mmol) was boiled under reflux with 3-chloroperoxybenzoic acid (85%; 1.44 g, 7 mmol) in CH_2Cl_2 (50 mL) for 8 h. The solution was washed with aqueous Na_2SO_3 (twice), saturated aqueous $NaHCO_3$ (twice), H_2O, and brine and was dried. The evaporation residue was purified by column chromatography ($CHCl_3$) to furnish **30** (240 mg, 33%) as a white solid: mp 120 °C dec; IR ν_{max} 3270, 2800–2550, 1650, 1620, 1560 cm^{-1}; NMR ($CDCl_3$/$(CD_3)_2SO$) δ 2.60 (dd, J = 4 and 2 Hz, 1 H) and 2.75 (t, J = 4 Hz, 1 H) (oxirane 3-H_2), 3.0–3.9 (m, 3 H, NCH_2CH), 6.65 (d, J = 16 Hz, 1 H) and 7.50 (d, J = 16 Hz, 1 H) (propenamide 2,3-H), 7.20 (d, J = 4 Hz, 1 H, thiophene 4-H), 7.85 (d, J = 4 Hz, 1 H, thiophene 3-H), 8.3 (br, 1 H, NH); MS (EI) m/z 254.0366 (M) ($C_{10}H_{10}N_2O_4S$ requires 254.0361).

3-Methyl-2-nitro-5-prop-2-enylthiophene (35). Method A. Allylmagnesium bromide (1.0 M in THF; 5 mL, 5 mmol) was added to **32** (710 mg, 5 mmol) in THF (20 mL) during 15 min at −50 °C. Stirring continued for a further 15 min at −50 °C. 5,6-Dichloro-2,3-dicyanobenzoquinone (1.7 g, 7.5 mmol) in THF (25 mL) was added and the solution was stirred at ambient temperature for 3 h before being poured into aqueous NH_4Cl and extracted three times with Et_2O. The combined extracts were dried. The evaporation residue was purified by column chromatography (EtOAc/hexane 1:4) and by radial PLC (EtOAc/hexane 1:5) to afford **35** (170 mg, 19%) as a colorless glass: NMR ($CDCl_3$) δ 2.55 (s, 3 H, CH_3), 3.50 (br d, J = 6 Hz, 2 H, thiophene CH_2), 5.0–6.2 (m, 3 H, $CH{=}CH_2$), 6.60 (br s, 1 H, thiophene 4-H).

Method B. Compound **32** (720 mg, 5 mmol) in THF (20 mL) was added during 30 min to allylmagnesium bromide in THF (1.0 M, 6 mL, 6 mmol) at −65 °C. After a further 30 min at this temperature, $BF_3{\cdot}Et_2O$ (1.0 mL) was added and the mixture was stirred at ambient temperature for 16 h before being poured onto aqueous NH_4Cl. The mixture was stirred for 1 h and was extracted four times with Et_2O. The combined extracts were dried. The evaporation residue was purified by column chromatography (CH_2Cl_2) to give **35** (220 mg, 24%) identical with the material described above. These materials were used for the preparation of **36** without further purification.

3-Methyl-2-nitro-5-(oxiranylmethyl)thiophene (36). Nitrothiophene **35** (250 mg, 1.4 mmol) was boiled under reflux with 3-chloroperoxybenzoic acid (55%; 2.52 g, 8 mmol) in CH_2Cl_2 (40 mL) for 12 h. The mixture was washed with saturated aqueous Na_2SO_3 (three times), saturated aqueous $NaHCO_3$ (twice), and brine before being dried. The evaporation residue was purified by radial PLC (CH_2Cl_2) to give **36** (110 mg, 41%) as a colorless gum: IR (liquid film) ν_{max} 1555 (w) cm^{-1}; NMR (270 MHz; $CDCl_3$) δ 2.58 (s, 3 H, CH_3), 2.60 (dd, J = 4.8 and 2.6 Hz, 1 H) and 2.87 (dd, J = 4.5 and 3.5 Hz, 1 H) (oxirane 3-H_2), 2.95 (dd, J = 15.8 and 6.0 Hz, 1 H) and 3.08 (dd, J = 15.8 and 4.0 Hz, 1 H) (thiophene CH_2), 3.19 (ddt, J = 6.0, 2.6, and 4.0 Hz, 1 H, oxirane 2-H), 6.76 (s, 1 H, thiophene 4-H); MS (EI) m/z 199.0297 (100) (M) ($C_8H_9NO_3S$ requires 199.0303), 182, 156, 126, 110.

5-(Dimethoxymethyl)-2-nitro-3-(prop-2-enyl)thiophene (40). 2-Nitrothiophene-5-carboxaldehyde (**24**) (314 mg, 2 mmol) was boiled under reflux with MeOH (5 mL) and ethereal HCl (1.0 M, 4 mL) for 45 min. The solvent and excess reagent were evaporated to give 5-(dimethoxymethyl)-2-nitrothiophene (**37**) (406 mg, quant.) as a colorless gum: NMR ($CDCl_3$) δ 3.40 (s, 6 H, 2 × OCH_3), 5.60 (s, 1 H, $CH(OMe)_2$), 7.00 (d, J = 4 Hz, 1 H, thiophene 4-H), 7.80 (d, J = 4 Hz, 1 H, thiophene 3-H). This acetal was treated with allylmagnesium bromide according to method A above to afford **40** (15%) as a colorless oil: IR (liquid film) ν_{max} 2950, 1500, 1330 cm^{-1}; NMR ($CDCl_3$) 3.35 (s, 6 H, 2 × OCH_3), 3.75 (br d, J = 6 Hz, 2 H, thiophene CH_2), 5.05 (br d, J = 16 Hz, 1 H) and 5.10 (br d, J = 11 Hz, 1 H) ($C{=}CH_2$), 5.50 (s, 1 H, $CH(OMe)_2$), 5.80 (ddd, J = 16, 11 and 6 Hz, 1 H, $CH{=}CH_2$), 6.85 (s, 1 H, thiophene 4-H).

Acknowledgment. Generous financial support from the British Technology Group (U.K.) and from the National Cancer Institute (U.S.A.) under Grant No. 5 RO1 CA44126 is gratefully acknowledged.

Registry No. 1, 13551-87-6; 4, 88876-88-4; 5, 129448-97-1; 7, 36050-35-8; 9, 133628-23-6; 10, 133628-24-7; 11, 133628-25-8; 12, 133628-26-9; 13, 133628-27-0; 15, 84624-27-1; 16, 133628-28-1; 17, 133628-30-5; 18, 133628-31-6; 19, 133628-32-7; 20, 133628-33-8; 21, 133628-34-9; 22, 25084-14-4; 23, 133628-35-0; 24, 4521-33-9; 25, 6317-37-9; 26, 133628-36-1; 27, 133628-37-2; 28, 50868-70-7; 29, 133628-38-3; 30, 133628-39-4; 32, 32059-75-9; 35, 133628-40-7; 36, 133628-41-8; 37, 17375-68-7; 40, 133628-42-9; 2-(dimethylamino)ethylamine, 108-00-9; 4-(2-aminoethyl)morpholine, 2038-03-1; 1-(2-aminoethyl)piperidine, 27578-60-5; (2-aminoethyl)-bis(1-methylethyl)amine, 121-05-1; *N*-(*tert*-butoxycarbonyl)lysine, 13734-28-6; fluoren-9-ylmethyl chloroformate, 40356-30-7.

Relative Structure–Inhibition Analyses of the *N*-Benzoyl and *N*-(Phenylsulfonyl) Amino Acid Aldose Reductase Inhibitors

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A number of *N*-benzoyl amino acids were synthesized and tested to compare structure–inhibition relationships with the isosteric *N*-(phenylsulfonyl) amino acid (PS-amino acid) aldose reductase inhibitors. Inhibition analyses with these series reveals that their kinetic mechanisms of inhibition are similar, but that significant differences in structure–inhibition relationships exist. For example, while the PS-alanines and PS-2-phenylglycines produce enantioselective inhibition ($S > R$), no consistent pattern of enantioselectivity is observed with the isosteric *N*-benzoylalanines and 2-phenylglycines. Also, *N*-methyl and *N*-phenyl substitution in the PS-amino acid series does not substantially alter inhibitory activity, while similar substitutions in the *N*-benzoyl series (particularly *N*-phenyl) results in a significant increase in inhibitory activity. Proton NMR analysis of the *N*-benzoylsarcosines reveals that these compounds exist as a mixture of rotamers in solutions including the enzyme assay buffer and that the preferred conformer is one in which the carboxymethyl moiety is trans to the aromatic ring. Similar analyses with the *N*-benzoyl-*N*-phenylglycines demonstrate that these derivatives exist exclusively in the trans rotameric conformation in solution. No such N-substituent effects on conformation were observed in the PS-amino acid series. These results suggest that the differences in structure–inhibition trends between these structurally related series may result from the effect of substituents on preferred conformation.

Introduction

Aldose reductase (alditol: $NADP^+$ oxidoreductase; EC 1.1.1.21; ALR2), an enzyme of the polyol pathway, catalyzes the NADPH-dependent reduction of glucose to sorbitol in a variety of mammalian tissues. During hyperglycemia there is increased flux of glucose through the polyol pathway in tissues such as the lens, retina, nerve, and kidney, and this is associated with several biochemical changes including intracellular sorbitol accumulation and *myo*-inositol depletion. Over the past decade, a significant

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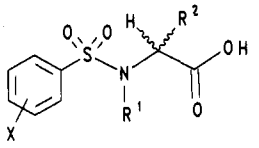
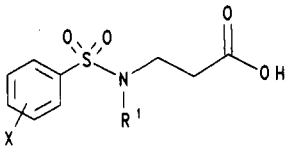
body of evidence has been amassed suggesting that these ALR2-mediated changes are ultimately expressed as chronic diabetic pathologies such as cataract, retinopathy, neuropathy, and nephropathy.[1-5]

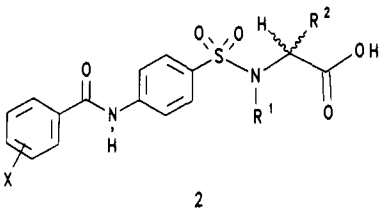
In earlier studies, we reported the synthesis and ALR2 inhibitory activities of a variety of *N*-(phenylsulfonyl) amino acid (PS-amino acids) derivatives **1**.[6-9] In these studies the following relative inhibitory potency trend was observed: (*S*)-PS-2-phenylglycines **1f** ≥ PS-*N*-phenylglycines **1c** ≥ PS-glycines **1a** ≥ PS-*N*-methylglycines **1b** > PS-β-alanines **1h** > (*S*)-PS-alanines **1d** > (*R*)-PS-alanines **1e** > (*R*)-PS-2-phenylglycines **1g**.[6-9] It was also



1a: $R^1 = R^2 = H$
1b: $R^1 = CH_3$, $R^2 = H$
1c: $R^1 = C_6H_5$, $R^2 = H$
1d: $R^1 = H$, $R^2 = CH_3$ (S-isomer)
1e: $R^1 = H$, $R^2 = CH_3$ (R-isomer)
1f: $R^1 = H$, $R^2 = C_6H_5$ (S-isomer)
1g: $R^1 = H$, $R^2 = C_6H_5$ (R-isomer)



1h



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noted that the most dramatic increase in inhibitory activity results when the *N*-(phenylsulfonyl) ring is substituted with a 4-(benzoylamino) moiety as in **2**.[6,9] In the present study a number of isosteric substituted *N*-benzoyl amino acids were synthesized to compare relative inhibitory trends and mechanisms of inhibition to the PS-amino acid series.

Discussion

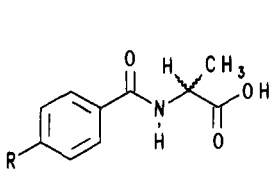
All of the *N*-benzoyl amino acids **3–5** were synthesized by the standard method involving reaction of commercially available benzoyl chlorides with the appropriate amino acids[11] in aqueous base or mixtures of organic solvent and aqueous base (Scheme I). The ring-substituted aroylamino derivatives **3d–3g**, **4i**, and **5i** were prepared by acylation of the corresponding *N*-(4-aminobenzoyl) amino acids in aqueous THF with sodium bicarbonate. The requisite 4-amino intermediates were prepared by catalytic reduction of the appropriate *N*-(4-nitrobenzoyl) amino acids. The physical data for these products are presented in Table I.

All of the *N*-benzoyl amino acids were initially tested for their ability to inhibit ALR2 isolated from rat lens using the methods outlined previously.[6,7] The compounds were evaluated at a minimum of four different concentrations, and log dose–response curves were constructed. Inhibitor IC_{50} values were calculated from the linear portions of these dose–response curves using the least-squares LINEFIT program of Barlow,[10] and these values are presented in Table II.

In an earlier study several *N*-benzoylglycines with simple ring substituents of varying electronic character (R = H, OCH_3, CH_3, NH_2, Cl, and NO_2) were evaluated and found to be relatively weak inhibitors of ALR2.[11] For example, in the rat lens assay, these compounds produce 50% enzyme inhibition only at concentrations exceeding 30 μM. Previous studies[6] with the isosteric *N*-(phenylsulfonyl)-glycines revealed that these derivatives are also relatively weak ALR2 inhibitors (IC_{50}s in the range of 10–100 μM), unless the aromatic ring contains a 4-(benzoylamino) substituent[6,9] (IC_{50}s of 0.5 μM or lower) or is replaced with a more lipophilic naphthalene ring system[6] (IC_{50}s of approximately 1 μM or lower). These observations prompted the synthesis and in vitro evaluation of similarly substituted analogues of the *N*-benzoylglycines. Interestingly, these naphthalene derivatives **3b** and **3c**, with IC_{50} values of 220 and 70 μM, respectively, are not significantly more inhibitory than *N*-benzoylglycine, demonstrating that naphthylene substitution does not significantly increase inhibitory activity in this series (Table II). However, addition of a 4-(benzoylamino) moiety in this series, as in **3d**, results in a substantial enhancement of inhibitory activity, as was observed in the PS-amino acid series. Moving this substituent to the 3-position (**3e**) or increasing the number of carbon atoms between the phenyl ring and amide carbonyl (**3f** and **3g**) of the 4-(benzoylamino) moiety results in a significant decrease in inhibitory activity. These results parallel those reported earlier for the PS-glycines.[7]

Earlier structure–inhibition studies with a series of *N*-PS-alanines **1d** and **1e** revealed that, even though the *S* enantiomers are more inhibitory than the *R* enantiomers, both isomers produce substantially less inhibition than the corresponding glycine derivatives **1a**.[6,9] Evaluation of a number of isosteric *N*-benzoylalanines **6** demonstrated that these derivatives are also somewhat less inhibitory than the glycines, but no consistent pattern of enantioselectivity was observed. For example, the enantiomeric 4-(benzoylamino) derivatives of **6** are very weak inhibitors of ALR2 with IC_{50}s of 600 and 300 μM for the *S* and *R* isomers, respectively. The enantiomeric 1-naphthalene derivatives of **6** also display low inhibitory activity, but in this case the IC_{50}s for enantiomers are essentially equal (280 μM for the *S* isomer and 260 μM for the *R* isomer).

(1) Dvornik, D. *Aldose Reductase Inhibition*; Biomedical Information Corp. (McGraw-Hill): New York, 1987.
(2) Larson, E. R.; Lipinski, C. A.; Sarges, R. *Med. Res. Rev.* **1988**, *8*, 159.
(3) Sarges, R. *Adv. Drug. Res.* **1989**, *18*, 139.
(4) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. *J. Med. Chem.* **1985**, *28*, 841.
(5) Humber, L. G. *Prog. Med. Chem.* **1987**, *24*, 299.
(6) DeRuiter, J.; Brubaker, A. N.; Garner, M. H.; Barksdale, J.; Mayfield, C. A. *J. Pharm. Sci.* **1987**, *76*, 149.
(7) Mayfield, C. A.; DeRuiter, J. *J. Med. Chem.* **1987**, *30*, 1595.
(8) DeRuiter, J.; Borne, R. F.; Mayfield, C. A. *J. Med. Chem.* **1989**, *32*, 145.
(9) Mayfield, C. A.; DeRuiter, J. *Int. J. Biochem.* **1989**, *21*, 1275.
(10) Barlow, R. B. *Biodata Handling with Microcomputers*; Elsevier Science: Amsterdam, The Netherlands, 1983; p 76.
(11) DeRuiter, J.; Swearingen, B. E.; Wandrekar, V.; Mayfield, C. A. *J. Med. Chem.* **1989**, *32*, 1033.



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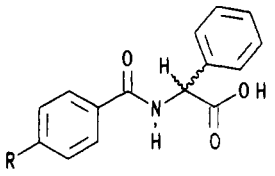

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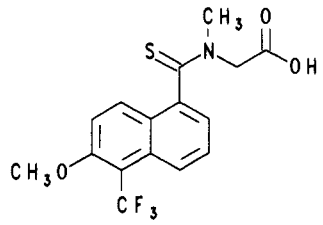

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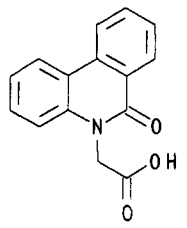

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Similar results were obtained upon analysis of several enantiomeric pairs of *N*-benzoyl-2-phenylglycines **7**. Again these compounds are very weak inhibitors of ALR2 and no enantioselectivity trend is apparent. For example, the *S* enantiomer of the 2-naphthalene derivative of **7** has an IC_{50} of 80 μM while the corresponding *R* enantiomer has an IC_{50} of 180 μM. But the 4-(benzoylamino) enantiomers of **7** display nearly equal inhibitory activities with IC_{50}s of 200 and 150 μM for the *S* and *R* enantiomers, respectively. These findings contrast significantly with results obtained with the isosteric PS-2-phenylglycines **1f** and **1g**. This series produces enantioselective inhibition with the *S* isomers **1f** ranging from 30 to 250 times more inhibitory than the *R* isomers **1g**.[8] Also, in the PS-2-phenylglycine series, the *S* isomers **1f** display inhibitory activities comparable to or greater than the corresponding PS-glycines **1a**. The significant differences in inhibitory trends observed between the *N*-benzoylalanines **6** and 2-phenylglycines **7** and the isosteric PS-amino acids could be interpreted to suggest that these two series, although very similar structurally, may not interact with ALR2 in precisely the same manner.

In the rat lens assay, most of the substituted *N*-benzoylsarcosine derivatives **4a–4f** were determined to be significantly more inhibitory than the *N*-benzoylglycines **3** or alanines **6** (Table II). The most inhibitory compound of this series, the 4-(benzoylamino) analogue **4i** produces 3.5 greater inhibition than the corresponding glycine derivative **3d**, suggesting that *N*-methyl substitution, like 4-(benzoylamino) substitution, contributes toward enhanced ALR2 affinity compared to *N*-benzoylglycine **3a**. Interestingly, in the isosteric *N*-PS-amino acid series, the sarcosine analogues **1b** were found to be no more inhibitory or slightly less inhibitory than the corresponding sarcosines.[6,9] Evaluation of the substituted *N*-benzoyl-*N*-phenylglycines **5a–5i** revealed that these compounds generally are even more inhibitory than the corresponding sarcosine derivatives **4** (Table II). While several derivatives of this series display IC_{50} values comparable to the corresponding sarcosines (**5d**, **5e**, and **5h**), others range from approximately 2 (**5c**) to 40 (**5f**) times as inhibitory. These data again contrast significantly with the isosteric PS-amino acids where the PS-*N*-phenylglycines **1c** are generally only slightly more inhibitory than the PS-glycines **1a**, which are more active than the PS-sarcosines **1b**.[8] The nonparallel structure-inhibition trends observed between the *N*-benzoylsarcosines **4** and *N*-phenylglycines **5** again could be interpreted as evidence that these two series of compounds may not be bound by ALR2 in exactly the same manner.

Proton NMR analysis of the benzoylsarcosine derivatives **4a–4i** reveals that these compounds exist as a mixiture of "cis" and "trans" rotamers in solution, resulting from hindered rotation about the amide C–N bond. As illustrated in Table III, both the sarcosine methylene protons and methyl protons give rise to two sets of signals (singlets) in deuterated acetone as well as the in vitro assay solvent (phosphate buffer, pH 6.2). The existence of such rotamerism was also reported earlier by Lee and Querijero[12] for the thioamide ALR2 inhibitor tolrestat **8**. They defined the rotamers as cis or trans based on the relative disposition of the bulkier naphthyl and carboxymethyl groups about the restricted C–N bond. In this study it was determined that in the less abundant cis rotameric form (26%), the methylene protons give rise to signals centered at 4.33 ppm while the *N*-methyl protons appear as a singlet at 3.85 ppm. In the more abundant trans rotamer (74%), the methylene proton signals are centered at 5.21 ppm while the methyl protons appear as a singlet at 3.23 ppm.

The assignments of cis and trans rotamers for the benzoylsarcosines **4** shown in Table III were established using the chemical shift data reported for tolrestat and related amides. For the spectra determined in deuterated acetone, the lower field methylene singlet (4.25–4.30 ppm) and higher field methyl singlet (3.03–3.10 ppm) result from the presence of the trans rotamer. Also the high-field methylene protons (4.07–4.12 ppm) and low-field methyl protons (>3.10 ppm) represent the presence of the cis rotameric forms. The relative integrals obtained from the spectra in acetone and phosphate buffer reveal that, in each case, the trans rotamer predominates, suggesting that this conformer is thermodynamically more stable in both solvents. Furthermore, the ratio of trans to cis rotamers does not vary significantly among different benzoylsarcosine derivatives. This ratio is approximately 65:35 in acetone and 60:40 in buffer. These data demonstrate that varying the electronic nature of ring substituents does not significantly influence conformation stability and thus does not affect the extent to which a particular rotamer exists. Therefore the subtle differences in IC_{50} values between sarcosine derivatives **4** with differing ring substituents do not appear to result from varying proportions of a particular rotamer.

To explore the rotameric nature of the benzoylsarcosines in more detail, rate constants and activation parameters ($E_a^{\ddagger}$, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$) were determined for the 4-fluoro derivative **4e** using temperature-dependent two-site exchange experiments with high-field NMR (Table IV). With this method spectra of samples of **4e** in deuterated DMSO were obtained to determine separation frequencies ($\Delta\upsilon$) at variable temperatures approaching the temperature of rotamer coalescence (T_c). From these data, the rate constants (k) were obtained for moderate exchange and these fitted by linear regression to a straight line according to the following equation:

$$\ln k' = -E_a^{\ddagger}/RT + \ln A$$

where E_a is the activation energy, R is the gas constant (1.987 kcal mol^{-1} K^{-1}), T is temperature (K), and A is the frequency factor. The correlation coefficient for this plot was −0.977. Using this equation, the activation energy for rotamer interconversion was determined to be 19.6 kcal/mol.

Using the activation energy determined from NMR exchange experiments and the standard thermodynamic

(12) Lee, H. K.; Querijero, G. *J. Pharm. Sci.* **1985**, *74*, 273.

Scheme I

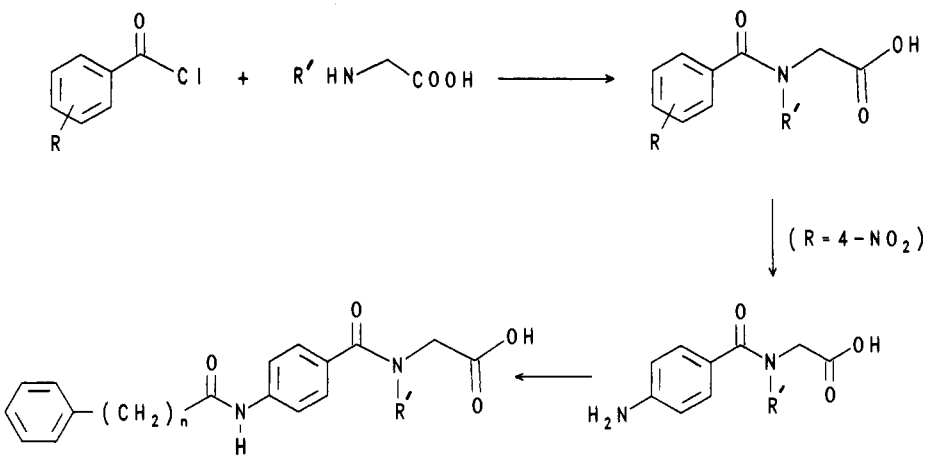
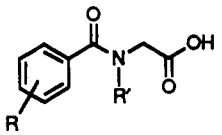


Table I. Physical Data for the Substituted *N*-Benzoyl Amino Acids



compd[a]	R	R′	recryst[b]	mp, °C	yield,[c] %	formula
3b	2,3-CHCHCHCH	H	A	146–150[d]	14	$C_{13}H_{11}NO_3$
3c	3,4-CHCHCHCH	H	A	168–170[e]	38	$C_{13}H_{11}NO_3$
3d	4-ArCONH	H	B	255–257	60	$C_{16}H_{14}N_2O_4$
3e	3-ArCONH	H	B	249–255	45	$C_{17}H_{16}N_2O_4$
3f	4-$ArCH_2CONH$	H	B	193–196	48	$C_{18}H_{18}N_2O_4$
3g	4-$ArCH_2CH_2CONH$	H	B	142–144	30	$C_{19}H_{20}N_2O_4$
4a	H	CH_3	C	102–104	16	$C_{10}H_{11}NO_3$
4b	4-CH_3	CH_3	A	156–160	60	$C_{11}H_{13}NO_3$
4c	4-OCH_3	CH_3	A	155–160	33	$C_{11}H_{13}NO_4$
4d	4-NH_2	CH_3	C	133–135	15	$C_{10}H_{12}N_2O_3$
4e	4-F	CH_3	A	97–101	13	$C_{10}H_{10}NO_3F$
4f	4-NO_2	CH_3	A	133–135	42	$C_{10}H_{10}N_2O_5$
4g	2,3-CHCHCHCH	CH_3	A	156–159	31	$C_{14}H_{13}NO_3$
4h	3,4-CHCHCHCH	CH_3	A	158–160	55	$C_{14}H_{13}NO_3$
4i	4-ArCONH	CH_3	B	227–229	26	$C_{17}H_{16}N_2O_4$
5a	H	C_6H_5	A	127–129	19	$C_{15}H_{13}NO_3$
5b	4-CH_3	C_6H_5	B	104–107	17	$C_{16}H_{15}NO_3$
5c	4-OCH_3	C_6H_5	A	158–163	28	$C_{16}H_{15}NO_4$
5d	4-NH_2	C_6H_5	A	167–169	34	$C_{15}H_{14}N_2O_4$
5e	4-F	C_6H_5	A	147–149	38	$C_{15}H_{12}NO_3F$
5f	4-NO_2	C_6H_5	A	172–175	32	$C_{15}H_{12}N_2O_5$
5g	2,3-CHCHCHCH	C_6H_5	A	150–152	15	$C_{19}H_{15}NO_3$
5h	3,4-CHCHCHCH	C_6H_5	A	114–116	35	$C_{19}H_{15}NO_3$
5i	4-ArCONH	C_6H_5	B	212–214	38	$C_{22}H_{18}NO_3$

[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 = EtOH; C = H_2O. [c] Percentages for all compounds except **3d–3g**, **4d**, **4i**, **5d**, and **5i** represent yields of the purified products obtained from the reaction of the substituted benzoyl chloride with the appropriate amino acid. The percentages for **3d–3g**, **4i**, and **5i** are the yields for acylation of the corresponding aniline intermediates, and the percentages for **4d** and **5d** are the yields from reduction of the corresponding nitro compounds **4f** and **5f**, respectively. [d] Lit. mp: 149–150 °C (ref 20). [e] Lit. mp: 169–170 °C (ref 21).

equations presented below, the enthalpy (ΔH^*), entropy (ΔS^*), and free energy (ΔG^*) of rotamer interconversion were determined (Table IV) as follows:

$$\Delta H^* = E_a - RT$$

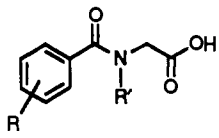
$$\Delta S^* = R(\ln A - \ln kT/h - 1)$$

$$\Delta G^* = \Delta H^* - T\Delta S^*$$

Based on these analyses, the average enthalpy of interconversion is approximately 19 kcal/mol, while the entropy of these processes is about 9 cal/mol K. Also, the free energy associated with rotamer interconversion appears to be near 16 kcal/mol. Since all of the benzoylsarcosine derivatives exist as nearly equal populations of trans and cis rotamers in solution, it is reasonable to assume that the activation parameters would be very similar for all members of this series. Furthermore, the activation energies and enthalpies of interconversion are lower for the benzoylsarcosine derivatives than those obtained for the rotamers of tolrestat.[12] These results seem reasonable since tolrestat bears a bulky naphthyl substituent which would be expected to present a greater barrier to rotamer interconversion than the aryl substituents of benzoylsarcosines such as the fluoro derivative **4e**.

Based on the presence of cis and trans rotamers of the *N*-benzoylsarcosines, even in this assay environment, it is reasonable to assume that ALR2 may bind, or at least have higher affinity for, one of the rotamers. If this assumption is accurate, then the IC_{50} values presented in Table II would significantly underestimate the potential inhibitory activities of these compounds. These conformational information provided by NMR may also account for the

Table II. Aldose Reductase Inhibitory Activity of Substituted *N*-Benzoyl Amino Acids



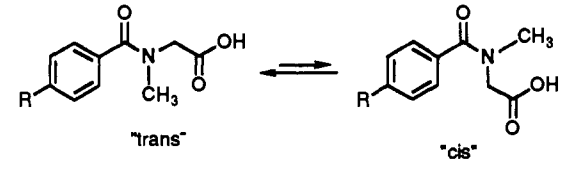
compd no.	R	R′	IC_{50}, μM (95% CI)[a]
3a	H	H	330 (280–490)
3b	2,3-CHCHCHCH	H	220 (100–450)
3c	3,4-CHCHCHCH	H	70 (34–160)
3d	4-ArCONH	H	9.6 (3.8–16)
3e	3-ArCONH	H	49 (21–110)
3f	4-$ArCH_2CONH$	H	21 (7.5–45)
3g	4-$ArCH_2CH_2CONH$	H	45 (22–90)
4a	H	CH_3	23 (10–65)
4b	4-CH_3	CH_3	13 (7.5–22)
4c	4-OCH_3	CH_3	6.7 (3.0–18)
4d	4-NH_2	CH_3	11 (6.2–21)
4e	4-F	CH_3	8.8 (3.6–30)
4f	4-NO_2	CH_3	26 (10–46)
4g	2,3-CHCHCHCH	CH_3	40 (25–81)
4h	3,4-CHCHCHCH	CH_3	19 (7.9–36)
4i	4-ArCONH	CH_3	2.8 (0.60–7.2)
5a	H	C_6H_5	6.3 (2.7–21)
5b	4-CH_3	C_6H_5	3.7 (1.6–10)
5c	4-OCH_3	C_6H_5	3.2 (1.6–6.5)
5d	4-NH_2	C_6H_5	12 (5.7–25)
5e	4-F	C_6H_5	8.2 (2.9–29)
5f	4-NO_2	C_6H_5	0.56 (0.21–1.4)
5g	2,3-CHCHCHCH	C_6H_5	8.5 (2.9–22)
5h	3,4-CHCHCHCH	C_6H_5	19 (6.9–29)
5i	4-ArCONH	C_6H_5	0.24 (0.09–0.90)
sorbinil			0.19 (0.08–0.39)[b]

[a] The IC_{50} values represent the concentration required to produce 50% inhibition of AR as determined by least-squares analyses of the linear portion of the log dose–response curves. The 95% confidence intervals (95% CI) were calculated from *T* values for *n* − 2 where *n* is the total number of determinations. [b] The literature IC_{50} value for sorbinil in the rat lens assay is 0.07 μM.[16]

differences in structure–inhibition trends observed between the isosteric *N*-benzoyl amino acids and *N*-PS-amino acids. The proton NMR spectra of the PS-sarcosines **1b** contain only a single set of signals for both the *N*-methyl and methylene protons, suggesting that these compounds exist exclusively in one rotameric form, or that there is free rotation about the S–N bond of the sulfonamide linkage at room temperature. This latter explanation appears more plausible since energy calculations using experimentally determined S–N bond stretching frequencies as well as bond lengths and angles[13–15] demonstrate that the barrier to rotation about this bond is substantially lower than rotational barriers of typical amides. Therefore, the increase in inhibitory activity observed for the *N*-benzoylsarcosines **4** versus the corresponding glycines **3** may simply result from the conformational effects imposed by the *N*-methyl substituent. Since the *N*-methyl moiety in the PS-sarcosine series **1b** does not influence conformational preference, it does not alter the affinity of ALR2 for these compounds compared to the corresponding glycines **1a**.

Proton NMR evaluation of the *N*-benzoyl-*N*-phenylglycines **5** demonstrates that these compounds exist as a single rotameric form in solution. Furthermore, based on comparison of the chemical shift data of these compounds

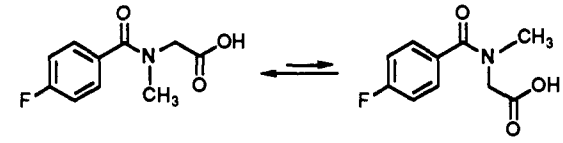
Table III. Proton NMR of Substituted *N*-Benzoylsarcosines[a]



	acetone-d_6			
	CH_2		CH_3	
compd	trans ppm (%)	cis ppm (%)	cis ppm (%)	trans ppm (%)
4b	4.26 (62)	4.08 (38)	3.05 (broad, 100)	
4c	4.25 (62)	4.12 (38)	3.09 (38)	3.06 (62)
4e	4.24 (66)	4.07 (34)	3.10 (broad, 100)	
4f	4.30 (63)	4.08 (37)	3.10 (37)	3.03 (63)
4h	4.33 (64)	4.14 (36)	3.13 (broad, 100)	
	deuterated phosphate buffer, pH 6.2			
	CH_2		CH_3	
compd	trans ppm (%)	cis ppm (%)	cis ppm (%)	trans ppm (%)
4c	4.12 (57)	3.92 (43)	3.06 (43)	3.04 (57)
4e	4.13 (61)	3.91 (39)	2.92 (39)	2.87 (61)
4f	4.18 (58)	3.87 (42)	2.98 (42)	2.89 (58)

[a] All spectra were run at 303 K on a Bruker AM400 spectrometer. The chemical shifts and relative proportions of signals do not change over time (48 h) at 303 K. The two sets of signals obtained for methylene and methyl protons converge into singlets at 324 K. For example, the methylene and methyl protons of the 4-methoxy derivative appear as singlets at 4.18 and 3.11 ppm, respectively.

Table IV. Separation Frequencies and Energy Activation Parameters as a Function of Temperature



temp, K	$(\Delta\upsilon_o)^2 - (\Delta\upsilon)^2$	k',[a] Hz	$E_a^{\ddagger}$,[b] kcal/mol	$\Delta H^{\ddagger}$,[c] kcal/mol	$\Delta S^{\ddagger}$,[d] cal/mol	$\Delta G^{\ddagger}$,[e] kcal/mol
320	536	51.43	19.7	19.1	8.61	16.34
322	950	68.47	19.6	19.6	8.61	16.83
324	1778	93.67	19.5	19.5	8.58	16.72
326	1909	97.06	19.6	19.0	8.56	16.21
328	2998	121.6	19.6	18.9	8.56	16.09
330	3634	133.91	19.6	18.9	8.54	16.08

[a] $k' = \pi\sqrt{1/2[(\Delta\upsilon_o)^2 - (\Delta\upsilon)^2]}$ where $\Delta\upsilon_o$ and $\Delta\upsilon$ are the separation frequencies for the methylene protons as a function of temperature and $\Delta\upsilon_o$ = 78.32 Hz. [b] $E_a^{\ddagger} = RT(\ln k' - \ln A)$. [c] $\Delta H^{\ddagger} = E_a^{\ddagger} - RT$. [d] $\Delta S^{\ddagger} = R(\ln A - \ln kT/h - 1)$ where *k* is Boltzmann's constant. [e] $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$.

to the corresponding sarcosines **4**, it appears that the *N*-phenylglycines **5** exist solely in a trans form; the chemical shifts of ca. 4.5 ppm for the methylene protons suggest that this moiety exists trans to the benzoyl ring, and therefore the *N*-phenyl ring is cis to the benzoyl group. Further evidence in support of these assignments is derived from both proton NMR and in vitro analyses of the phenanthridinone *N*-acetic acid **9**, which represents a rigid analogue of the *N*-benzoyl-*N*-phenylglycines **5** locked in the trans conformation. In the proton NMR, the methylene protons appear at 4.7 ppm. Also, in the rat assay, this compound has an IC_{50} of 10 μM, nearly identical with that of the unrestricted derivative **5a** (IC_{50} = 9.5 μM[11]). Furthermore, the fact that the *N*-phenyl derivatives **5**, which exist solely in the trans conformation, are more inhibitory than the sarcosines **4** suggests that ALR2 may have higher affinity for the trans rotamers of the sarcosines.

The kinetics of inhibition of one of the more potent *N*-benzoyl amino acids, compound **5f**, was studied as a

(13) Hakkinen, A.-M.; Ruostesuo, P.; Kivekas, R. *J. Chem. Soc., Perkin Trans. 2* 1988, 815.
(14) DeBenedetti, P. G.; Iarossi, D.; Menziani, M. C.; Frassineti, C.; Benedetti, A. *J. Mol. Struct.* 1989, *175*, 37.
(15) Kim, B. S.; Lee, S. H.; Chung, U. T.; Kang, Y. K. *J. Pharm. Soc. Korea* 1989, *33*, 350.

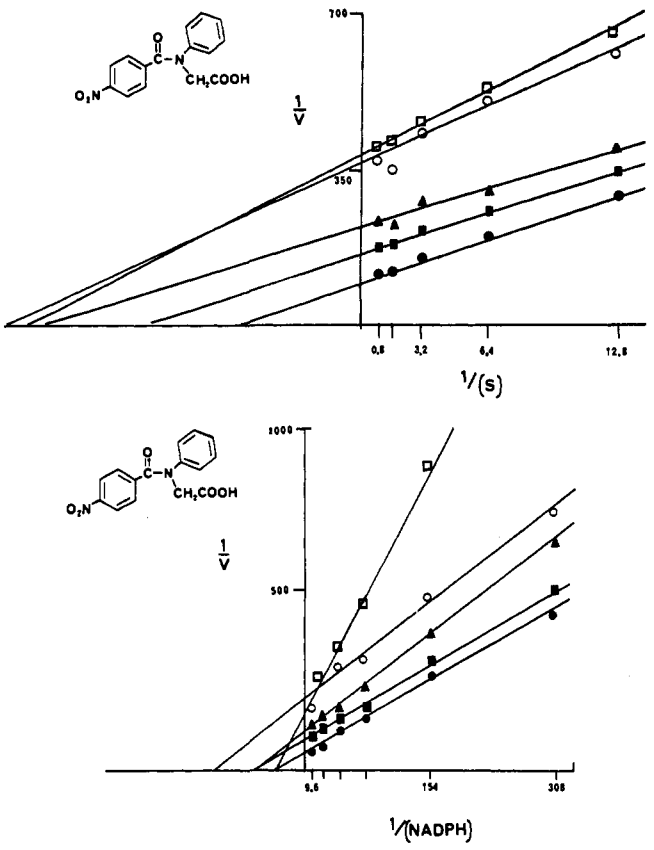


Figure 1. (a, Top): Double-reciprocal plot of initial enzyme velocity versus concentration of substrate (DL-glyceraldehyde) with and without inhibitor **5f**: no inhibitor (●) and 0.5 μM (■), 1.0 μM (▲), 5.0 μM (○), and 10 μM (□) inhibitor. (b, Bottom): Double-reciprocal plot of initial enzyme velocity versus concentration of cofactor (NADPH) with and without inhibitor **5f**: no inhibitor (●) and 0.5 μM (■), 1.0 μM (▲), 5.0 μM (○) and 10 μM (□) inhibitor.

function of inhibitor concentration relative to both substrate and cofactor. The Lineweaver–Burk plots obtained from analysis of **5f** versus the substrate DL-glyceraldehyde (Figure 1a) demonstrate that this compound produces uncompetitive inhibition at lower concentrations (0.5 and 1.0 μM) and mixed-type inhibition at higher concentrations (5.0 and 10.0 μM). A similar dependence of kinetics on inhibitor concentration was observed in the cofactor studies (Figure 1b), and these results are very similar to those obtained with the PS-amino acids.[7,9] It has been reported that sorbinil, alrestatin, tolrestat, and ponalrestat also produce noncompetitive or uncompetitive inhibition of ALR2 relative to substrate and cofactor, and these results have been interpreted as evidence that the inhibitors are not bound by the catalytic or cofactor site of the enzyme.[3] Furthermore, Kador and Sharpless proposed that ALR2 inhibitors bind at a common site on the enzyme based on evidence obtained from competition studies and the identification of electronic and steric similarities in a variety of inhibitors.[16] The similarities in kinetic profiles between the *N*-benzoyl amino acids and PS-amino acids, as well as other known inhibitors of ALR2, suggests that these compounds share a common kinetic mechanism of inhibition, and possibly, a common site of interaction on ALR2. Such a hypothesis can be rationalized for the *N*-benzoyl amino acids and PS-amino acids, in spite of the significant differences in the structure–inhibition trends between these series, by consideration of the effects of substituents on conformational preferences in each series. For example, in the PS-amino acid series, the *N*-methyl (**1b**) and *N*-phenyl (**1c**) substituents do not appear to interact directly with complimentary binding sites on ALR2[8,9] and do not influence conformational preference. Therefore these substituents do not substantially alter affinity in the PS-amino acid series. In the *N*-benzoyl amino acid series the *N*-methyl (**4**) and *N*-phenyl (**5**) substituents again may not interact directly with ALR2 but may influence the efficacy of interaction with ALR2 by affecting the preferred conformation and hence the relative disposition of key functionalities required for binding.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. For structure determination, ^{1}H NMR spectra were recorded on a Varian EM360 NMR spectrometer in $CDCl_3$ or Me_2SO-d_6, 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 used in the syntheses were purchased from the Aldrich Chemical Co., Milwaukee, WI, and were used as received. DL-Glyceraldehyde and NADPH (type I) used for the enzyme assay were purchased from Sigma Chemical Co.

Synthesis of the *N*-Benzoyl Amino Acids. Benzoyl chlorides (15 mmol) obtained commercially or by treatment of available benzoic acids with thionyl chloride were added portionwise over a 15–30-min period to a cold (0–10 °C) solution of amino acid (18 mmol) and NaOH (33 mmol) in water (50 mL). After the addition was complete, the reaction mixture was stirred for 30 min at room temperature and then filtered. The filtrate was cooled (ice bath) and acidified to pH 1 with concentrated HCl. The resultant solid was isolated by filtration, washed with water, and recrystallized from mixtures of aqueous ethanol.

Synthesis of the *N*-(4-(Benzoylamino)benzoyl) Amino Acids. A solution of *N*-(4-nitrobenzoyl) amino acid (10 mmol) in ethanol (100 mL) containing 5% Pd–C (0.5 g) was shaken under a H_2 atmosphere (initial psi 45) on a Parr apparatus until the consumption of H_2 ceased (ca. 30 min). Filtration, followed by evaporation of the filtrate solvent, gave the intermediate amines, which were recrystallized from aqueous ethanol. A solution of benzoyl chloride (5.0 mmol) in THF (15 mL) was then added to a solution of the intermediate amine (5.0 mmol) and $NaHCO_3$ (10 mmol) in water (25 mL). After the addition was complete the reaction mixture was stirred at room temperature for 1 h and then evaporated to dryness. The resulting solid was suspended in water (25 mL) and acidified to pH 1 with concentrated HCl to yield the product as a solid. The product was isolated by filtration, washed with water, and recrystallized.

Rotamer Studies. The conformer composition of the *N*-benzoylsarcosines was determined by ^{1}H NMR using a Bruker AM-400 MHz spectrometer with a variable temperature-controlled probe. The sarcosine derivatives (10 mg) were dissolved in acetone-d_6, Me_2SO-d_6, or deuterated phosphate buffer, pH 6.2 (0.5–0.7 mL); acetone and DMSO solutions were used to determine the presence of cis and trans rotamers in organic media, while phosphate buffer solutions were used to determine the rotameric behavior of the sarcosines under the in vitro enzyme assay con-

(16) Kador, P. F.; Sharpless, N. E. *Mol. Pharmacol.* **1983**, *24*, 521.
(17) Dvornik, D. Cooperative Cataract Research Group Meeting, Orlando, FL, May 1980 (also see ref 1, pp 233–238).
(18) Kador, P. F.; Kinoshita, J. H.; Tung, W. H.; Chylack, L. T. *Invest. Ophthalmol. Vis. Sci.* **1980**, *19*, 980.
(19) Muller, P.; Hockwin, O.; Ohrloff, C. *Ophthalmic Res.* **1985**, *17*, 115.
(20) Cioranesca, E.; Birladeanu, L.; Frangopol, P. T.; Balaban, A. T. *Rev. Chim. Acad. Rep. Populair Roumaine* **1962**, *7*, 755; *Chem. Abstr.* **1964**, *61*, 4333f.
(21) Friedmann, E. *Biochem. Z.* **1913**, *55*, 463.

ditions. The solvent Me_2SO-d_6 was selected for variable-temperature studies to determine activation parameters since this solvent has a higher boiling point.

Enzyme Assays. Frozen rat eyes were purchased from Charles River Breeding Labs, Inc., Wilmington, MA. The lenses were dissected from the partially thawed eyes and kept frozen until used for enzyme isolation. Crude enzyme supernatant was prepared by homogenizing 100 lenses in distilled water (20 mL) and then centrifuging the crude homogenate at 10000 rpm for 15 min while maintaining an ambient temperature of 5 °C. The supernatant was then isolated and ammonium sulfate was added to achieve 40% saturation, and this solution centrifuged at 10000 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 measuring the decrease in NADPH concentration at 340 nm with a Shimadzu UV-160 spectrophotometer equipped with a thermocontrolled multicell positioner. The control reaction mixture contained 0.1 M phosphate buffer, pH 6.2; 0.104 mM NADPH and 10 mM DL-glyceraldehyde and 0.2 mL of the enzyme supernatant in a total volume of 2.0 mL. A reference blank containing all of the above reagents except the substrate glyceraldehyde was used to correct for oxidation of NADPH not associated with reduction of the substrate. The reactions were initiated by addition of glyceraldehyde and were monitored for 3 min after a 45-s incubation period. Enzyme activity was adjusted by dilution of the supernatant with distilled water so that 0.2 mL of supernatant gave an average reaction rate for control reactions of 0.0120 ± 0.002 absorbance units per minute. The inhibitory activity of the *N*-benzoyl amino acids was determined by including 0.2 mL of an aqueous solution of the inhibitor at the desired concentrations in the reaction mixture. For IC_{50} determinations, each inhibitor was tested at no fewer than six different concentrations with a minimum of two determinations at each concentration. The percent inhibition for each inhibitor was calculated at all concentrations by comparing the rate of reactions containing inhibitor to that of control reactions with no inhibitor. Inhibitor IC_{50} value were then obtained by least-squares analyses of the linear portion of log dose–response curves using the LINEFIT program of Barlow.[10]

Kinetic Studies. Kinetic analyses (Figure 1) with **5f** were conducted using a minimum of four concentrations (0.5, 1.0, 5.0, and 10.0 μM) of the inhibitor. For substrate kinetics, the concentrations of DL-glyceraldehyde ranged from 1.25 to 0.078 mM, and the concentration of cofactor was held constant at 0.104 mM. For cofactor kinetics, the concentrations of NADPH were varied from 3.25 to 105 μM and the substrate concentration held constant at 10 mM. The nature of inhibition produced by each concentration of inhibitor was determined by analysis of double reciprocal plots of enzyme velocity versus DL-glyceraldehyde or NADPH concentration as generated by least-squares fit of the data using the program of Barlow.[10]

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Registry No. **3a**, 495-69-2; **3b**, 75446-60-5; **3c**, 69826-63-7; **3d**, 133604-60-1; **3e**, 133604-61-2; **3f**, 133604-62-3; **3g**, 133604-63-4; **4a**, 2568-34-5; **4b**, 133604-64-5; **4c**, 133604-65-6; **4d**, 133604-66-7; **4e**, 133604-67-8; **4f**, 35876-37-0; **4g**, 133604-68-9; **4h**, 133604-69-0; **4i**, 133604-70-3; **5a**, 119656-49-4; **5b**, 133604-71-4; **5c**, 35876-73-4; **5d**, 133604-72-5; **5e**, 2995-56-4; **5f**, 35876-34-7; **5g**, 119656-55-2; **5h**, 133604-73-6; **5i**, 133604-74-7; ALR2, 9028-31-3; H-Gly-OH, 56-40-6; H-Sar-OH, 107-97-1; PhCOCl, 98-88-4; 4-MeC_6H_4COCl, 874-60-2; 4-$MeOC_6H_4COCl$, 100-07-2; 4-FC_6H_4COCl, 403-43-0; 4-$O_2NC_6H_4COCl$, 122-04-3; 4-$O_2NC_6H_4CO$-Gly-OH, 2645-07-0; 4-$H_2NC_6H_4CO$-Gly-OH, 61-78-9; Ph-Gly-OH, 103-01-5; 1-naphthylcarbonyl chloride, 879-18-5; 2-naphthylcarbonyl chloride, 2243-83-6.

Synthesis and in Vivo Photodynamic Activity of Some Bacteriochlorin Derivatives against Bladder Tumors in Rodents

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Bacteriochlorins have been suggested as potential photosensitizers for use in photodynamic therapy. We have shown that bacteriochlorin-like macrocycles can be generated through cyclization of either 5,10- or 5,15-bis[(ethoxycarbonyl)vinyl]porphyrins; however, the resulting products are rapidly decomposed on exposure to air. More stable systems can be generated by Diels–Alder reactions between dienophiles such as dimethyl acetylenedicarboxylate or tetracyanoethylene, and vinylporphyrinones. Although spectroscopic properties of these latter products resemble those of porphyrinones rather than bacteriochlorins, in vivo studies using the *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide-induced rat bladder tumor (AY-27) transplanted into Fisher CDF (F344)/CrlBr rats demonstrated a powerful photodynamic response.

Introduction

The bacteriochlorins (A; Figure 1) are a series of tetrapyrrolic macrocycles that differ from porphyrins (B; Figure 1) only in the reduced state of two opposite pyrrole rings. This difference is suffice to alter dramatically the electromagnetic spectrum of the molecule, with the longer absorbing (Q) band of the porphyrin near 630 nm and the corresponding absorption of the tetrahydro species red-shifted by some 150 nm.[1] Bacteriochlorins have therefore been proposed as potentially useful candidates for use in photodynamic therapy where strong absorptions in the visible spectrum can be used to photoactive dyes previously located in target (neoplastic) tissues.[2] Ensuing energy transfer or electron transfer can then generate cytotoxic species in situ that ultimately lead to tumor necrosis.[3]

Although bacteriochlorophylls, naturally occurring bacteriochlorins, have been shown to photosensitize cells in vitro[4] and have also shown photodynamic activity in

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(1) Smith, K. M. In *Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon Press: New York, 1984; Vol. 4, p 385.
(2) Bonnett, R.; Nizhnik, A. N.; Berenbaum, M. C. *J. Chem. Soc., Chem. Commun.* **1989**, 1822.
(3) Girotti, A. W. *Photochem. Photobiol.* **1983**, *38*, 745.
(4) Beems, E. M.; Dubbelman, T. M. A. R.; Lugtenburg, I.; Van Best, J. A.; Smeets, M. F. M. A.; Boegheim, J. P. S. *Photochem. Photobiol.* **1987**, *46*, 639.

0022-2623/91/1834-2126$02.50/0