5; $PhCH_2OCH_2COCl$, 19810-31-2; $PhCH_2OCOCl$, 501-53-1; PhNHAc, 103-84-4; 4-$AcNHC_6H_4SO_2Cl$, 121-60-8; PhNCO, 103-71-9; PhNCS, 103-72-0; PhCHO, 100-52-7; HCHO, 50-00-0; aldose reductase, 9028-31-3.