N- and 2-Substituted N-(Phenylsulfonyl)glycines as Inhibitors of Rat Lens Aldose Reductase

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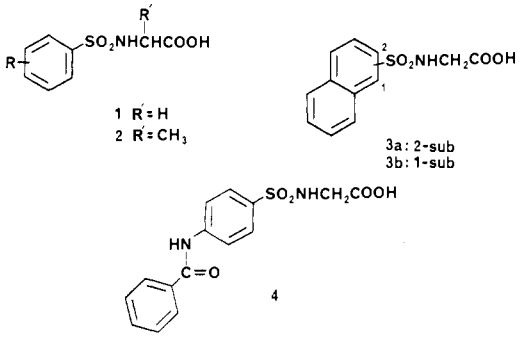
A variety of N-(phenylsulfonyl)-N-phenylglycines **5**, N-(phenylsulfonyl)-2-phenylglycines **6**, and N-(phenylsulfonyl)anthranilic acids **7** were prepared as analogues of the N-(phenylsulfonyl)glycine 1 aldose reductase inhibitors. In the rat lens assay, several derivatives of **5** display greater inhibitory activity than the corresponding glycines 1, suggesting that N-phenyl substitution enhances affinity for aldose reductase. Enzyme kinetic evaluations of the 4-benzoylamino analogues of **5** and 1 demonstrate that these compounds produce inhibition by the same mechanism. However, the significant differences in relative inhibitory potencies between compounds of series **5** and 1 may indicate that these compounds do not interact with the inhibitor binding site in precisely the same manner. Evaluation of the individual enantiomers of series **6** reveals that the S isomers are substantially more active than the corresponding R isomers. Also, with the exception of the naphthalene analogue **6n**, the S stereoisomers of this series display greater inhibitory potencies than the glycines 1. The anthranilates **7** generally are less active than the glycines 1, demonstrating that direct incorporation of an aromatic ring in the glycine side chain may result in a decrease in affinity for aldose reductase.

The relationship between increased polyol pathway activity and the development of chronic diabetic pathologies such as cataracts, retinopathy, neuropathies, and nephropathies has been well documented over the past decade.[1-5] During hyperglycemia the polyol pathway is activated in ocular, nerve, and renal tissue, resulting in reduction of the excess glucose to sorbitol, a reaction catalyzed by the NADPH-dependent enzyme aldose reductase. Once formed, sorbitol accumulates intracellularly due to its inability to diffuse out of the cell and inefficient oxidation to fructose by sorbitol dehydrogenase.[6,7] As sorbitol accumulates in tissues such as the lens, a hyperosmotic state results and this ultimately leads to disruption of cell integrity and tissue degeneration. It also has been proposed that the increased activity of aldose reductase during hyperglycemia may deplete intracellular NADPH, a cofactor required for other key biochemical processes such as glutathione production. In the lens glutathione functions as a free-radical scavenger, protecting crystallin proteins from oxidative polymerization.[8,9] Therefore the depletion of glutathione associated with enhanced consumption of NADPH by aldose reductase may accelerate lens cataract formation and contribute to other tissue pathologies during diabetes. Furthermore, in recent years increased aldose reductase activity has been linked to the depletion of *myo*-inositol in nerve tissue.[10-12] *myo*-Inositol serves as a precursor of diacylglycerol, a positive modulator of protein kinase which, in turn, activates Na^+/K^+ ATPase. Thus increased activity of the polyol pathway and aldose reductase may result in decreased Na^+/K^+ ATPase activity, ultimately producing the decreased nerve conduction velocities and nerve degeneration associated with diabetic neuropathies.

Since the hyperosmotic effect resulting from intracellular sorbitol accumulation as well as the depletion of NADPH and *myo*-inositol associated with increased aldose reductase activity has been implicated in the pathogenesis of a number of diabetic complications, considerable attention has been focused on the development of aldose reductase inhibitors.[13-17] Previously we described the synthesis and evaluation of a number of N-(phenylsulfonyl)amino acids as inhibitors of this enzyme.[16,17] In these studies it was observed that the N-(phenylsulfonyl)glycines 1 are 5 to 25 times as potent as the corresponding N-(phenylsulfonyl)alanines **2**, and more than 100 times as potent as



the corresponding N-(phenylsulfonyl)prolines as inhibitors of aldose reductase isolated from rat lens.[16] It was also determined that replacement of the phenyl ring of 1 with a more lipophilic naphthalene ring as in **3a** and **3b**, or substitution of a 4-aroylamino substituent as in **4** results in a dramatic increase in inhibitory activity.[17] These observations prompted us to investigate the effect of incor-

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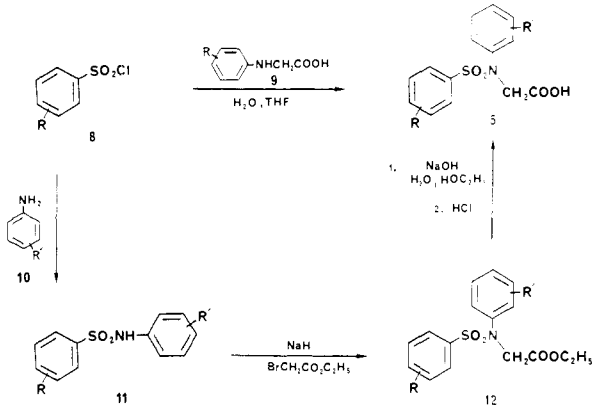
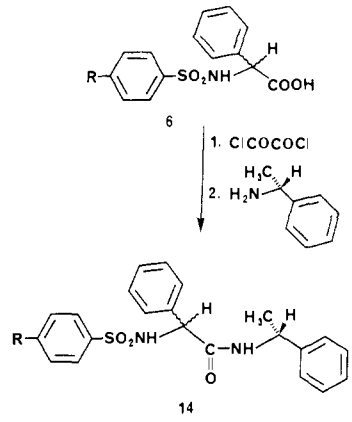
Scheme I



Scheme II



poration of an additional lipophilic moiety in the amino acid portion of **1** on aldose reductase inhibitory potency. Therefore, a series of *N*-(phenylsulfonyl)-*N*-phenylglycines **5**, *N*-(phenylsulfonyl)-2-phenylglycines **6**, and *N*-(phenylsulfonyl)anthranilic acids **7** were synthesized and evaluated as inhibitors of aldose reductase obtained from rat lens.

Chemistry

The *N*-(phenylsulfonyl)-*N*-phenylglycines **5** (Table I) were synthesized from commercially available arenesulfonyl chlorides **8** by two different routes as shown in Scheme I. The *N*-(substituted-phenylsulfonyl)-*N*-phenylglycines can be obtained directly by reaction of **8** with *N*-phenylglycine **9** in aqueous THF containing 2 equiv of base. Alternatively, the *N*-(phenylsulfonyl)-*N*-(substituted-phenyl)glycines were prepared in a multistep sequence via the intermediate sulfonamides **11** (Scheme I). Reaction of **8** with anilines **10** in dichloromethane using triethylamine base provided the intermediate sulfonamides **11**. Treatment of **11** with sodium hydride followed by alkylation with ethyl bromoacetate gave the esters **12**. Base-catalyzed hydrolysis of the esters **12**, followed by neutralization with HCl, afforded the products **5**. The amino derivatives **5d–f** were prepared by reduction of the corresponding nitro compounds **5i–j**. The *N*-(4-aminophenyl) analogue **5w** was synthesized by reduction of the intermediate nitro ester **12** (R′ = NO_2), followed by hydrolysis.

The *N*-(phenylsulfonyl)-2-phenylglycines **6** (Table I) were synthesized by reaction of available arenesulfonyl chlorides **8** with (*R*)- or (*S*)-2-phenylglycine in water or a water–THF solvent mixture. To determine if the configuration at C-2 was retained during the course of these reactions, each 2-phenylglycine product **6** was derivatized and analyzed by HPLC as described by Clark and Barksdale.[25] The derivatization process involved conversion of each 2-phenylglycine product **6** to the corresponding acid chloride **13**, followed by treatment with (*R*)-α-methylbenzylamine to yield the amides **14** (Scheme II). With this method, 2-phenylglycines **6**, which racemized upon formation, would form a pair of diastereomeric amides (*S,R* and *R,R*) upon derivatization and these diastereomers could be detected by HPLC. However, if configuration at C-2 is retained, then each 2-phenylglycine product **6** would yield only single amide **14** upon derivatization—the *S,R* diastereomer from the (*S*)-2-phenylglycines **6** and the *R,R* diastereomer from the (*R*)-2-phenylglycines. The reverse-phase HPLC analysis of the amide derivatives **14** obtained from the 4-fluoro analogues **6i** and **6j** is shown in Figure 1. A single amide derivative was obtained from each 2-phenylglycine product (Figures 1B and 1C) and these derivatives have different

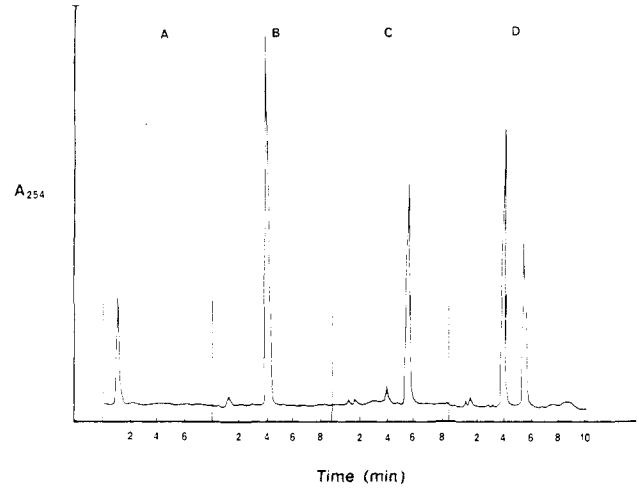


Figure 1. Reverse phase HPLC (Hypersil C18) analysis of the diastereomeric amides (**14**) obtained upon derivatization of **6i** and **6j**. A = *S* acid **6j**; B = amide from **6i** (*R,R* diastereomer); C = amide from **6j** (*S,R* doastereomer); D = mixture of amides from **6i** and **6j**. Mobile phase: 40% acetonitrile and 60% water.

retention properties (Figure 1D) demonstrating their diastereomeric relationship. Furthermore, similar results were obtained upon normal phase HPLC analysis of the amide derivatives formed from **6a** and **6b** (Figure 2) as well as the other 2-phenylglycine products. These data demonstrate that racemization did not occur during the reactions to form **6a** and therefore the configuration at C-2 in products **6a–l** is retained.

The anthranilates **7** were prepared as described in an earlier publication,[18] by reaction of anthranilic acid or *N*-methylanthranilates with arenesulfonyl chlorides **8** in aqueous base. Again the amino analogue **7d** was obtained upon catalytic reduction of the corresponding nitro derivative **7g**.

Results and Discussion

All of the *N*-(phenylsulfonyl)-*N*-phenylglycines **5**, *N*-(phenylsulfonyl)-2-phenylglycines **6**, and *N*-(phenylsulfonyl)anthranilic acids **7** were tested for their ability to inhibit aldose reductase obtained from rat lens as described previously.[16,17] These compounds were initially screened at a concentration of 100 μM and those derivatives found to produce greater than 25% inhibition at this concentration were tested at additional concentrations to generate log dose–response curves. Inhibitor IC_{50} values were then

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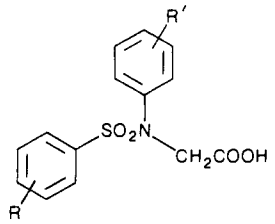
Table I. Physical Data for the *N*-(Phenylsulfonyl)-*N*-phenylglycines and *N*-(Phenylsulfonyl)-2-phenylglycines[a]



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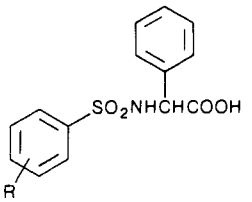

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compd[b]	R	R'	recryst[c]	yield,[d] %	mp, °C
5a	H	H	A	76	137–145
5b	4-CH_3	H	E	20	178–183
5c	4-OCH_3	H	B	42	170–172
5d	2-NH_2	H	A	91	151–152
5e	3-NH_2	H	C	76	201–202
5f	4-NH_2	H	A	82	92–96
5g	4-F	H	A	29	196–198
5h	4-Cl	H	B	69	208–210
5i	2-NO_2	H	D	22	163–164
5j	3-NO_2	H	A	55	153–154
5k	4-NO_2	H	A	70	221–223
5l	3,4-CH=CHCH=CH	H	B	72	194–196
5m	4-C_6H_5CONH	H	A	21	229–231
5n	H	2-CH_3	A	33	152–153
5o	H	3-CH_3	A	58	140–141
5p	H	4-CH_3	A	33	168–169
5q	H	2-Cl	A	22	142–143
5r	H	3-Cl	A	35	174–178
5s	H	4-Cl	A	41	163–164
5t	H	4-F	A	37	145–147
5u	H	4-OCH_3	B	13	138
5v	H	3-NO_2	A	19	161–162
5w	H	4-NH_2	D	11	163–164
6a	H (*R*)		D	40	173–175
6b	H (*S*)		D	36	183–186
6c	4-CH_3 (*R*)		A	46	171–173
6d	4-CH_3 (*S*)		A	51	175–176
6e	4-OCH_2 (*R*)		A	16	183–185
6f	4-OCH_3 (*S*)		A	14	185–187
6g	4-Cl (*R*)		D	54	159–161
6h	4-Cl (*S*)		D	42	160–161
6i	4-F (*R*)		A	60	157–159
6j	4-F (*S*)		A	61	158–160
6k	4-NO_2 (*R*)		A	17	105
6l	4-NO_2 (*S*)		A	16	105
6m	3,4-CH=CHCH=CH (*R*)		A	49	189–190
6n	3,4-CH=CHCH=CH (*S*)		A	56	189–190

[a] The synthesis and characterization of **1a–l** and **7a–l** were described previously.[16,18] [b] All products exhibited IR and ^1H NMR spectra consistant with the assigned structures and gave satisfactory C, H, and N combustion analyses. [c] Recrystallization solvents: A = EtOH/H_2O; B = EtOH; C = H_2O; D = acetone/H_2O; E = EtOH/EtOAc. [d] Percentages given for **5d–f** represent yields for the reduction reaction from the corresponding nitro compounds **5i–k**. Percentage given for **5v** is the yield obtained from reduction of the intermediate ester **12**, while the percentage for **5k** represents the yield for hydrolysis of the corresponding ester. All other percentages represent the final yields obtained from the intermediate amides **11** or from *N*-phenylglycine.

calculated from the linear portion of the log dose–response curves by linear regression (Table II).[19]

The *N*-(phenylsulfonyl)-*N*-phenylglycines **5a–l**, which represent analogues of **1** containing an aromatic substituent on the glycine nitrogen atom, have IC_{50}s in the range of 0.4 to 70 μM (Table II). Therefore a number of these compounds, particularly **5c** and **5k**, are significantly more potent than the corresponding *N*-(phenylsulfonyl)glycines (**1c** and **1j**, respectively) suggesting that *N*-phenyl substitution may result in enhanced affinity for aldose reductase. Comparison of the relative potencies of derivatives of **5** and **1**, however, reveals that these compounds do not display completely parallel structure–activity relationships. For example, in series **1**, the β-naphthyl (**1k**) and 4-benzoylamino (**1l**) derivatives are significantly more active than the 4-methoxy (**1c**), 2-nitro (**1i**), or 4-nitro (**1j**) analogues. In the *N*-phenyl series **5a–l**, however, the β-naphthyl (**5l**), 4-benzoylamino (**5m**), 4-methoxy (**5c**), 2-nitro (**5i**), and 4-nitro (**5k**) derivatives display comparable inhibitory potencies (Table II). Earlier we reported that the 4-benzoylamino analogue **11** at IC_{50} concentrations produces uncompetitive inhibition relative to the substrate glyceraldehyde.[17] Kinetic evaluation of **5m**, the *N*-phenyl derivative of **11**, reveals that this compound also produces uncompetitive inhibition of aldose reductase with respect to substrate at inhibitor concentrations approximating the IC_{50} value (Figure 3) and noncompetitive inhibition at higher concentrations. On the basis of this similarity in kinetic profiles, both **11** and **5m** appear to inhibit the enzyme by a similar mechanism involving interaction at a site distinct from the substrate binding site. However, the significant variation in relative inhibitory potencies be-

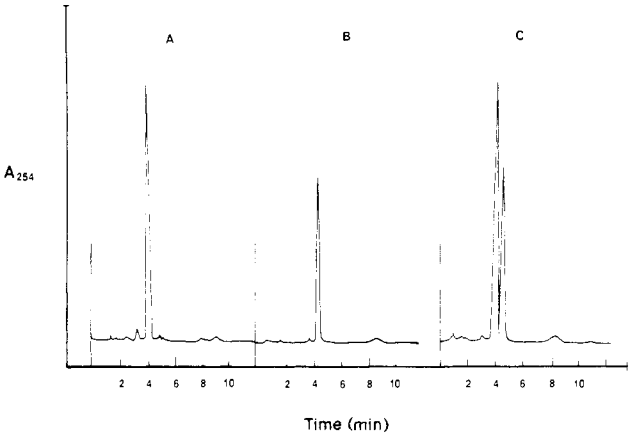

Figure 2. Normal phase HPLC (Hypersil Silica) analysis of the diastereomeric amides (**14**) obtained upon derivatization of **6a** and **6b**. A = amide from **6a** (*R,R* diastereomer); B = amide from **6b** (*S,R* diastereomer); C = mixture of amides from **6a** and **6b**. Mobile phase: 90% chloroform and 10% heptane.

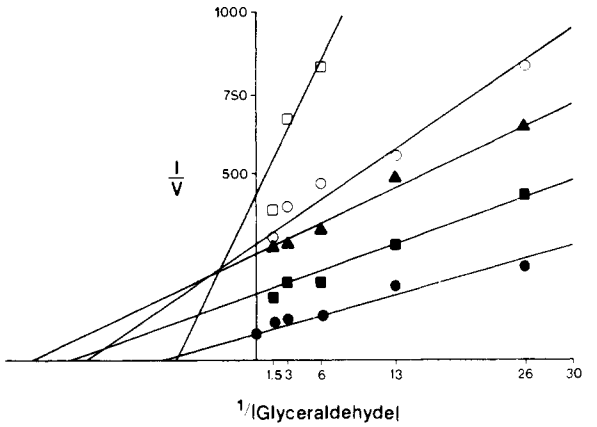

Figure 3. Double reciprocal plot of initial enzyme velocity versus concentration of substrate (DL-glyceraldehyde) in the presence or absence of inhibitor **5m**: no inhibitor (●), 1.0 μM (■), 2.5 μM (▲), 5.0 μM (○), 10 μM (□).

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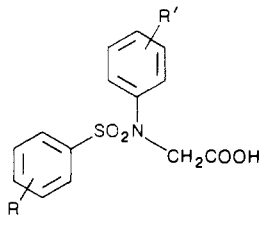
Table II. Aldose Reductase Inhibitory Activity of the *N*-(Phenylsulfonyl)-*N*-phenylglycines, *N*-(Phenylsulfonyl)-2-phenylglycines, and *N*-(Phenylsulfonyl)anthranilic Acids[a]



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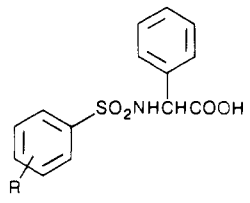

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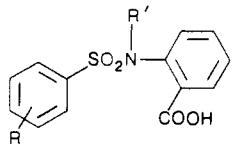

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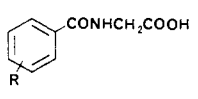
cmpd	R	R′	IC_{50},[b] μM (95% Cl)	cmpd	R	R′	IC_{50},[b] μM (95% Cl)
1a	H	H	130 (9–1200)	**5t**	H	4-F	22 (3–150)
1b	4-CH_3		32 (11–90)	**5u**	H	4-OCH_3	38 (2–78)
1c	4-OCH_3		31 (10–86)	**5v**	H	3-NO_2	8.7 (0.9–100)
1d	2-NH_2		37 (22–65)	**5w**	H	4-NH_2	68 (27–160)
1e	3-NH_2		29 (15–60)	**6a**	H (*R*)		460 (130–2800)
1f	4-NH_2		16 (6–40)	**6b**	H (*S*)		11 (5.6–23)
1g	4-F		43 (18–100)	**6c**	4-CH_3 (*R*)		90 (21–390)
1h	4-Cl		130 (12–1600)	**6d**	4-CH_3 (*S*)		8.9 (3.0–25)
1i	2-NO_2		13 (5–35)	**6e**	4-OCH_3 (*R*)		36 (5.3–60)
1j	4-NO_2		79 (24–270)	**6f**	4-OCH_3 (*S*)		13 (2.8–101)
1k	3,4-CH=CHCH=CH		0.40 (0.1–1.1)	**6g**	4-Cl (*R*)		340 (110–1300)
1l	4-C_6H_5CONH		0.88 (0.40–1.8)	**6h**	4-Cl (*S*)		7.1 (2.4–20)
5a	H	H	29 (3–290)	**6i**	4-F (*R*)		960 (300–5500)
5b	4-CH_3	H	14 (6–35)	**6j**	4-F (*S*)		25 (13–50)
5c	4-OCH_3	H	1.8 (0.4–7)	**6k**	4-NO_2 (*R*)		980 (190–2700)
5d	2-NH_2	H	67 (11–160)	**6l**	4-NO_2 (*S*)		8.6 (4.9–15)
5e	3-NH_2	H	27 (4–200)	**6m**	3,4-CH=CHCH=CH (*R*)		230 (61–1000)
5f	4-NH_2	H	9.3 (1–57)	**6n**	3,4-CH=CHCH=CH (*S*)		2.5 (0.9–6.9)
5g	4-F	H	26 (7–80)	**7a**	H	H	65 (32–130)
5h	4-Cl	H	16 (2–250)	**7b**	4-CH_3	H	69 (31–150)
5i	2-NO_2	H	3.6 (0.2–36)	**7c**	4-OCH_3	H	130 (57–330)
5j	3-NO_2	H	1.3 (0.2–5)	**7d**	4-NH_2	H	7.9 (0.2–98)
5k	4-NO_2	H	4.4 (0.2–9)	**7e**	4-Cl	H	130 (46–480)
5l	3,4-CH=CHCH=CH	H	4.5 (0.8–25)	**7f**	4-Br	H	85 (34–220)
5m	4-C_6H_5CONH	H	0.38 (0.10–1.4)	**7g**	4-NO_2	H	390 (240–700)
5n	H	2-CH_3	340 (45–3800)	**7h**	3,4-CH=CHCH=CH	H	5.7 (0.4–76)
5o	H	3-CH_3	27 (8–108)	**7i**	2,3-CH=CHCH=CH	H	8.3 (0.6–29)
5p	H	4-CH_3	26 (10–70)	**7j**	4-NO_2	CH_3	>2000
5q	H	2-Cl	7.6 (3–24)	**7k**	4-OCH_3	CH_3	>2000
5r	H	3-Cl	13 (1–140)	**7l**	3,4-CH=CHCH=CH	CH_3	400 (61–3200)
5s	H	4-Cl	27 (9–75)				

[a] Sorbinil was used as a standard for the assay and found to have an IC_{50} of 0.18 μM compared to a literature value of 0.07 μM.[24] [b] The IC_{50} values represent the concentration required to produce 50% enzyme inhibition ad determined by least-squares analyses of the linear portion of the log dose–response curves. The 95% confidence limits (95% CL) were calculated from *T* values for $n - 2$ where n is the total number of determinations.

tween derivatives of series **1** and **5** may suggest these compounds do not interact with the inhibitor binding site of aldose reductase in precisely the same manner.

The increase in inhibitory activity associated with *N*-phenyl substitution in the *N*-(phenylsulfonyl)glycine series **1** is consistant with structure–activity data obtained with the isosteric *N*-benzoylglycines **15**. Simple ring-substi-



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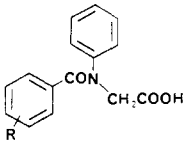


16

tuted derivatives of **15** are very weak inhibitors of aldose reductase, producing 50% enzyme inhibition only at concentrations of approximately 100 μM or greater.[20] Substitution of a phenyl moiety on the glycine nitrogen of these compounds to yield the *N*-phenyl-*N*-benzoylglycines **16**, however, results in a dramatic increase in activity; in the rat lens assay, derivatives of **16** have IC_{50} values of 0.3 to 10 μM.[20] Comparison of relative inhibitory potencies among series **15** and **16** also reveals significant differences in structure–activity relationships, again suggesting the possibility that while *N*-phenyl substitution may not change the mechanism of inhibition, it can alter the mode of interaction with aldose reductase.

A number of *N*-(phenylsulfonyl)-*N*-phenylglycine derivatives with varying substituents in the *N*-phenyl ring (**5n–w**) were also prepared and evaluated in the rat lens assay (Table II). With the exception of the 2-methyl

(20) Unpublished results presented at the International Workshop on Aldose Reductase Inhibitors, Honolulu, Hawaii, Dec. 1987.

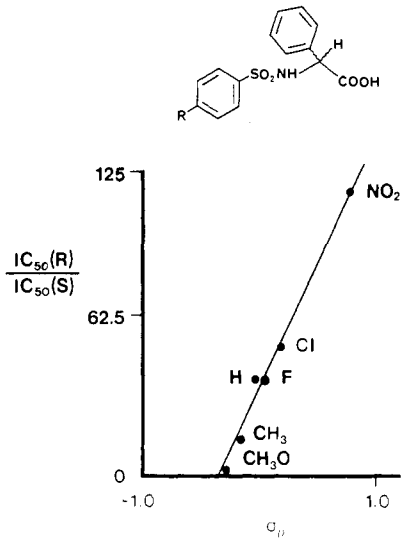
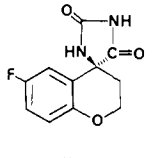


Figure 4. Relationship between enantiomeric potency ratios ($IC_{50}(R)/IC_{50}(S)$) and Hammett σ constants for *N*-(phenylsulfonyl)-2-phenylglycines **6a**–**l** (r = 0.99).

analogue **5n**, these compounds display inhibitory potencies comparable to or greater than the *N*-(phenylsulfonyl)-glycines **1**. Also, most of these derivatives display inhibitory activity of the same order as the unsubstituted *N*-phenyl derivative **5a**. Perhaps the most interesting compounds of this series are the 2-methyl and 2-chloro derivatives, **5n** and **5q**, respectively. The 2-methyl analogue (IC_{50} of 340 μM) is the least active compound of this series while the 2-chloro derivative (IC_{50} of 7.6 μM) is among the most potent (Table II). These data demonstrate that substitution at the 2-position can significantly alter affinity for the enzyme, depending on the electronic and steric nature of the substituent.

The *N*-(phenylsulfonyl)-2-phenylglycines **6a**–**n** were designed to explore the effect of phenyl substitution on the 2-carbon of the glycine side chain of **1**. Since these derivatives contain a chiral center, the relationship between aldose reductase inhibitory activity and stereoisomerism was also investigated. In earlier studies it has been demonstrated that the inhibitor binding site of aldose reductase can recognize inhibitors stereospecifically. For example, the *S* enantiomer of sorbinil (**17**) is approximately 30 times as potent as the *R* isomer,[21] and (*R*)-α-methylalrestatin (**18**) is twice as active its *S* isomer.[22] Also, we have reported



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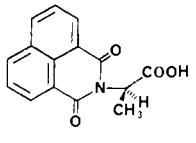

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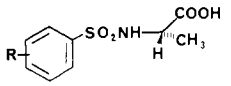

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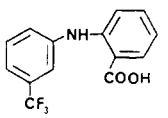
that the *S* enantiomers of *N*-(phenylsulfonyl)alanines **2** are two to four times more active than the *R* isomers.[16] Evaluation of the individual enantiomers of **6** reveals that inhibitory activity resides primarily in the *S* stereoisomers as was also observed in the *N*-(phenylsulfonyl)alanines **2**; the *S* isomers range from 3 (**6f**) to 110 (**6l**) times as potent as the *R* enantiomers. Therefore, 2-phenyl substitution produces a substantially greater separation of enantiomeric potencies than does 2-methyl substitution as in the alanines **2**. Furthermore, comparison of the enantiomeric pairs of 4-substituted derivatives of **6** suggests that the differences in enantiomeric potencies are dependent upon the electronic nature of the ring substituent. For example, a linear relationship (r = 0.99) appears to exist between enantiomeric potency ratios ($IC_{50}(R)/IC_{50}(S)$) and Hammett σ constants, with enantioselectivity increasing as the electron-withdrawing nature of the 4-substituent increases (Figure 4). While the significance of this relationship remains to be determined, it is clear from derivatization and HPLC analysis that this relationship is not the result of varying degrees of racemization in **6a**–**l**. Finally, with the exception of the naphthalene derivative **6n**, the *S* stereoisomers are more active than the corresponding *N*-(phenylsulfonyl)glycines **1** and generally display similar relative inhibitory activities, suggesting that 2-phenyl substitution enhances affinity for aldose reductase without altering the mode of interaction with the enzyme.

The *N*-(phenylsulfonyl)anthranilates **7** represent derivatives of **1** in which an aromatic ring has been incorporated directly in the amino acid side chain; in these compounds the amino and carboxylate moieties are separated by two carbons which are part of an aromatic ring. These compounds are also structurally related to the *N*-arylanthranilate antiinflammatory agent flufenamic acid (**19**), which has been reported to inhibit aldose reductase



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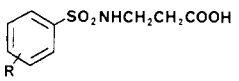

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(50% inhibition at 100 μM).[23] Anthranilates **7a**–**i**, with IC_{50}s of 6 to 390 μM (Table II), are generally less active than the corresponding glycine derivatives **1**; the one exception to this trend is the amino derivative **7d**, which displays inhibitory potency comparable to the 4-amino derivative of **1**. These observations are consistant with earlier studies with the structurally related *N*-(phenylsulfonyl)-β-alanines **20** where it was demonstrated that insertion of an additional carbon atom between the glycine nitrogen and carboxyl moieties resulted in a decrease in inhibitory potency.[16] The *N*-(arylsulfonyl)anthranilates, however, do display structure–activity relationships that parallel those observed in the *N*-(phenylsulfonyl)glycine series **1**. For example: (1) the naphthalene analogues **7h** and **7i** are the most potent inhibitors of this series, (2) the amino derivative **7d** is the most active 4-substituted analogue, and (3) *N*-methyl substitution, as in **7j**–**l**, results in a decrease in inhibitory activity (Table II). Therefore from these data it is apparent that anthranilate substitution may result in a decrease in affinity for aldose reductase but does not alter the mode of interaction with the enzyme.

The importance of the relative positions of the amino and carboxylate moieties in **7h** was examined by evaluation

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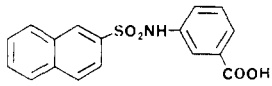
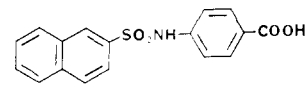
of the corresponding meta (**7m**) and para (**7n**) regioisomers. These compounds produce only 26% and 41% en-



7m



7n

zyme inhibition at concentrations of 100 μM, respectively. Therefore, altering the positions of these key moieties results in a further decrease in affinity for the enzyme.

In summary, addition of an aromatic substituent in the glycine moiety of **1** has variable effects on aldose reductase inhibitory activity in vitro. Direct incorporation of a phenyl ring between the glycine amino and carboxylate moieties as in the anthranilates **7** apparently results in decreased affinity for the enzyme without altering the mode of interaction. In some instances, *N*-phenyl substitution, as in derivatives of **5**, appears to enhance enzyme affinity but may also alter the mode of interaction as suggested by the significant differences observed in relative inhibitory activity between these compounds and the parent *N*-(phenylsulfonyl)glycines **1**. Also, substitution of a 2-phenyl moiety in the proper stereochemical orientation as in **6b**, **6d**, **6h**, **6j**, **6l**, and **6n** appears to increase inhibitor affinity and to increase stereospecificity compared to the *N*-(phenylsulfonyl)alanines **2**. The degree of stereospecificity within the 2-phenyl series is dependent upon the electronic nature of the substituent present on the phenylsulfonyl moiety.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian T-60A NMR spectrometer with a $CDCl_3$ solvent or a solvent mixture of $CDCl_3$ and DMSO-d_6 and Me_4Si as an internal standard. IR spectra were recorded on a Beckman 4230 infrared spectrophotometer as Nujol mulls. UV spectra and enzyme reactions were recorded with a Shimadzu UV-120 spectrophotometer equipped with a CPS kinetic program and thermocontrolled multicell positioner. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are within 0.4 of theoretical percentages. Common reagent grade chemicals for the syntheses were purchased from the Aldrich Chemical Co. and were used as received. DL-Glyceraldehyde and NADPH (type 1) used in the biochemical studies was obtained from the Sigma Chemical Co.

Synthesis of the *N*-(Phenylsulfonyl)-*N*-phenylglycines 5. Method A. Arenesulfonyl chloride (20 mmol) was added portionwise over a period of 15 min to a stirred solution of *N*-phenylglycine (3.02 g, 20 mmol) and NaOH (1.6 g, 40 mmol) in water (20 mL) or water (10 mL)/THF (10 mL) at 70–80 °C. The mixture was stirred at 70–80 °C for 30 min and then cooled in an ice bath. Acidification to pH 1 with concentrated HCl provided the crude product as a thick precipitate. The product was isolated by filtration, washed with water (2 × 10 mL), and recrystallized (see Table I for recrystallization solvents, yields, and melting points).

Method B. Arenesulfonyl chloride (40 mmol) was added portionwise over 30 min to a stirred solution (room temperature) of the aniline derivative (40 mmol) and triethylamine (6.3 mL, 45 mmol) in dichloromethane (100 mL). After the addition was complete the reaction mixture was stirred at room temperature overnight and then washed successively with 10% HCl (2 × 100 mL) and H_2O (100 mL). Evaporation of the dichloromethane solvent yielded the crude (arylsulfonyl)anilides **11**, which were recrystallized from aqueous ethanol and dried in vacuo. Sodium hydride (0.17 g, 12 mmol) was added portionwise to a stirred solution of the intermediate (arylsulfonyl)anilides **11** (10 mmol) in DMF (25 mL), and the mixture was stirred at room temperature for 30 min. Ethyl bromoacetate (1.5 mL, 14 mmol) was then added and the reaction mixture allowed to stir at room temperature overnight. The mixture was then evaporated in vacuo to yield the crude ester **12** as an oil or a solid. The ester was dissolved in a solvent mixture of ethanol (50 mL) and H_2O (20 mL) containing NaOH (0.6 g, 15 mmol) and the resulting mixture stirred at reflux for 1 to 2 h. The mixture was then cooled and evaporated to dryness under reduced pressure. The remaining solid was suspended in H_2O (75 mL) was washed with dichloromethane (2 × 50 mL). The basic aqueous solution was then acidified to pH 1 with concentrated HCl and the resulting precipitate isolated by filtration and washed with distilled H_2O (2 × 25 mL). The solid was then dissolved in saturated $NaHCO_3$ (50 mL) and washed with $CHCl_3$ (2 × 50 mL). The $NaHCO_3$ solution was then cooled (ice bath) and carefully acidified to pH 1. The resulting precipitate was isolated by filtration, washed with H_2O (2 × 20 mL), and recrystallized from aqueous ethanol to give **5a–v** (see Table I for recrystallization solvents, yields, and melting points).

Reduction of the Nitro Derivatives 5i–k. A solution of the nitro compounds **5i–k** (3.0 g, 9 mmol) in ethanol (100 mL) containing 5% palladium on carbon (0.5 g) was shaken under a H_2 atmosphere (initial psi of 45) until the consumption of H_2 ceased (within 2 h). Filtration followed by evaporation of the filtrate solvent provided the crude amines **5d–e**, which were purified by recrystallization (see Table I for recrystallization solvents, yields, and melting points).

Synthesis of the *N*-(Phenylsulfonyl)-2-phenylglycines 6. A solution of 2-phenylglycine (20 mmol), arenesulfonyl chloride (20 mmol), and NaOH (2.0 g, 50 mmol) in water (20 mL) or a mixture of water (10 mL) and THF (10 mL) was stirred at 70–80 °C for 30 min. The reaction mixture was then cooled (ice bath) and acidified to pH 1 with concentrated HCl to yield the crude product as a precipitate. The product was isolated by filtration, washed with water (2 × 25 mL), and recrystallized (see Table I for recrystallization solvents, yields, and melting points).

Synthesis of the Diastereomeric α-Methylbenzylamine Amides 14. A mixture of the acid **6** (0.03 mmol) and oxalyl chloride (1.0 mL) in $CHCl_3$ (5 mL) was stirred at room temperature for 15 min and then evaported to dryness in vacuo. The resultant acid chloride was dissolved in $CHCl_3$ (5 mL) and (*R*)-α-methylbenzylamine (0.1 mL) added. This mixture was stirred at room temperature for 15 min and then evaporated under reduced pressure to yield the crude diastereomeric amide **14**. The crude product was dissolved in ether (10 mL) and washed successively with 10% HCl (2 × 10 mL), H_2O (10 mL), and saturated $NaHCO_3$ (2 × 10 mL). Evaporation of the ether solvent, followed by heptane recrystallization of the resultant residue, provided the diastereomeric amide products.

HPLC Analysis of the Diastereomeric α-Methylbenzylamine Amides 14. The liquid chromatography was a modular isocratic system consisting of a Waters 510 pump, U6K injector, and 440 absorbance detector. Normal phase separations were achieved with a 150 × 4.6 mm id Hypersil-Silica column (Phenomenex), with mobile phases consisting of various mixtures of $CHCl_3$ and heptane at a flow rate of 1.5 mL/min. Reverse-phase separations were accomplished on a 150 × 4.6 mm Hypersil-C18 column (Phenomenex) with mobile phases consisting of mixtures of acetonitrile and water at a flow rate of 1.0 mL/min. For all separations the UV detector was set at 254 nm and 0.01 AUFS.

Aldose Reductase Enzyme Assay. Frozen rat eyes were purchased from Charles River Breeding Laboratories and the lenses were dissected from the partially thawed eyes and then stored at −5 °C until used for the assay. Enzyme supernatant was prepared by homogenizing 100 lenses in 20 mL of distilled water followed by centrifugation of the homogenate at 10000 rpm for 15 min at −5 °C. Ammonium sulfate was then added to the supernatant to 40% saturation and the supernatant centrifuged at 10000 rpm for an additional 15 min.

Aldose reductase activity of the freshly prepared 40% ammonium sulfate supernatant was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm in a Shimadzu UV-120 spectrophotometer. The reaction mixture contained 0.1 M phosphate buffer (pH 6.2), 0.104 mM NADPH (Sigma type I), 10 mM DL-glyceraldehyde, and 0.2 mL of enzyme supernatant in a total volume of 2.0 mL. The reference blank contained all of the above reagents except for glyceraldehyde to correct for oxidation of NADPH not associated with the reduction of substrate. The reaction was initiated by the addition of the substrate and was monitored for 3.0 min after a 45-s initiation period. Enzyme activity was adjusted by dilution of the enzyme

supernatant with distilled water so that 0.2 mL of supernatant gave an average reaction rate for the control reaction of 0.0100 + 0.0010 absorbance units per min. The effects of the inhibitors on enzyme activity were determined by including 0.2 mL of an aqueous solution of the inhibitor at the desired concentration in the reaction mixture. Each compound was tested at least three different concentrations with a minimum of two determinations per concentration. The percent inhibition for each compound at all concentrations was then calculated by comparing the reaction rate of the solutions containing inhibitor to that of control reactions with no inhibitor and log dose–response curves constructed. Inhibitor IC_{50} values were then obtained by least-squares analyses of the linear portions of the log dose–response curves with use of the LINEFIT linear regression program of Barlow.[19]

Kinetic studies were performed with four concentrations (10, 5.0, 2.5, and 1.0 μM) of inhibitor **5m**. For these studies, the concentrations of the substrate DL-glyceraldehyde were varied (1.25, 0.625, 0.313, 0.156, 0.078 mM) while inhibitor and cofactor concentrations (0.104 mM) were held constant. The nature of inhibition produced by each concentration of **5m** was then determined by analyzing double-reciprocal plots of enzyme velocity versus glyceraldehyde concentration. The double reciprocal plots were generated by least-squares fit of the data using the LINEFIT program of Barlow.[19]

Acknowledgment. This research was supported in part by a Faculty Research Grant from the University of Mississippi, by the Research Institute of Pharmaceutical Sciences, and by a grant from the Vicksburg Hospital Medical Foundation. The technical assistance provided by R. L. Peden, G. M. Worsham, B. E. Swearingen, Kevin Kerr, Jackie Johnson, and Tina Artale is gratefully acknowledged.

Registry No. **1a**, 5398-96-9; **1b**, 1080-44-0; **1c**, 13029-74-8; **1d**, 13514-59-5; **1e**, 109065-68-1; **1f**, 5616-30-8; **1g**, 13029-71-5; 1h, 13029-72-6; **1i**, 15054-42-9; **1j**, 15054-44-1; **1k**, 92740-48-2; 1l, 109065-69-2; **5a**, 59724-82-2; **5b**, 80271-12-1; **5c**, 117309-27-0; **5d**, 117309-28-1; **5e**, 117309-29-2; **5f**, 92192-48-8; **5g**, 117309-30-5; **5h**, 92290-91-0; **5i**, 117309-31-6; **5j**, 117309-32-7; **5k**, 117309-33-8; 5l, 117309-34-9; **5m**, 117309-35-0; **5n**, 117309-36-1; **5o**, 117309-37-2; **5p**, 117309-38-3; **5q**, 117309-39-4; **5r**, 117309-40-7; **5s**, 117309-41-8; **5t**, 117309-42-9; **5u**, 117309-43-0; **5v**, 117309-44-1; **5w**, 117309-45-2; **6a**, 96686-11-2; **6b**, 105441-57-4; **6c**, 60712-47-2; **6d**, 111524-98-2; **6e**, 96686-12-3; **6f**, 117309-46-3; **6g**, 96686-13-4; **6h**, 117309-47-4; **6i**, 117309-48-5; **6j**, 117309-49-6; **6k**, 117309-50-9; **6l**, 117309-51-0; **6m**, 96686-17-8; **6n**, 117406-01-6; **7a**, 34837-67-7; **7b**, 6311-23-5; **7c**, 38957-44-7; **7d**, 530-73-4; **7e**, 51012-31-8; **7f**, 51012-29-4; **7g**, 38957-45-8; **7h**, 117309-52-1; **7i**, 117309-53-2; **7j**, 117309-54-3; **7k**, 117309-55-4; **7l**, 117309-56-5; **14a**, 117309-57-6; **14b**, 117309-58-7; **14i**, 117309-59-8; **14j**, 117309-60-1; $PhSO_2Cl$, 98-09-9; 4-$MeC_6H_4SO_2Cl$, 98-59-9; 4-$MeOC_6H_4SO_2Cl$, 98-68-0; 4-$ClC_6H_4SO_2Cl$, 98-60-2; 4-$FC_6H_4SO_2Cl$, 349-88-2; 4-$O_2NC_6H_4SO_2Cl$, 98-74-8; 2-naphthalenesulfonyl chloride, 93-11-8; (*R*)-2-phenylglycine, 875-74-1; (*S*)-2-phenylglycine, 2935-35-5; (*R*)-α-methylbenzylamine, 3886-69-9; aldose reductase, 9028-31-3.

Synthesis and Activity of Nonhydrolyzable Pseudomonic Acid Analogues

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Several series of pseudomonic acid analogues have been prepared that incorporate modified functionalities in place of the C1–C3 α,β-unsaturated ester group. The inhibition of isoleucyl-*t*RNA synthetase and the in vitro activity of these compounds against various Gram-positive and Gram-negative strains are described. Several derivatives showed enzyme inhibition equivalent to or better than that of methyl pseudomonate (**3**), while lacking the hydrolyzable ester group at C1. These analogues include the corresponding phenyl ketone and the ether **12**. The long-chain ketone **24** exhibited similar in vitro activity as the parent ester.

Pseudomonic acid A, **1**, is a novel Gram-positive antibiotic that was isolated in 1971 from *Pseudomona fluorescens*.[1] Its structure was determined[2] in 1977, and a year later the absolute stereochemistry was defined by Alexander.[3] In 1982 Beecham Co. marketed the sodium salt **2** as a topical agent under the trade name Bactroban.[4] Studies of the in vivo efficacy of this antibiotic showed its short half-life was due to metabolic inactivation. The major metabolite was discovered to be monic acid A, **4** (Scheme I), which itself shows little antibacterial activity and is rapidly cleared in the urine.[5] Since that time several attempts to slow or halt the enzymatic hydrolysis by varying the structure and function of the C1–C3 fragment have been carried out, including preparation of the 2-halo and 2-alkyl derivatives and the formation of amides at C1.[6] Synthesis of the corresponding alkyl ketones by Beecham led to compounds retaining good in vitro activity.[7] We report in this paper our attempts to study the effect of several functional and structural modifications of the C1–C3 moiety on the enzyme inhibition and in vitro activity in hopes of deriving an in vivo active analogue of this antibiotic.

Chemistry

Initially our approach involved ascertaining the general structure–activity relationships of the various segments of pseudomonic acid. A description of standard substitution of the hydroxyl groups with hydrogen, fluoro, and amino groups will be published in due course. However, since the functionality paramount to activity is thought to be unsaturated grouping at C1–C3, we focused our efforts there, and this paper will disclose results from this study. Previous reports indicated a marked sensitivity to change of this molecular unit. For example, 1,2- or 1,4-reduction of this group produced the C2–C3 dihydro ester

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