presented on a variable-interval schedule of 2 min. The session consisted of sixty trials. Animals were run two to three times weekly with control sessions always preceding a drug run, and with at least 1 day intervening. Compounds were administered ip or po at a pretreatment time of 30 min to a minimum of five rats at each dose level (20 or 40 mg/kg) or over a range of doses. The following experimental parameters were recorded by computer: (1) the number of intertrial interval responses, (2) the number of avoidance responses, (3) the number of escape responses, and (4) the number of trials in which no response occurred. These data were used to calculate the percent difference from control values previously determined. For active compounds, response counts were summed over all subjects at a given dose. The number of trials in which rats failed to exhibit an avoidance response (avoidance block, AB) was determined at each dose. This number was expressed as a percentage of the total trials. Control performance was arbitrarily set at 100% for avoidance responding, and the dose calculated to produce a 50% block in avoidance responding (AB_{50}) was obtained from a dose effect regression line fitted by the method of least squares.

Receptor Binding Assays. D_2 and 5-HT_{1A} receptor binding assays were performed as previously described.[6] Binding assays to other receptor sites were performed as described by Muth et al.[25]

Serotonin Syndrome and Rotorod Tests. In vivo serotonin syndrome and rotorod tests were performed according to an adaptation of the methods of Smith and Peroutka[17] and Malick et al.[19]

Acknowledgment. Thanks are due to B. Hofmann and his staff for ^1H NMR determinations, Alice Conti for octanol–water partition coefficient determinations, Charles Kuhlman and his staff for HPLC and microanalytical work, Joseph Tokolics for providing compounds **50**, **55**, and **56**, and Marilyn Winkler for typing the manuscript.

Registry No. **9**, 119639-24-6; **10**, 26479-40-3; **11**, 119717-60-1; **12**, 69729-80-2; **13**, 69729-75-5; **14**, 112978-09-3; **15**, 112977-69-2; **16**, 119717-61-2; **17**, 112978-13-9; **18**, 113033-94-6; **19**, 112978-14-0; **20**, 119639-25-7; **21**, 119717-62-3; **22**, 119717-63-4; **22**·2HCl, 119785-05-6; **23**, 119717-64-5; **23**·HCl, 119785-06-7; **24**, 119718-82-0; **24**·HCl, 119785-07-8; **25**, 119717-65-6; **25**·2HCl, 119785-08-9; **26**, 119639-26-8; **26**·2HCl, 119717-69-0; **27**, 119639-27-9; **27**·2HCl, 119639-50-8; **28**, 119639-28-0; **28**·2HCl, 119639-51-9; **29**, 119717-66-7; **29**·2HCl, 119785-09-0; **30**, 119717-67-8; **30**·HCl, 119785-10-3; **31**, 119639-29-1; **31**·2HCl, 119717-70-3; **32**, 119639-30-4; **32**·2HCl, 119717-71-4; **33**, 119639-31-5; **33**·2HCl, 119717-72-5; **34**, 112977-76-1; **34**·2HCl, 112977-93-2; **35**, 112977-77-2; **35**·HCl, 112977-94-3; **36**, 112977-85-2; **36**·HCl, 112978-03-7; **37**, 112977-87-4; **37**·2HCl, 112978-04-8; **38**, 119639-32-6; **38**·HCl, 119639-52-0; **39**, 119639-33-7; **39**·2HCl, 119717-73-6; **40**, 113033-90-2; **40**·HCl, 113083-05-9; **41**, 119639-34-8; **41**·HCl, 119717-74-7; **42**, 119717-68-9; **42**·2HCl, 119785-11-4; **43**, 112977-70-5; **43**·HCl, 113033-91-3; **45**, 119639-35-9; **45**·2HCl, 119717-75-8; **46**, 119639-36-0; **46**·2HCl, 119717-76-9; **47**, 119639-37-1; **47**·2HCl, 119639-53-1; **48**, 112977-80-7; **48**·HCl, 112977-97-6; **49**, 112977-78-3; **49**·HCl, 112977-95-4; **50**, 119639-38-2; **50**·HCl, 119639-54-2; **51**, 119639-39-3; **51**·2HCl, 119639-55-3; **52**, 112977-79-4; **52**·HCl, 112977-96-5; **53**, 119656-10-9; **53**·HCl, 112977-98-7; **54**, 119639-40-6; **54**·2HCl, 119656-12-1; **55**, 119639-41-7; **55**·HCl, 119639-56-4; **56**, 119656-11-0; **56**·2HCl, 119656-13-2; **57**, 119639-42-8; **57**·HCl, 119717-77-0; **58**, 119639-43-9; **58**·HCl, 119717-78-1; **59**, 119639-44-0; **59**·HCl, 119639-44-0; **60**, 119639-45-1; **60**·HCl, 119639-57-5; **61**, 119639-46-2; **61**·HCl, 119639-58-6; **62**, 114895-53-3; **62**·2HCl, 114895-57-7; **63**, 114895-51-1; **63**·2HCl, 114895-58-8; **64**, 114895-52-2; **64**·2HCl, 114895-56-6; **65**, 119639-47-3; **65**·2HCl, 119639-59-7; **66**, 119639-48-4; **66**·HCl, 119639-60-0; **67**, 36935-58-7; furan, 110-00-9; cyclopentadiene, 542-92-7; 1,3-cyclohexadiene, 592-57-4; 2,3-dimethyl-1,3-butadiene, 513-81-5; 1,3-cycloheptadiene, 4054-38-0; 1,3,5,7-cyclooctatetraene, 629-20-9; 1,4-dibromobutane, 110-52-1; (3aα,4β,7β,7aα)-hexahydro-2-(4-bromobutyl)-4,7-epoxy-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide, 119717-59-8; 1-(6-chloro-2-pyrazinyl)piperazine, 64022-27-1; 1-(2-pyrimidinyl)piperazine, 20980-22-7; 1-(6-chloro-3-pyridizinyl)piperazine, 56392-83-7; 1-(3-trifluoromethylphenyl)-piperazine, 15532-75-9; 1-(3-chlorophenyl)piperazine, 6640-24-0; 1-(2-pyrazinyl)piperazine, 34803-68-4; 1-(3-chloro-2-pyrazinyl)-piperazine, 85386-99-8; 1-(1-methyl-5-tetraazolyl)piperazine, 119639-49-5; 1,2,3,4-tetrahydropyrrole[1,2-*a*]pyrazine, 71257-38-0; 1-benzylhexahydropyrrolo[3,4-*c*]pyrrole, 86732-22-1; 4-pyridinylbutyl bromide hydrobromide, 107266-09-1; 2,6-dichloropyrazine, 4774-14-5; 1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide, 81-07-2; thieno[3,4-*d*]isothiazol-3(2*H*)-one 1,1-dioxide, 59337-79-0; 1-amino-1-cyclohexane carboxylic acid, 2756-85-6; 1,2-benzisothiazol-3(2*H*)-one 1-oxide, 14599-38-3; 1-(4-aminobutyl)-4-(2-pyrimidinyl)piperazine, 33386-20-8; spiro[4,5]decan-2,4′-oxazolidine-2,5′-dione, 3253-43-8; 2-(4-bromobutyl)-5,6-dihydro-5-methyl-2*H*-1,2,6-thiadiazin-3(4*H*)-one 1,1-dioxide, 114895-54-5; 2-amino-*N*-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]cyclohexanecarboxamide, 119639-61-1.

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Synthesis and in Vitro Aldose Reductase Inhibitory Activity of Compounds Containing an *N*-Acylglycine Moiety

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A number of *N*-benzoylglycines (**6**), *N*-acetyl-*N*-phenylglycines (**7**), *N*-benzoyl-*N*-phenylglycines (**8**), and tricyclic *N*-acetic acids (**9–12**) were synthesized as analogues of the *N*-acylglycine-containing aldose reductase inhibitors alrestatin and 2-oxoquinoline-1-acetic acid. Derivatives of **6**, which represent ring-simplified analogues of alrestatin, are very weak inhibitors of aldose reductase obtained from rat lens, producing 50% inhibition only at concentrations exceeding 100 μM. Compounds of series **7** were designed as ring-opened analogues of the 2-oxoquinolines. While these derivatives are more potent than compounds of series **6** (IC_{50}s of 6–80 μM), they are less active than the corresponding 2-oxoquinolines. Analogues of series **8** were designed as hybrid structures of both alrestatin and the 2-oxoquinoline-1-acetic acids. These compounds are substantially more potent than compounds of series **6** and **7** and display inhibitory activities comparable to or greater than alrestatin or the 2-oxoquinolines (IC_{50}s of 0.1–10 μM). Of the rigid analogues of **8**, the most potent derivative is benzoxindole (**12**) with an IC_{50} of 0.67 μM, suggesting that fusion of the two aromatic rings of **8** in a coplanar conformation may optimize affinity for aldose reductase in this series.

Over the past decade, a number of structurally diverse compounds have been reported to inhibit the enzyme aldose reductase and therefore possess potential utility for the prevention of some pathologies of chronic diabetes.[1-3]

0022-2623/89/1832-1033$01.50/0

Chart I

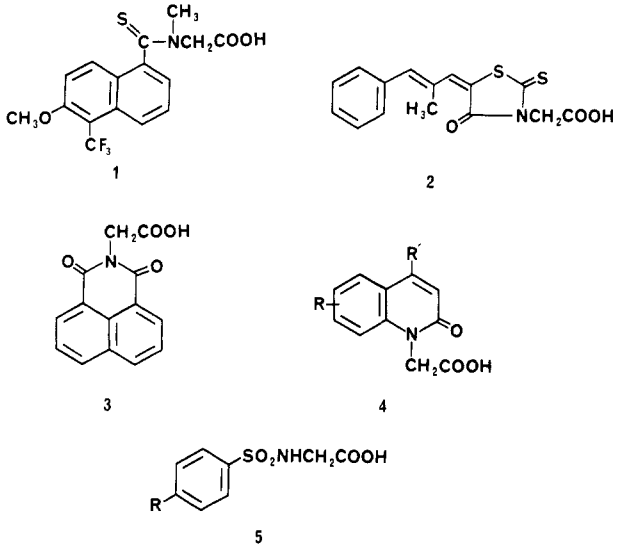
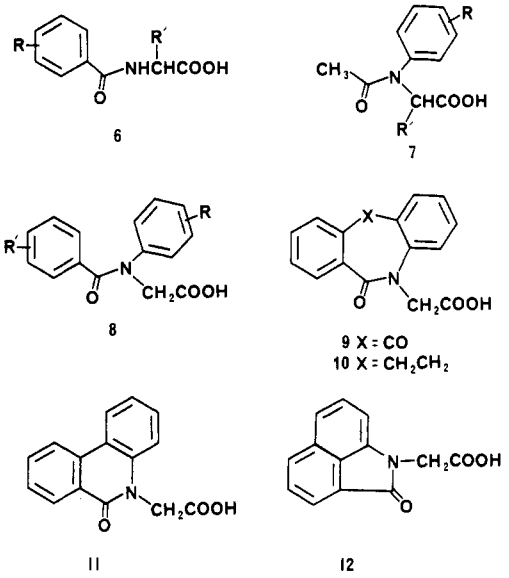
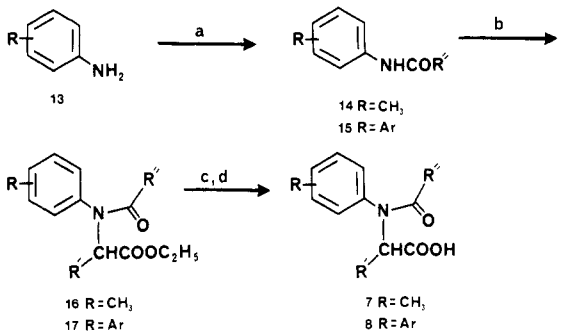


Chart II



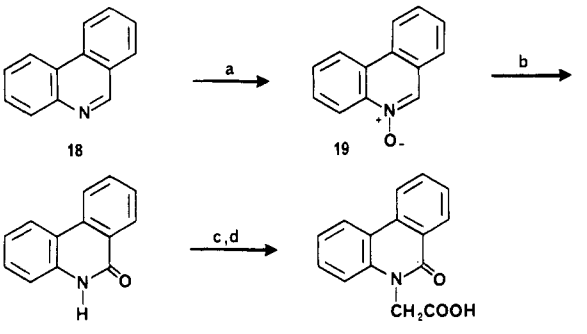
Many of these compounds, such as tolrestat (1; see Chart I) ONO-2235 (2), and alrestatin (3), contain a heterocyclic *N*-acetic acid fragment, *N*-benzoylglycine moiety, or isosteric fragments which may function as the basic pharmacophores required for aldose reductase inhibitory activity. In previous publications,[4-7] we described the synthesis and aldose reductase inhibitory activity of a variety of 2-oxoquinoline-1-acetic acids **4** and *N*-(phenylsulfonyl)glycines **5** as compounds that possess these key structural moieties. Several of these derivatives were found to be relatively potent inhibitors in the rat lens assay with IC_{50}s in the 5.0–0.3 μM range. As part of our continuing studies with compounds of this type, we now report the synthesis and aldose reductase activity of a variety of

Scheme I[a]



[a] (a) Ac_2O, HOAc, or C_6H_5COCl, Et_3N, CH_2Cl_2; (b) NaH, DMF, $R'CHBrCO_2Et$; (c) NaOH, H_2O, EtOH; (d) HCl.

Scheme II[a]



[a] (a) 30% H_2O_2, HOAc, then NH_4OH; (b) Ac_2O, then NH_4OH; (c) NaH, DMF, $BrCH_2CO_2Et$; (d) NaOH, then HCl.

analogues, including (1) *N*-benzoylglycines **6** (see Chart II) which represent ring-opened derivatives of alrestatin in which one of the benzoyl moieties has been eliminated, (2) *N*-acetyl-*N*-phenylglycines **7** designed as analogues of **4** in which the quinoline ring has been opened between C-4 and C-4a and the 3,4 double bond eliminated, (3) *N*-benzoyl-N-phenylglycines **8** designed as hybrid structures that contain the *N*-phenylglycine and unsaturated amide moieties present in **4** and the *N*-benzoylglycine fragment found in **3**, and (4) tricyclic *N*-acetic acids **9–12** which represent analogues of **4** containing an additional aromatic ring, as well as conformationally restricted derivatives of the *N*-benzoyl-*N*-phenylglycines **8**.

Chemistry

The *N*-benzoylglycine derivatives **6** as well as several alanines **6m–o** were prepared by reaction of substituted benzoyl chlorides with the appropriate amino acid in aqueous base.[8] The requisite benzoyl chlorides were obtained commercially or prepared by treatment of available benzoic acids with oxalyl chloride.[9] The *N*-acetyl- (**7**) and *N*-benzoyl-*N*-phenylglycines (**8**) and several *N*-acetyl-*N*-phenylalanines (**7l–o**) were synthesized as outlined in Scheme I. Reaction of commercially available anilines **13** with acetic anhydride or benzoyl chloride afforded the acetanilides **14** and benzanilides **15**, respectively.[10] Alkylation of the sodium salts of anilides (**14** and **15**) with ethyl bromoacetate or ethyl 2-bromopropionate gave the intermediate esters **16** and **17**, which were hydrolyzed with NaOH in aqueous ethanol to give the products **7** and **8** (Table I).[11] Attempts to prepare the 4-nitro derivatives

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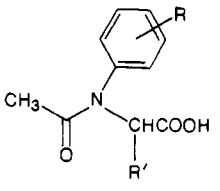
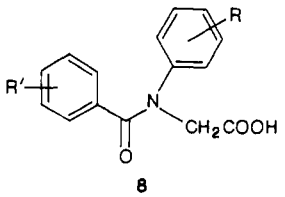
Table I. Physical Data for the *N*-Acetyl-*N*-arylglycines, *N*-Benzoyl-*N*-phenylglycines, and Tricyclic *N*-Acetic Acids



7



8

compd[a]	R	R'	recryst[b]	yield,[c] %	mp, °C	formula
7a	H	H	A	47	193–195	$C_{10}H_{11}NO_3$
7b	2-CH_3	H	A	56	210–215	$C_{11}H_{13}NO_3$
7c	3-CH_3	H	A	24	146–150	$C_{11}H_{13}NO_3$
7d	4-CH_3	H	B	49	174–176	$C_{11}H_{13}NO_3$
7e	4-OCH_3	H	A	28	186–189	$C_{11}H_{13}NO_4$
7f	4-OH	H	C	5	159–162	$C_{10}H_{11}NO_4$
7g	4-NH_2	H	A	52	190–192	$C_{10}H_{12}N_2O_3$
7h	4-Cl	H	A	45	175–178	$C_{10}H_{40}ClNO_3$
7i	4-NO_2	H	A	39	187–189	$C_{10}H_{10}N_2O_5$
7j	3-NO_2	H	A	10	166–168	$C_{10}H_{10}N_2O_5$
7k	2,3-CHCHCHCH–	H	D	15	208–212	$C_{14}H_{13}NO_3$
7l	H	CH_3	A	15	147–149	$C_{11}H_{13}NO_3$
7m	4-CH_3	CH_3	A	44	167–168	$C_{12}H_{15}NO_3$
7n	4-OCH_3	CH_3	A	56	195–196	$C_{12}H_{15}NO_4$
7o	4-Cl	CH_3	A	18	119–121	$C_{11}H_{12}ClNO_3$
8a	H	H	A	30	161–164	$C_{15}H_{13}NO_3$
8b	4-CH_3	H	A	32	152–154	$C_{16}H_{15}NO_3$
8c	4-OCH_3	H	B	35	126–129	$C_{16}H_{15}NO_4$
8d	4-Cl	H	C	60	146–148	$C_{15}H_{12}ClNO_3$
8e	3-NO_2	H	A	42	181–183	$C_{15}N_{12}N_2O_5$
8f	4-NO_2	3-NO_2	A	69	161–164	$C_{15}H_{11}N_3O_7$
8g	2,3-CHCHCHCH–	H	D	15	209–211	$C_{19}H_{15}NO_3$
8h	3,4-CHCHCHCH–	H	D	10	105–108	$C_{19}H_{15}NO_3$
8i	H	2,3-CHCHCHCH–	A	1,	110–113	$C_{19}H_{15}NO_3$
9			A	73	240–242	$C_{16}H_{11}NO_4$
10			A	53	140–143	$C_{17}H_{15}NO_3$
11			A	20	>200	$C_{15}H_{11}NO_3$
12			A	41	258–262	$C_{13}H_9NO_3$

[a] All products exhibited IR and H NMR spectra consistent with the assigned structures and gave satisfactory C, H, and N combustion analyses. [b] Recrystallization solvents: A = EtOH/H_2O; B = EtOAc/CCl_4; C = EtOH/pet ether; D = acetone/H_2O. [c] Percentages for all compounds except **7g**, **7i**, and **8f** represent yields for the N-alkylation–hydrolysis sequence. Percentages for **7i** and **8f** are for the nitration reaction, and the percent given for **7g** represents the yield from reduction of the nitro compound **7i**.

of **7** and **8** by this method were unsuccessful. In these instances, the presence of an electron-withdrawing 4-nitro moiety hindered N-alkylation and facilitated amide hydrolysis in the last step of the reaction sequence. Therefore, these compounds were prepared directly from the unsubstituted *N*-acyl-*N*-phenylglycines **7a** and **8a** by nitration (Scheme I); nitration of **8a** yielded the dinitro product **8f**.

The tricyclic *N*-acetic acids **9–12** were synthesized by the same alkylation–hydrolysis procedure described above. All of the tricyclic amides required for these syntheses, except for the phenanthridinone **20**, were obtained commercially. The phenanthridinone **20** was prepared from phenanthridine **18** by a reaction sequence involving N-oxidation,[11] treatment with acetic anhydride, and base-catalyzed hydrolysis (Scheme II).

Results and Discussion

All of the *N*-benzoylamino acids **6**, *N*-acetyl-*N*-phenylamino acids **7**, *N*-benzoyl-*N*-phenylglycines **8**, and tricyclic *N*-acetic acids **9–12** were tested for their ability to inhibit partially purified aldose reductase obtained from rat lens.[5-7] Those compounds producing greater than 50% enzyme inhibition at 100 μM were tested at lower concentrations and log dose–response curves constructed. Inhibitor IC_{50} values were then calculated from the linear portion of the log dose–response curves by using the LINEFIT[12] linear regression program. The results of these evaluations are presented in Table II.

All of the substituted *N*-benzoylglycines **6a–l** are substantially less potent than alrestatin **3** as inhibitors of rat lens aldose reductase; the most active compounds of this series **6c**, **6h**, **6k**, and **6l** have IC_{50}s of 30–70 μM (Table II), while alrestatin produces 50% inhibition at a concentration of 1.5 μM.[1] Therefore, elimination of one of the benzoyl moieties of alrestatin appears to result in a significant decrease in enzyme affinity. The *N*-benzoylglycines **6** are also less effective as inhibitors of aldose reductase than the isosteric *N*-(phenylsulfonyl)glycines **5** reported earlier.[5] Derivatives of **5** have IC_{50} values of 0.4–100 μM,[5] suggesting that replacement of the amide carbonyl moiety of **6** with a sulfonyl group as in **5** may enhance affinity for the enzyme. Interestingly, while the benzoyl series **6** and sulfonyl series **5** do not display identical relative potencies, there are several general structure–activity similarities, including the following: (1) elimination or esterification of the carboxylate moiety in each series results in a loss of inhibitory potency, (2) substitution of an α-methyl moiety decreases inhibitory activity, and (3) replacement of the *N*-benzoyl moiety (**6a**) with a naphthoyl moiety (**6l**) significantly enhances inhibitory potency.

The *N*-acetyl-*N*-phenylglycines **7** are substantially more potent than the *N*-benzoylglycines **6**, but less active than

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Table II. Aldose Reductase Inhibitory Activity

compd	R	R′	% inhibn at 100 μM (SEM)	IC_{50}, μM (95% CL)[a]
6a	H	H		330 (280–490)
6b	4-CH_3	H		220 (110–430)
6c	4-OCH_3	H		71 (35–160)
6d	4-NH_2	H	22 (4)	ND
6e	3-NH_2	H	36 (2)	ND
6f	2-NH_2	H	16 (2)	ND
6g	4-Cl	H		550 (300–1100)
6h	2-Cl	H		31 (3–300)
6i	4-NO_2	H		280 (140–580)
6j	3-NO_2	H	32 (7)	ND
6k	2-NO_2	H		58 (32–110)
6l	3,4-CHCHCHCH–	H		40 (25–64)
6m	H	CH_3	14 (1)	ND
6n	4-CH_3	CH_3	19 (2)	ND
6o	4-OCH_3	CH_3	38 (4)	ND
7a	H	H		21 (5–97)
7b	2-CH_3	H	14 (2)	ND
7c	3-CH_3	H	10 (1)	ND
7d	4-CH_3	H		15 (7–33)
7e	4-OCH_3	H		80 (7–130)
7f	4-OH	H		34 (10–130)
7g	4-NH_2	H		55 (20–160)
7h	4-Cl	H		25 (9–69)
7i	4-NO_2	H		5.8 (0.6–47)
7j	3-NO_2	H		27 (10–42)
7k	2,3-CHCHCHCH–	H	20 (3)	ND
7l	H	CH_3		200 (10–1300)
7m	4-CH_3	CH_3		128 (16–360)
7n	4-OCH_3	CH_3		950 (300–2000)
7o	4-Cl	CH_3		110 (17–720)
8a	H	H		9.5 (6–14)
8b	4-CH_3	H		3.0 (0.5–16)
8c	4-OCH_3	H		0.96 (0.1–7)
8d	4-Cl	H		5.0 (1.5–16)
8e	3-NO_2	H		0.86 (0.3–2.3)
8f	4-NO_2	3-NO_2		0.13 (0.02–0.64)
8g	2,3-CHCHCHCH–	H		230 (50–650)
8h	3,4-CHCHCHCH–	H		220 (40–680)
8i	H	2,3-CHCHCHCH–		5.8 (1.0–34)
9				65 (8.5–410)
10				79 (10–520)
11				10.7 (4.9–23)
12				0.67 (0.15–3.3)
sorbinil				0.23 (0.07–1.2)

[a] The IC_{50} values represent the concentration required to produce 50% enzyme inhibition as 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.

alrestatin and the 2-oxoquinoline-1-acetic acids **4** (Table II). For example, benzene ring substituted derivatives of **4** produce 50% enzyme inhibition at concentrations of 6.0–0.4 μM,[4] while analogues of **7** have IC_{50}s of 6 μM or greater. These data demonstrate that opening the quinoline ring of **4** at C_4 and/or elimination of the 3,4 double bond results in a decrease in affinity for the inhibitor binding site of aldose reductase. It is important to note that the *N*-acetyl-*N*-phenylglycines and 2-oxoquinoline-1-acetic acids **4** generally display parallel structure–activity relationships. For example, among the 4-substituted analogues of **7**, the nitro derivative **7i** is most potent, while the nitro derivative of the analogous 6-substituted derivatives of **4** is also the most active of this series. Furthermore, substitution of an α-methyl as in **7l–o** results in a dramatic decrease in inhibitory activity (Table II) as was also observed[4] upon α-substitution in the quinoline series **4**.

It has been proposed that addition of an appropriately positioned lipophilic moiety in inhibitor compounds can increase inhibitory activity via direct interactions with a hydrophobic region present on the enzyme.[3] This observation prompted the synthesis of **7k** in which the *N*-phenyl ring has been replaced with a more lipophilic naphthalene system. However, in the rat lens assay, **7k** is substantially less potent than most other ring-substituted *N*-acetyl-*N*-arylglycines (Table II), producing only 20% inhibition at a concentration of 100 μM. The low activity of this compound, as well as the 2- and 3-methyl analogues **7b** and **7c**, suggests that additional lipophilic (bulky) moieties at the 2- and/or 3-positions are not accommodated at the binding site of aldose reductase.

The *N*-benzoyl-*N*-phenylglycines **8** were designed as hybrid derivatives containing the *N*-phenylglycine fragment of **4** and the *N*-benzoylglycine moiety in **3**. These derivatives also represent hybrid analogues of **4** and **7**; conformationally, they resemble the ring-opened analogues **7**, but since they contain a site of unsaturation adjacent to the amide carbonyl, these compounds may mimic the electronic properties of quinolines **4**. Generally, the *N*-benzoyl-*N*-phenylglycines display significantly greater inhibitory potencies than the *N*-benzoylglycines **6** (Table II), demonstrating that substitution of a phenyl moiety on the glycine nitrogen of **6** greatly improves affinity for the inhibitor binding site of aldose reductase. These analogues are also 2–80 times as active as their corresponding *N*-acetyl derivatives **7**, suggesting that replacement of the acetyl methyl of **7** with a phenyl ring enhances binding.

The *N*-benzoyl-*N*-phenylglycines and *N*-acetyl-*N*-phenylglycines **8** and *N*-acetyl derivatives **7**, however, do not display completely parallel structure–activity relationships. For example, among the 4-substituted derivatives of **7** the order of potency is nitro (**7i**) > methyl (**7d**), unsubstituted (**7a**), or chloro (**7h**) > methoxy (**7e**), while the relative potencies of the similarly substituted analogues of **8** is nitro (**8f**) > methoxy (**8c**) > methyl (**8b**) or chloro (**8d**) > unsubstituted (**8a**).

To explore the effect of increasing the lipophilic character of the *N*-benzoyl-*N*-arylglycines **8** on aldose reductase inhibitory activity, several derivatives were synthesized in which either the *N*-benzoyl moiety or the *N*-phenyl moiety is replaced by a naphthalene ring system. As was observed in the *N*-acetyl series **7**, replacement of the *N*-phenyl ring with a naphthalene moiety as in **8g** and **8h** results in a substantial decline in inhibitory potency; these derivatives have IC_{50}s of 230 and 220 μM, respectively (Table II). Substitution of an *N*-naphthoyl moiety for the *N*-benzoyl group as in **8i** however, produces a 2-fold increase in inhibitory activity compared to the unsubstituted *N*-benzoyl derivative **8a** (Table II). Therefore, increasing the size and/or lipophilic nature of the *N*-phenyl substituent of **8** may interfere with enzyme binding, while increasing the lipophilic character of the *N*-benzoyl moiety may result in a modest increase in affinity for aldose reductase.

In their inhibitor pharmacophore hypothesis for aldose reductase, Kador and co-workers[3] proposed that the inhibitor binding site consisted of a charge-transfer pocket positioned to bind a carbonyl-like moiety and two ("primary" and "broad secondary") hydrophobic regions capable of interaction with aromatic moieties present on inhibitors. Furthermore, they postulated that the hydrophobic binding regions of the enzyme are coplanar and therefore optimal interactions occurs when an inhibitor molecule contains two appropriately positioned coplanar aromatic moieties. While the *N*-benzoyl-*N*-arylglycines **8** possess two aromatic ring systems, these rings would not exist preferentially in a coplanar conformation. Therefore, these compounds may not be capable of optimal interaction with the inhibitor binding site of aldose reductase. To test these hypotheses, a number of rigid analogues of the *N*-benzoyl-*N*-arylglycines, including **9–12**, were synthesized and evaluated. In the dibenzazepinone **9** and dibenzazocinone **10** the two rings are locked in an angular conformation in which they may more closely approximate coplanarity than they do in the *N*-benzoyl-*N*-arylglycines **8**. Also, in the phenanthridinone derivative **11**, the two rings are linked by an ortho–ortho bond and exist in a nearly coplanar conformation, while in the benzoxindole analogue **12** the two rings are fused in a coplanar arrangement. The nonplanar tricycles **9** and **10** are relatively weak inhibitors of aldose reductase, producing 50% enzyme inhibition only at concentrations exceeding 60 μM (Table II). Therefore, locking the aromatic rings of **8** in an angular conformation appears to decrease affinity for the enzyme. The phenanthridinone **11** is equipotent with the unsubstituted *N*-benzoyl-*N*-phenylglycine **8a**, indicating that fixing the aromatic rings of **8a** in a coplanar conformation does not result in enhanced affinity for aldose reductase. The benzoxindole **12**, with an IC_{50} of 0.67 μM, is more than 10 times as potent as **8a**, demonstrating that the two rings fused together in a coplanar conformation may optimize affinity for the enzyme in this series. Analysis of the data obtained with the *N*-benzoyl-*N*-arylglycines **8** and the tricyclic *N*-acetic acids **9–12** may suggest that the two aromatic rings present in these compounds interact with only one of the two proposed[3] ring binding sites present on aldose reductase and that this interaction is optimized when these rings are placed in close proximity and in a coplanar relative conformation.

In conclusion, analysis of the inhibitory data obtained for the *N*-benzoylglycines (**6**), *N*-acetyl-*N*-phenylglycines (**7**), *N*-benzoyl-*N*-phenylglycines (**8**), and tricyclic *N*-acetic acids (**9–12**) allows for several general structure conclusions: (1) The *N*-benzoylglycine moiety alone is not sufficient to produce significant inhibition of aldose reductase; (2) addition of a second aromatic ring on the *N*-benzoylglycine fragment as in derivatives of series **8** results in a substantial increase in inhibitory activity; compounds of series **8** display inhibitory potencies comparable to or greater than alrestatin (**3**) or the 2-oxoquinoline-1-acetic acids **4**; and (3) inhibitory activity in the *N*-benzoyl-*N*-phenylglycine series may be optimized when the two rings are fused together in a coplanar conformation as in **12**.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas–Hoover melting apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian T-60A NMR spectrometer with $CDCl_3$ or $CDCl_3$/DMSO-d_6 solvent mixtures 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-160 equipped with a CPS kinetics program and a thermocontrolled multicell positioner. Elemental analyses were performed by Atlantic Microlab, 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, except for the anilines, which were recrystallized or distilled prior to use. The solvent DMF (Fisher) was also dried and distilled prior to use. Phosphate buffer, DL-glyceraldehyde, and NADPH (type 1) used in the enzyme assays were obtained from the Sigma Chemical Co.

Synthesis of the Benzoyl Chlorides. DMF (1–3 drops) was added to a solution of the benzoic acid derivative (10–20 mmol) and oxalyl chloride (3–4 mL) in dry benzene (10–20 mL), and the reaction solution was stirred at room temperature for 2–10 h. The progress of acid chloride formation was monitored by IR by observing the disappearance of the acid carbonyl moiety. Upon completion, the reaction solutions were evaporated to dryness to give the crude acid chlorides which were used without further purification in subsequent reactions.

Synthesis of the *N*-Benzoylglycines (6). Glycine or alanine (20 mmol) was added to a solution of H_2O (20 mL) and NaOH (40 mmol) at room temperature. The reaction mixture was then warmed to 70–80 °C and benzoyl chloride (20 mmol) added portionwise over a 10–20-min period. After the addition was complete, the reaction mixture was stirred with warming for an additional 15–30 min. The solution was then cooled (ice bath) and acidified to pH 1 with concentrated HCl. The resultant precipitate was isolated by filtration, washed with water (25–50 mL), and recrystallized.

Synthesis of the Intermediate Acetanilides (14). Substituted anilines (20–120 mmol) were added to a mixture of glacial acetic acid (15–50 mL) and acetic anhydride (10–30 mL), and the resulting solution was stirred at reflux for 2–4 h. The reaction mixture was then cooled to room temperature and poured into a boiling mixture of water (10–50 mL) and ethanol (10–50 mL). The resulting solution was stirred at room temperature for 30 min–1 h and then cooled (0–5 °C) overnight. The crystalline products that formed were isolated by filtration, washed with water (2 × 25 mL), and dried in vacuo.

Synthesis of the Intermediate Benzanilides (15). A solution of benzoyl chloride (1.1 molar excess) in CH_2Cl_2 (50–100 mL) was added dropwise over a period of 20–30 min to a stirred solution of the aniline (20–120 mmol) and triethylamine (1.1 molar excess) in CH_2Cl_2 (50–200 mL). After the addition was complete, the reaction mixture was stirred at reflux overnight. The reaction mixture was then cooled to room temperature and washed with H_2O (1 × equal volume), saturated $NaHCO_3$ (2 × equal volume), H_2O (1 × equal volume), and 3 N HCl (2 × equal volume). The

CH_2Cl_2 solution was then evaporated to dryness under reduced pressure, and the resulting oil or solid was crystallized and recrystallized (Table I).

Synthesis of the *N*-Acyl-*N*-arylglycines (7 and 8). Sodium hydride (1.1 molar excess of a 60% oil dispersion) was added portionwise to a solution of the intermediate acetanilide or benzanilide (20–120 mmol) in dry DMF (20–250 mL), and the reaction mixture was stirred at 60–70 °C for 1 h. The mixture was then cooled to room temperature, and ethyl bromoacetate (1.2 molar excess) added. This mixture was stirred at 80 °C overnight and then cooled to room temperature and evaporated under reduced pressure. the resultant crude ester was dissolved in a solution of ethanol (50–250 mL) and H_2O (20–150 mL) containing NaOH (1.5 molar excess) and stirred at reflux for 0.5–4 h. The reaction mixture was cooled to room temperature and evaporated under reduced pressure. The resultant oil or solid was suspended in H_2O (25–100 mL) and washed with CH_2Cl_2 (2 × equal volume of H_2O). The aqueous layer was then cooled (ice bath) and acidified to pH 1 with concentrated HCl. The solid precipitate which formed was isolated by filtration and washed with H_2O and recrystallized (Table I).

Synthesis of the *N*-Acyl-*N*-(4-nitrophenyl)glycines (7i and 8f). Cold concentrated HNO_3 (3.4 mL) was added dropwise over a period of 30 min to a cold (ice bath) solution of the *N*-acyl-*N*-phenylglycine (10 mmol) in concentrated H_2SO_4 (7.0 mL). After the addition was complete, the reaction mixture was stirred with cooling for an additional hour and then was quenched by addition of crushed ice (ca. 30 mL). The precipitate that formed was isolated by filtration, thoroughly washed with H_2O (3 × 15 mL), and recrystallized (Table I).

***N*-Acetyl-*N*-(4-aminophenyl)glycine (7g).** The nitro compound **7i** (15 mmol) was dissolved in absolute ethanol (100 mL), and 5% palladium on carbon (500 mg) was added. This mixture was then shaken under a H_2 atmosphere (initial pressure of 40 psi) on a Parr apparatus until the uptake of H_2 ceased (within 1 h). The reaction mixture was then filtered, and the catalyst was washed with hot ethanol (2 × 50 mL). The combined filtrate and washings were evaporated under reduced pressure, and the product amine was recrystallized (Table I).

Synthesis of the *N*-Acetyl-*N*-arylalanines (7l–o). These compounds were prepared by alkylation of the intermediate acetanilides **14** with ethyl 2-bromopropionate by the general method described above (Table I).

6(5*H*)-Phenanthridinone (20). A mixture of phenanthridine (2.0 g, 11 mmol), 30% H_2O_2 (3 mL), and glacial acetic acid (14 mL) was stirred at 80 °C for 20 h and then cooled (ice bath) and neutralized with NH_4OH to yield the intermediate *N*-oxide. The crude *N*-oxide was dissolved in acetic anhydride (3 mL) and stirred at 75 °C for 18 h. The reaction solution was then poured over crushed ice (50 mL) and neutralized with NH_4OH. The resulting product was isolated by filtration, dried in vacuo, and used without further purification.

Synthesis of the Tricyclic *N*-Acetic Acids (9–12). The tricyclic amides (40 mmol) were dissolved in dry DMF (50 mL), and sodium hydride (1.8 g, 44 mmol of a 60% oil dispersion) was added portionwise. The resulting suspension was stirred at 80 °C for 1 h and then allowed to cool to room temperature. Ethyl bromoacetate (4.9 mL, 44 mmol) was added and the mixture stirred at 90 °C overnight. After cooling, the reaction mixture was evaporated to dryness in vacuo and the resulting oil was dissolved in a solution of ethanol (50 mL) and H_2O (25 mL) containing NaOH (1.8 g, 44 mmol). This mixture was stirred at reflux for 2 h, cooled to room temperature, and evaporated to dryness under reduced pressure. The resulting oil was suspended in H_2O (50 mL) and washed with $CHCl_3$ (3 × 25 mL). The aqueous layer was then cooled (ice bath) and acidified to pH 1 with concentrated HCl. The solid that formed was isolated by filtration, washed with water (25 mL), and recrystallized (Table I).

Aldose Reductase Enzyme Assay. Frozen rat eyes were purchased from Charles River Breeding Labs, Inc., Wilmington, MA, and the lenses dissected and stored at 6 °C until used for the assay. Crude enzyme supernatant was prepared by homogenizing 100 lenses in 20 mL of distilled water and centrifuging the crude homogenate at 10 000 rpm for 15 min, maintaining an ambient temperature of 0–5 °C. The supernatant was then isolated and ammonium sulfate added to achieve 40% saturation, and this solution was centrifuged at 10 000 rpm for 15 min at 5 °C.

Aldose reductase activity of the freshly prepared 40% ammonium sulfate supernatant was assayed spectrophotometrically at 30 °C 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 enzyme supernatant in a total volume of 2.0 mL. The reference blank contained all of the above reagents except the substrate glyceraldehyde to correct for oxidation of NADPH not associated with reduction of substrate. The enzyme-catalyzed reaction was initiated by addition of glyceraldehyde and was monitored for 3.0 min after a initiation period of 1.0 min. Enzyme activity was adjusted by diluting the supernatant with distilled water so that 0.2 mL of supernatant gave an average reaction rate for the control sample of 0.0100 ± 0.0020 absorbance units/min. The inhibitory activity of the compounds was determined by including 0.2 mL of an aqueous solution of each inhibitor at the desired concentration in the enzyme reaction mixture. The percent inhibition produced by each compound at each test 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. All compounds were initially screened at a concentration of 100 μM. To generate IC_{50} values, compounds were tested at at least four concentrations with a minimum of three determinations at each concentration. Log dose–response curves were then constructed from the inhibitory data and IC_{50} values calculated by least-squares analysis of the linear portions of log dose–response curves.[12]

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Registry No. **6a**, 495-69-2; **6b**, 27115-50-0; **6c**, 13214-64-7; **6d**, 61-78-9; **6e**, 20938-64-1; **6f**, 526-21-6; **6g**, 13450-77-6; **6h**, 16555-60-5; **6i**, 2645-07-0; **6j**, 617-10-7; **6k**, 10167-23-4; **6l**, 69826-63-7; **6m**, 2198-64-3; **6n**, 62107-96-4; **6o**, 93709-64-9; **7a**, 579-98-6; **7b**, 119656-39-2; **7c**, 119656-40-5; **7d**, 119656-41-6; **7e**, 71456-28-5; **7f**, 119656-42-7; **7g**, 119656-43-8; **7h**, 99420-98-1; **7i**, 99072-44-3; **7j**, 119656-44-9; **7k**, 119656-45-0; **7l**, 28750-70-1; **7m**, 119656-46-1; **7n**, 119656-47-2; **7o**, 119656-48-3; **8a**, 119656-49-4; **8b**, 119656-50-7; **8c**, 28794-25-4; **8d**, 28794-28-7; **8e**, 119656-51-8; **8f**, 119656-52-9; **8g**, 119656-53-0; **8h**, 119656-54-1; **8i**, 119656-55-2; **9**, 119656-56-3; **10**, 119656-57-4; **11**, 37046-34-7; **12**, 39273-46-6; **13** (R = H), 62-53-3; **13** (R = 2-Me), 95-53-4; **13** (R = 3-Me), 108-44-1; **13** (R = 4-Me), 106-49-0; **13** (R = 4-OMe), 104-94-9; **13** (R = 4-Cl), 106-47-8; **13** (R = 3-NO_2), 99-09-2; **13** (R = 2,3-CHCHCHCH–), 134-32-7; **13** (R = 3,4-CHCHCHCH–), 91-59-8; **14** (R = H), 103-84-4; **14** (R = 2-Me), 120-66-1; **14** (R = 3-Me), 537-92-8; **14** (R = 4-Me), 103-89-9; **14** (R = 4-OMe), 51-66-1; **14** (R = Cl), 539-03-7; **14** (R = 3-NO_2), 122-28-1; **14** (R = 2,3-CHCHCHCH–), 575-36-0; **15** (R = H, R″ = Ph), 93-98-1; **15** (R = 4-Me, R″ = Ph), 582-78-5; **15** (R = 4-OMe, R″ = Ph), 7472-54-0; **15** (R = 4-Cl, R″ = Ph), 2866-82-2; **15** (R = 3-NO_2, R″ = Ph), 4771-08-8; **15** (R = 2,3-CHCHCHCH–, R″ = Ph), 634-42-4; **15** (R = 3,4-CHCHCHCH–, R″ = Ph), 18271-22-2; **16** (R = H, R′ = H), 83949-33-1; **16** (R = 2-Me, R′ = H), 119656-58-5; **16** (R = 3-Me, R′ = H), 119656-59-6; **16** (R = 4-Me, R′ = H), 119656-60-9; **16** (R = 4-OMe, R′ = H), 100610-39-7; **16** (R = 4-Cl, R′ = H), 119656-61-0; **16** (R = 3-NO_2, R′ = H), 3589-61-5; **16** (R = 2,3-CHCHCHCH–, R′ = H), 119656-62-1; **16** (R = H, R′ = Me), 119656-63-2; **16** (R = 4-Me, R′ = Me), 119656-64-3; **16** (R = 4-OMe, R′ = Me), 119656-65-4; **16** (R = 4-Cl, R′ = Me), 119656-66-5; **17** (R = H, R′ = H, R″ = Ph), 75277-57-5; **17** (R = 4-Me, R′ = H, R″ = Ph), 69825-52-1; **17** (R = 4-OMe, R′ = H, R″ = Ph), 69825-55-4; **17** (R = 4-Cl, R′ = H, R″ = Ph), 69825-53-2; **17** (R = 3-NO_2, R′ = H, R″ = Ph), 119656-67-6; **17** (R = 2,3-CHCHCHCH–, R′ = H, R″ = Ph), 119656-68-7; **17** (R = 3,4-CHCHCHCH–, R′ = H, R″ = Ph), 119656-69-8; **18**, 229-87-8; **19**, 14548-01-7; **20**, 1015-89-0; $BrCH_2CO_2Et$, 105-36-2; $MeCHBrCO_2Et$, 535-11-5; aldose reductase, 9028-31-3.