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Synthesis of potential aldose reductase inhibitors based on minimal pharmacophore requirements

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Abstract

A series of 17 compounds were synthesized based on the premise that the minimal pharmacophore for aldose reductase inhibition requires the presence of both an aryl group and polar group connected by a linking structure. Three groups of compounds were synthesized, the first possessing an aniline-4-(2′-6′-methylbenzothiazole) or 2-aminobenzothiazole group as the aryl group, the second possessing a 2-naphthyl as the aryl group and the third possessing either a 4-(2-phenylthiazole) or 2-(5-2′-nitrophenylfuran) as the aryl group. In all three of these groups the carboxylate or its methyl ester are linked to the aryl group through various lengths of methylene carbons and amide or cinnamide groups. Optimal activity was observed when the carboxylic group was separated from the aryl group by a linking structure of five atoms in length. Both a double bond and an amide moiety are well tolerated in the linking structure.

Introduction

Although progress has been made in the treatment of diabetes mellitus with insulin or oral anti-hyperglycaemics, this current therapy cannot prevent the development of long-term, diabetes-related complications. These complications, which include neuropathy, nephropathy and retinopathy, not only considerably diminish the quality of life of diabetics but also reduce their lifespan. In tissues possessing insulin-independent glucose uptake, elevated blood-glucose concentrations lead to an increased flow of glucose into the sorbitol pathway. In the sorbitol pathway glucose is reduced to sorbitol by the enzyme aldose reductase using NADPH as hydride donor. Under diabetic conditions the increased flow of glucose through the sorbitol pathway results in elevated intracellular sorbitol concentrations in tissues developing diabetic complications and altered redox changes associated with the increased utilization of nucleotides. This intracellular increase of sorbitol can lead to osmotic effects and eventually the loss of membrane integrity. Both membrane permeability changes and redox changes have been linked to the molecular cause of the clinically manifest symptoms (Kador et al 1985; Kador 1988; Tomlinson et al 1992; Yabe-Nishimura 1998). Therefore, inhibition of aldose reductase has been considered an important target for drug development. The efforts of several industrial and academic research groups have resulted in the identification of numerous structurally diverse aldose reductase inhibitors (Larson et al 1988; Sarges 1989), which include the carboxylic acids and hydantoins summarized in Figure 1.

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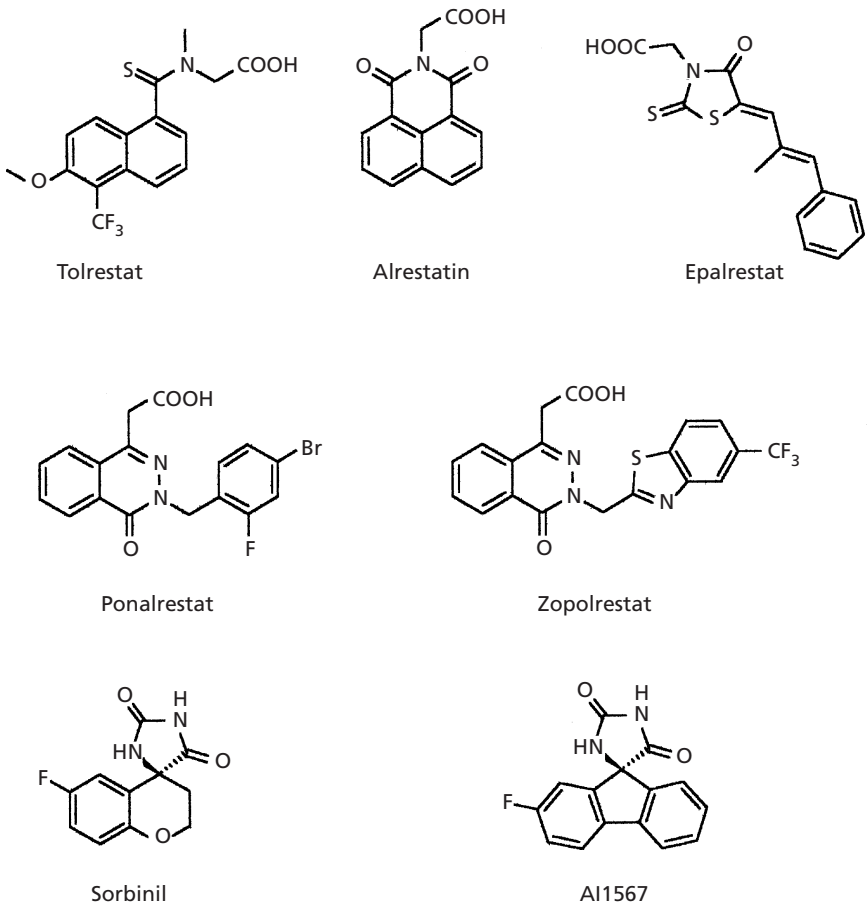

Figure 1 Representative examples of known aldose reductase inhibitors.

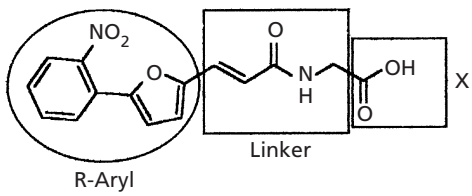

Figure 2 Pharmacophore template for the design of aldose reductase inhibitors exemplified with compound **16**.

Examination of a number of these structurally diverse inhibitors has resulted in the hypothesis that the minimum pharmacophore requirements for an aldose reductase inhibitor consist of a hydrophobic aromatic structure (R-Aryl) and a polar or ionic group (X) linked by a flexible chain of suitable length (linker) (Kador et al 1985; Lee et al 1994, 1998). To test this basic premise a number of compounds were prepared according to the suggested general lead structure the basic features of which are outlined in Figure 2.

Material and Methods

Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Jeol JMN-GX-400 and a Jeol JMN-LA-500 spectrometer. Mass spectra were obtained with a Vacuum Generators VG 7070 H using a Vector 1 data acquisition system from Teknivent or an AutoSpec mass spectrometer from Micromass. High resolution mass spectroscopy was carried out as an additional structural proof except for those compounds in which the M^{+} signal was either too weak or not recordable. IR spectra were recorded on a Nicolet 510P FTIR spectrometer. Microanalyses were obtained from a CH analyser according to Dr Salzer from Labormatic and from a Hewlett Packard CHN-analyser type 185. Column chromatography was carried out using silica gel 60 (0.062–0.200 mm) from Merck.

Abbreviations: MS, mass spectroscopy; HRMS, high resolution mass spectroscopy; EI, electron impact ionization.

Method 1. Preparation of amides of dicarbonic acid methyl esters 1, 2, and 5

An equimolar mixture of an aromatic amine and malonic acid methyl ester chloride or succinic acid methyl ester chloride was refluxed in dry tetrahydrofuran (THF) for 2 h. The reaction mixture was evaporated to dryness and the resulting residue was purified by recrystallization.

N-[4-(6-*Methylbenzothiazole*-2-*yl*)-*phenyl*]-*malonamic acid methyl ester* (**1**)
Recrystallization from dioxane/methanol. Yield 0.52 g (76%), white crystals; mp 187°C. IR: $\nu = 3020, 1750, 1685, 1590, 1525\ cm^{-1}$; ^1H NMR (d_6-DMSO): δ 10.69 (s, 1H, *NH*), 8.36–8.14 (m, 2H, aromatic), 7.90–7.88 (m, 2H, aromatic), 7.81–7.79 (m, 2H, aromatic), 7.34–7.32 (m, 1H, aromatic), 3.68 (s, 3H, OCH_3), 3.57 (s, 2H, CH_2), 2.45 (s, 3H, *Ar*-CH_3); ^{13}H NMR (d_6-DMSO): δ = 167.8, 165.6, 164.3 (2 × *CO* and *benzothiazole-C-2*), 151.6, 141.3, 134.9, 134.3, 127.9, 127.7, 122.0, 121.6, 119.3 (aromatic), 51.8 (OCH_3), 43.4 (CH_2), 20.9 (*Ar*-CH_3); MS: m/z (%) = 340 (100) [M^+], 266 (33), 240 (87); HRMS calculated for $C_{18}H_{16}N_2O_3S$: 340.0882; found 340.0892; elemental analysis calculated for $C_{18}H_{16}N_2O_3S$: C 63.51, H 4.74, N 8.23; found C 63.84, H 5.09, N 7.98.

N-[4-(6-*Methylbenzothiazole*-2-*yl*)-*phenyl*]-*succinamic acid methyl ester* (**2**)
Recrystallization from dioxane/methanol. Yield 0.62 g (87%), white crystals; mp 209°C; IR: $\nu = 3330, 1735, 1690, 1590\ cm^{-1}$; ^1H NMR (d_6-DMSO): δ 10.32 (s, 1H, *NH*), 8.01–7.98 (m, 2H, aromatic), 7.89–7.87 (m, 2H, aromatic), 7.78–7.76 (m, 2H , aromatic), 7.34–7.32 (m, 1H, aromatic), 3.61 (s, 3H, OCH_3), 2.70–2.60 (m, 4H, CH_2-CH_2), 2.45 (s, 3H, *Ar*-CH_3); ^{13}H NMR (d_6-DMSO): δ 172.6, 172.0, 170.1 (2 × *CO* and *benzothiazole-C-2*), 151.0, 141.0, 134.8, 134.3, 127.8, 127.6, 127.4, 122.0, 121.6, 119.0 (aromatic), 51.2 (OCH_3), 30.9, 28.3 (CH_2-CH_2), 20.9 (*Ar*-CH_3); MS: m/z (%) = 354 (2) [M^+], 323 (100); elemental analysis calculated for: C 64.39, H 5.12, N 7.90; found C 64.19, H 5.19, N 7.86.

N-*Benzothiazole*-2-*yl-succinamic acid methyl ester* (**5**)
Recrystallization from THF. Yield 0.35 g (70%), white crystals; mp 179°C; IR: $\nu = 3095, 1735, 1720, 1560\ cm^{-1}$; ^1H NMR (d_6-DMSO): δ 7.96–7.93 (m, 1H, aromatic), 7.74–7.72 (m, 1H, aromatic), 7.42 (m, 1H, aromatic), 7.29 (m, 1H, aromatic), 3.61 (s, 3H, OCH_3), 2.77 (t, J = 7 Hz, 2H, CH_2-CH_2), 2.67 (t, J = 7 Hz, 2H, CH_2-CH_2); ^{13}H NMR (d_6-DMSO): δ 172.4, 170.9 (2 × *CO*), 157.7, 148.4, 131.4, 125.9, 123.3, 121.5, 120.4 (aromatic *C*), 51.3 (OCH_3), 30.0, 28.0 (CH_2-CH_2); MS (EI): m/z (%) = 264 (8) [M^+], 177 (100), 150 (79); HRMS calculated for $C_{12}H_{12}N_2O_3S$: 264.0569; found 264.0571; elemental analysis calculated for $C_{12}H_{12}N_2O_3S$: C 54.53, H 4.58, N 10.60; found C 54.92, H 4.61, N 10.29.

Method 2. Preparation of amides 3, 4, 6 and 8 from amines and succinic anhydride or glutaric anhydride

An equimolar mixture of an aromatic amine and succinic anhydride or glutaric anhydride was refluxed in dry THF for 2 h. The reaction mixture was evaporated to dryness and the resulting residue was purified by recrystallization.

N-[4-(6-*Methylbenzothiazole*-2-*yl*)-*phenyl*]-*succinamic acid* (**3**)
Recrystallization from dioxane. Yield 0.384 g (64%), white crystals; mp 228°C; IR: $\nu = 3330, 1735, 1690, 1590\ cm^{-1}$; ^1H NMR (d_6-DMSO): δ 10.23 (s, 1H, *NH*), 8.01–7.99 (m, 2H, aromatic), 7.89–7.87 (m, 2H, aromatic), 7.78–7.77 (m, 2H, aromatic), 7.34–7.32 (m, 1H, aromatic), 2.61 (m, 2H, CH_2-CH_2), 2.54 (m, 2H, CH_2-CH_2), 2.45 (s, 3H, *Ar*-CH_3); ^{13}H NMR (d_6-DMSO): δ 173.5, 170.4, 165.7 (2 × *CO* and *benzothiazole-C-2*), 151.7, 141.7, 134.8, 134.3, 127.8, 127.6, 127.4, 121.9, 121.5, 119.0 (aromatic), 31.1, 28.6 (CH_2-CH_2), 20.9 (*Ar*-CH_3); MS (EI): m/z (%) = 340 (3) [M^+], 322 (100), 240 (45), 97 (59); elemental analysis calculated for $C_{18}H_{16}N_2O_3S$: C 63.51, H 4.74, N 8.23; found C 63.87, H 4.54, N 8.58.

N-[4-(6-*Methylbenzothiazole*-2-*yl*)-*phenyl*]-*glutaramic acid* (**4**)
Recrystallization from dioxane. Yield 0.560 g (69%), white crystals; mp 231°C; IR: $\nu = 3310, 1705, 1665, 1525\ cm^{-1}$; ^1H NMR (d_6-DMSO): δ = 10.20 (s, 1H, *NH*), 8.01–7.98 (m, 2H, aromatic), 7.89–7.87 (m, 2H, aromatic), 7.78–7.77 (m, 2H , aromatic), 7.34–7.32 (m, 1H, aromatic), 2.45 (s, 3H, *Ar*-CH_3), 2.41 (m, 2H, CH_2-CH_2-CH_2), 2.29 (m, 2H, CH_2-CH_2-CH_2), 1.84 (m, 2H, CH_2-CH_2-CH_2); ^{13}H NMR (d_6-DMSO): δ = 173.9, 170.1, 165.7 (2 × *CO* and *benzothiazole-C-2*), 151.7, 141.8, 134.8, 134.3, 127.8, 127.6, 127.4, 121.9, 121.5, 119.1 (aromatic), 35.4, 32.9, 20.2 (CH_2-CH_2-CH_2), 20.8 (*Ar*-CH_3); MS: m/z (%) = 354 (45) [M^+], 336 (63), 240

(100); HRMS calculated for $C_{19}H_{18}N_2O_3S$: 354.1038; found 354.1028; elemental analysis calculated for $C_{19}H_{18}N_2O_3S$: C 64.39, H 5.12, N 7.90; found C 64.62, H 5.16, N 7.75.

N-*Benzothiazole*-2-*yl-succinamic acid* (**6**)
Recrystallization from dioxane/ethanol/pentane. Yield 0.6 g (80 %), white crystals; mp 250°C; IR: ν = 3185, 1735, 1690, 1610, 1565 cm^{-1}; ^1H NMR (d_6-DMSO): δ = 12.26 (s, 1H, *COOH*), 7.95–7.93 (m, 1H, aromatic), 7.73–7.71 (m, 1H, aromatic), 7.42 (m, 1 H, aromatic), 7.31 (m, 1H, aromatic), 2.74 (t, J = 6 Hz, 2H, CH_2-CH_2), 2.59 (t, J = 6 Hz, 2H, CH_2-CH_2); ^{13}H NMR (d_6-DMSO): δ 173.3, 171.2 (2 × *CO*), 157.7, 148.5, 131.4, 125.9, 123.3, 121.5, 120.3 (aromatic), 30.2, 28.3 (CH_2-CH_2); MS: m/z (%) = 250 (9) [M^+], 232 (91), 177 (71), 150 (100); HRMS calculated for $C_{11}H_{10}N_2O_3S$: 250.0412; found 250.0406; elemental analysis calculated for $C_{11}H_{10}N_2O_3S$: C 52.79, H 4.03, N 11.19; found C 52.79, H 4.01, N 10.82.

N-*Benzothiazole*-2-*yl-glutaramic acid* (**8**)
Recrystallization from dioxane/ethanol. Yield 1.04 g (84 %), white crystals; mp 232°C; IR: ν = 2960, 1690, 1605, 1555 cm^{-1}; ^1H NMR (d_6-DMSO): δ 7.96–7.94 (m, 1H, aromatic), 7.73–7.71 (m, 1H, aromatic), 7.42 (m, 1H, aromatic), 7.29 (m, 1H, aromatic), 2.54 (m, 2H, CH_2-CH_2-CH_2), 2.29 (m, 2H, CH_2-CH_2-CH_2), 1.86 (m, 2H, CH_2-CH_2-CH_2); ^{13}H NMR (d_6-DMSO): δ 173.8, 171.7 (2 × *CO*), 157.7, 148.5, 131.4, 125.9, 123.3, 121.5, 120.3 (aromatic), 34.2, 32.8, 19.8 (CH_2-CH_2-CH_2); MS: m/z (%) = 190 (65), 121 (100); elemental analysis calculated for $C_{12}H_{12}N_2O_3S$: C 54.53, H 4.58, N 10.60; found C 54.63, H 4.44, N 10.50.

N-*Benzothiazole*-2-*yl-glutaramic acid methyl ester* (**7**)
Compound **8** (0.925 g; 3.5 mmol) was dissolved in 50 mL methanol. After addition of 0.7 mL thionyl chloride the mixture was heated to reflux for 2 h. After concentration and cooling the product precipitated as white crystals. Yield 0.61 g (63 %), white crystals; mp 142°C; IR: ν = 3100, 3045, 1725, 1715, 1565 $cm^{-1;}$ ^1H NMR (d_6-DMSO): δ 7.96–7.93 (m, 1H, aromatic), 7.73–7.71 (m, 1H, aromatic), 7.42 (m, 1H , aromatic), 7.29 (m, 1H, aromatic), 3.59 (s, 3H, OCH_3), 2.55 (m, 2H, CH_2-CH_2-CH_2), 2.38 (m, 2 H, CH_2-CH_2-CH_2), 1.89 (m, 2H, CH_2-CH_2-CH_2); ^{13}H NMR (d_6-DMSO): δ = 172.7, 171.5 (2 × *CO*), 157.7, 148.4, 131.3, 125.8, 123.2, 121.4, 120.3 (aromatic), 51.1 (OCH_3), 34.0, 32.4, 19.7 (CH_2-CH_2-CH_2); MS: m/z (%) = 278 (47) [M^+], 150 (100); HRMS calculated for $C_{13}H_{14}N_2O_3S$: 278.0725; found 278.0729; elemental analysis calculated for $C_{13}H_{14}N_2O_3S$: C 56.10, H 5.07, N 10.06; found C 55.81, H 5.34, N 9.89.

Method 3. Preparation of *N*-acyl amino acids

To 1 mmol amino acid dissolved in 4 mL water, 3 mmol $KHCO_3$ was added and the mixture was cooled to 0°C. The appropriate acid chloride (1 mmol) in 4 mL acetone was added and the mixture was stirred for 1 h at 0°C and then for an additional 1 h at room temperature. After removal of the acetone on a rotary evaporator, the reaction mixture was cooled and acidified to pH 1 with concentrated hydrochloric acid. The resulting solid was collected by suction and washed with water. The dried product was purified by recrystallization.

N-2-*Naphthylacetyl-β-alanine* (**9**)
Recrystallization from toluene/dioxane. Yield 0.50 g (97 %), white crystals; mp 232°C; IR: ν = 3245, 3070, 1715, 1630, 1565 cm^{-1}; ^1H NMR (d_6-DMSO): δ 12.10 (s, 1H, *COOH*), 8.13 (s, 1H, *NH*), 7.87–7.82 (m, 3H, aromatic), 7.74 (s, 1H, aromatic), 7.51–7.40 (m, 3H, aromatic), 3.58 (s, 2H, *Ar-CH_2-CO*), 3.28 (q, J = 7 Hz, 2H, *β-ala-β-H*), 2.40 (q, J = 7 Hz, 2H, *β-ala-α-H*); ^{13}H NMR (d_6-DMSO): δ 172.7, 170.0 (2 × *CO*), 134.0, 132.9, 131.7, 127.4, 127.3, 127.2, 125.9, 125.3 (aromatic), 42.3 (*β-ala-β-C*), 34.9 (*β-ala-α-C*) 33.8 (*Ar-CH_2-CO*); MS: m/z (%) = 257 (7) [M^+], 141 (33), 59 (100); elemental analysis calculated for $C_{15}H_{15}NO_3$: C 70.02, H 5.88, N 5.44; found C 69.95, H 5.82, N 5.40.

N-3-(2-*Phenyl-thiazole*-4-*yl*)-*acryloyl-glycine* (**12**)
Recrystallization from toluene/dioxane. Yield 0.33 g (57 %), white crystals; mp 169°C; IR: ν = 3320, 1745, 1735, 1660, 1625 cm^{-1}; ^1H-NMR (d_6-DMSO): δ 12.52 (s, 1H, *COOH*), 8.55 (s, 1H, *NH*), 7.89 (m, 3H, aromatic), 7.54–7.52 (m, 3H, aromatic), 7.47 (d, J = 15 Hz, 1H, vinylic), 7.00 (d, J = 15 Hz, 1H, vinylic), 3.90 (d, J = 6 Hz, 2H, *gly-α-H*); ^{13}H NMR (d_6-DMSO): δ 171.1, 167.5 (2 × *CO*), 165.3, 152.5, 132.7, 131.8, 130.5, 129.2, 126.3, 123.7, 121.9 (aromatic, vinylic), 40.8 (*gly-α-C*); MS: m/z (%) = 288 (30) [M^+], 215 (56), 214 (100), 187 (40); HRMS calculated for $C_{14}H_{12}N_2O_3S$: 288.0569; found 288.0569; elemental analysis calculated for $C_{14}H_{12}N_2O_3S$: C 58.32, H 4.20, N 9.72; found C 58.51, H 4.50, N 9.36.

N-3-(2-*Phenyl-thiazole*-4-*yl*)-*acryloyl-β-alanine* (**13**)
Recrystallization from toluene/dioxane. Yield 0.41 g (68 %), white crystals; mp 148°C; IR: ν = 3315, 2960, 1715, 1650, 1600 cm^{-1}; ^1H NMR (d_6-DMSO): δ 12.18 (s, 1H, *COOH*), 8.30 (t, *J* = 5 Hz, 1H, *NH*), 7.98–7.95

(m, 3H, aromatic), 7.56–7.52 (m, 3H, aromatic), 7.44 (d, J = 15 Hz, 1H, vinylic), 6.95 (d, J = 15 Hz, vinylic), 3.39 (q, J = 7 Hz, 2H, *β-ala-β-H*), 2.50 (q, J = 7 Hz, 2H, *β-ala-α-H*); ^{13}H NMR (d_6-DMSO): δ 172.6, 167.4 (2 × *CO*), 165.9, 152.5, 132.6, 131.1, 130.4, 129.1, 126.2, 124.1, 121.6 (aromatic, vinylic), 34.9, 33.7 (*ala-C*); MS: m/z (%) = 302 (52) [M^+], 215 (56), 214 (100), 187 (57); HRMS calculated for $C_{15}H_{14}N_2O_3S$: 302.0725; found 302.0711; elemental analysis calculated for $C_{15}H_{14}N_2O_3S$: C 59.59, H 4.67, N 9.27; found C 59.16, H 5.03, N 9.22.

N-3-(2-*Phenyl-thiazol*-4-*yl*)-*acryloyl-alanine* (**14**)
Recrystallization from toluene/dioxane. Yield 0.55 g (91 %), white crystals; mp 159°C; IR: ν = 3300, 3095, 1715, 1665, 1630 cm^{-1}; ^{1}H NMR (d_6-DMSO): δ 12.45 (s, 1H, *COOH*), 8.52 (d, J = 7 Hz, 1H, *NH*), 8.01–7.96 (m, 3H, aromatic), 7.53 (m, 3H, aromatic), 7.46 (d, J = 15 Hz, 1H, vinylic), 7.01 (d, J = 15 Hz, vinylic), 4.35 (m, 1H, *ala-α-H*), 2.50 (d, J = 8 Hz, 3H, *ala-β-H*); ^{13}H NMR (d_6-DMSO): δ 173.9, 167.4 (2 × *CO*), 164.6, 152.5, 132.6, 130.6, 129.2, 126.3, 123.8, 121.8 (aromatic, vinylic), 47.6 (*ala-α-C*), 17.1 (*ala-β-C*); MS: m/z (%) = 302 (38) [M^+], 215 (83), 214 (100), 187 (80); HRMS calculated for $C_{15}H_{14}N_2O_3S$: 302.0725; found 302.0714; elemental analysis calculated for $C_{15}H_{14}N_2O_3S$: C 59.59, H 4.67, N 9.27; found C 59.58, H 4.52, N 9.53.

N-3-[5-(2-*Nitrophenyl*)-*furan*-2-*yl*]-*acryloyl-glycine* (**16**)
Recrystallization from toluene/dioxane. Yield 0.20 g (42 %), yellow crystals; mp 202°C; IR: ν = 3390, 3085, 2940, 1725, 1655, 1625, 1530, 1240 cm^{-1}; ^{1}H NMR (d_6-DMSO): δ = 12.53 (s, 1H, *COOH*), 8.57 (t, J = 7 Hz, 1H, *NH*), 7.91 (m, 2H, aromatic), 7.75 (m, 1H, aromatic), 7.61 (m, 1H, aromatic), 7.26 (d, J = 16 Hz, 1H, vinylic), 7.04 (d, J = 3 Hz, 1H, furylic), 6.94 (d, J = 3 Hz, 1H, furylic), 6.46 (d, J = 16 Hz, 1H, vinylic), 3.87 (d, J = 6 Hz, 2H, *gly-α-H*). ^{13}H NMR (d_6-DMSO): δ 171.1, 164.7 (2 × *CO*), 152.0, 149.1, 146.9, 132.3, 129.6, 128.8, 125.8, 123.9, 122.0 (aromatic, vinylic), 40.8 (*gly-α-C*). MS (EI): m/z (%) = 316 (48) [M^+], 242 (41), 170 (83), 139 (82), 110 (100); HRMS calculated for $C_{15}H_{12}N_2O_6$: 316.0695; found 316.0697; elemental analysis calculated for $C_{15}H_{12}N_2O_6$: C 56.97, H 3.82, N 8.86; found C 56.75, H 3.83, N 8.55.

(±)-N-3-[5-(2-*Nitrophenyl*)-*furan*-2-*yl*]-*acryloyl-phenylalanine* (**17**)
Recrystallization from toluene/dioxane. Yield 0.37 g (61 %), yellow crystals; mp 180°C; IR: ν = 3385, 3065, 3030, 1715, 1655, 1625, 1530, 1210 cm^{-1}; ^{1}H NMR (d_6-DMSO): δ 12.70 (s, 1H, *COOH*), 8.59 (d, J = 8 Hz, 1H, *NH*), 7.91 (m, 2H, aromatic), 7.76 (m, 1H, aromatic), 7.62 (m, 1H, aromatic), 7.30–7.17 (m, 6H, aromatic, vinylic), 7.01 (d, J = 3 Hz, 1H, furylic), 6.92 (d, J = 3 Hz, 1H, furylic), 6.44 (d, J = 16 Hz, 1H, vinylic), 4.55 (d, J = 6 Hz, 1H, *phe-α-H*), 3.12 (m, 1H, *phe-β-H*), 2.92 (m, 1H, *phe-β-H*); ^{13}H NMR (d_6-DMSO): δ = 172.9, 164.4 (2 × *CO*), 152.1, 149.1, 146.9, 137.6, 132.5, 129.6, 128.9, 128.1, 126.3, 125.8, 124.0, 122.2, 119.9, 115.9, 112.3 (aromatic, vinylic), 53.7 (*phe-α-C*), 36.7 (*phe-β-C*); MS: m/z (%) = 406 (37) [M^+], 297 (44), 242 (100), 212 (54); HRMS calculated for $C_{22}H_{18}N_2O_6$: 406.1165; found 406.1171; elemental analysis calculated for $C_{22}H_{18}N_2O_6$: C 65.02, H 4.46, N 6.89; found C 65.18, H 4.55, N 6.82.

Method 4. Preparation of *N*-acyl amino acid methyl esters

The appropriate carboxylic acid was dissolved in dry dimethylformamide (DMF) in a flame-dried flask under an Ar atmosphere. After addition of 3 mmol *N*-methylmorpholine the solution was cooled to −15°C and 1.3 mmol isobutyl chloroformate was added. After 5 min, a solution containing 1 mmol of the amino acid ester hydrochloride and 1 mmol of *N*-methylmorpholine in dry DMF was added. The mixture was left to warm to room temperature overnight and then poured into brine (400–800 mL). The aqueous mixture was extracted with ethyl acetate (3 × 100 mL) and the combined organic extracts were washed successively with 0.67 M citric acid, sat. $NaHCO_3$ solution and NaCl solution and dried with $MgSO_4$. The residue obtained after filtration and removal of the solvent was purified as described below.

N-3-(2-*Naphthyl*)-*acryloyl-glycine methyl ester* (**10**)
Recrystallization from toluene. Yield 0.30 g (66 %), white crystals; mp 115°C; IR: ν = 3290, 3055, 1745, 1660, 1625 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.87–7.77 (m, 5H, aromatic), 7.62 (m, 1H, aromatic), 7.50–7.44 (m, 2H, aromatic, vinylic), 6.58 (d, J = 16 Hz, 1 H, vinylic), 6.36 (s, 1H, *NH*), 4.20 (d, J = 5 Hz, 2H, *gly-α-H*), 3.78 (s, 3H, OCH_3); ^{13}H NMR ($CDCl_3$): δ 170.6, 166.1 (2 × *CO*), 142.0, 134.1, 133.4, 132.1, 129.6, 128.6, 128.5, 127.8, 127.1, 126.7, 123.6, 120.0 (aromatic, vinylic), 52.5 (OCH_3), 401.6 (*gly-α-C*); MS: m/z (%) = 269 (52) [M^+], 181 (100), 152 (64); HRMS calculated for $C_{16}H_{15}NO_3$: 269.1052; found 269.1050; elemental analysis calculated

for $C_{16}H_{15}NO_3$: C 71.36, H 5.61, N 5.20; found C 70.99, H 5.61, N 5.24.

N-3-(2-*Phenyl-thiazole*-4-*yl*)-*acryloyl-glycine methyl ester* (**11**)
Recrystallization from toluene. Yield 0.20 g (57%), white crystals; mp 128°C; IR: ν = 3370, 3090, 1740, 1670, 1635 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 7.98–7.94 (m, 2H, aromatic), 7.59 (d, J = 15 Hz, 1 H, vinylic), 7.47–7.41 (m, 3H, aromatic), 7.35 (s, 1H, thiazol), 6.95 (d, J = 15 Hz, 1H, vinylic), 6.29 (s, 1H, *NH*), 4.19 (d, J = 5 Hz, 2H, *gly-α-H*), 3.78 (s, 3H, OCH_3); ^{13}H NMR ($CDCl_3$): δ 170.4, 168.6 (2 × *CO*), 166.1, 152.8, 133.3, 130.6, 129.1, 126.8, 122.5, 121.0 (aromatic, vinylic), 52.5 (OCH_3), 41.6 (*gly-α-C*); MS: m/z (%) = 302 (31) [M^+], 215 (29), 214 (100); HRMS calculated for $C_{15}H_{14}N_2O_3S$: 301.0725; found 302.0717; elemental analysis calculated for $C_{15}H_{14}N_2O_3S$: C 59.59, H 4.67, N 9.27; found C 59.22, H 4.30, N 9.58.

N-3-[5-(2-*Nitrophenyl*)-*furan*-2-*yl*]-*acryloyl-glycine methyl ester* (**15**)
Recrystallization from toluene. Yield 0.30 g (61%), yellow crystals; mp 134°C; IR: ν = 3355, 2950, 1740, 1675, 1635, 1530, 1210 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.73–7.67 (m, 2H, aromatic), 7.58 (m, 1H, aromatic), 7.43 (m, 1H, aromatic), 7.39 (d, J = 15 Hz, 1H, vinylic), 6.71 (d, J = 4 Hz, 1H, furylic), 6.62 (d, J = 4 Hz, 1H, furylic), 6.37 (d, J = 15 Hz, 1H, vinylic), 4.17 (d, J = 5 Hz, 2H, *gly-α-H*), 3.77 (s, 3H, OCH_3); ^{13}H NMR ($CDCl_3$): δ 170.4, 165.5 (2 × *CO*), 152.20, 149.7, 147.6, 131.9, 129.0, 128.9, 127.9, 124.0, 123.4, 118.6, 116.0, 112.0 (aromatic, vinylic), 52.5 (OCH_3), 41.6 (*gly-α-C*); MS: m/z (%) = 330 (63) [M^+], 242 (94), 170 (84), 110 (100); HRMS calculated for $C_{16}H_{14}N_2O_6$: 330.0852, found 330.0834; elemental analysis calculated for $C_{16}H_{14}N_2O_6$: C 58.18, H 4.27, N 8.48; found C 58.15, H 4.31, N 8.25.

Biological methods

Enzyme purification
Recombinant rat lens aldose reductase was purified by a series of chromatographic procedures as previously described (Old et al 1990).

Enzyme assay
The aldose reductase inhibitory assay was conducted as previously described (Old et al 1990). Briefly, the decrease in the absorption of NADPH at 340 nm was followed over a 4-min period with DL-glyceraldehyde as substrate on a Shimadzu UV2100U spectrophotometer. Each 1-mL cuvette contained enzyme (0.0025 units), 0.1 M Na,K phosphate buffer, pH 6.2, 0.3 mM NADPH with or without 10 mM substrate and the inhibitor. One unit of enzyme activity was defined as the activity consuming 1 μM of NADPH per min. Compounds were dissolved in either weak base or DMSO (up to 5% (v/v) total solution). Appropriate controls containing enzyme solution with NADPH and inhibitor (with or without DMSO), but no substrate, were run to negate potential changes in the absorption of nucleotide at 340 nm not due to direct enzymatic reaction. Activity with inhibitor dissolved in DMSO was directly compared with activity with similar enzyme solution containing DMSO but no inhibitor. All measurements were conducted in triplicate and the activity reported reflects the average of three independent measurements. IC50 values were calculated by linear regression.

Results and Discussion

The amides of the dicarbonic acid methyl esters **1**, **2** and **5** were prepared by refluxing the aromatic amine with one equivalent of malonic acid methyl ester chloride and succinic acid methyl ester chloride, respectively (Method 1). The glutaric acid ester **7** was obtained from the corresponding free acid **8** by treatment with methanol/thionyl chloride. The succinic and glutaric acid amides **3**, **4**, **6** and **8** were prepared from the aromatic amines and the corresponding acid anhydrides by refluxing in THF (Method 2). For the preparation of the amino acid derivatives carrying a free carboxyl group (**9**, **12**, **13**, **15**, **16** and **17**) appropriate acid chlorides were reacted with the unprotected amino acids in water/acetone using potassium carbonate as a base (Method 3). The *N*-acyl amino acid methyl esters **10**, **11** and **15** were obtained from the acrylic acid derivatives activated as mixed anhydrides using isobutyl chloroformate and the appropriate amino acid methyl ester hydrochloride (Method 4) (Figure 3). Arylacrylic acids were prepared from the corresponding aldehydes via Knoevenagel condensation (Jones 1967).

The compounds prepared according to the proposed lead structure (Kador et al 1985; Lee et al 1994, 1998) and their biological activity are summarized in Figure 4 and Table 1, respectively. The compounds were assayed against rat lens aldose reductase as previously described (Old et al 1990). In general these compounds can be classified into three groups: one possessing an aniline-4-(2′-6′-methylbenzothiazole) or 2-aminobenzothiazole group as the aryl group; one possessing a 2-naphthyl

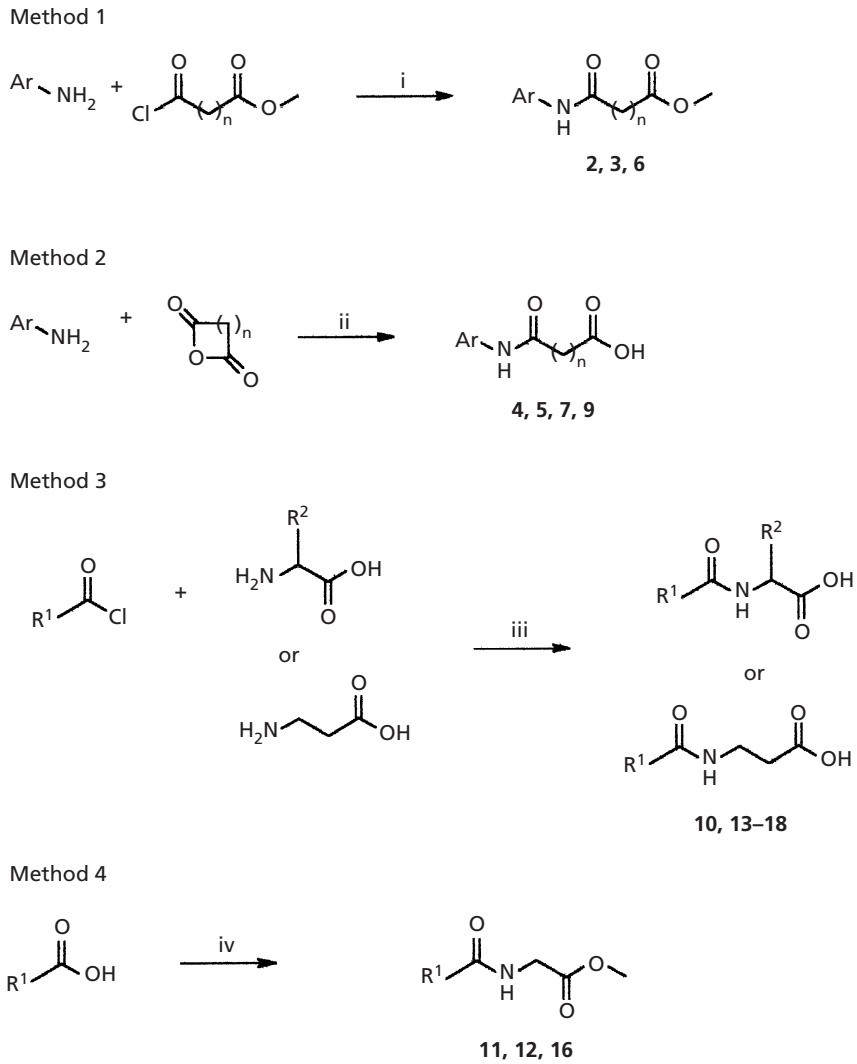

Figure 3 Summary of methods employed for the synthesis of new aldose reductase inhibitors **2**–**18**: (i) Tetrahydrofuran (THF), reflux, 2 h; (ii) THF, reflux, 2 h; (iii) K_2CO_3, water/acetone 0°C → rt, 2 h; (iv) (a) iso-butyl chloroformate, *N*-methylmorpholine, dimethylformamide (DMF), −15°C, 5 min., (b) glycine methyl ester hydrochloride, *N*-methylmorpholine, DMF, −15°C → rt, overnight.

as the aryl group; and one possessing either a 4-(2-phenylthiazole) or 2-(5–2′-nitrophenylfuran) as the aryl group. In these groups the carboxylate or its methyl ester is linked to the aryl group through various lengths of methylene carbons and amide or cinnamide groups.

The establishment of correct structure–activity relationships among the groups was hampered by insufficient solubility of at least one compound within each series of structurally related groups. Two of the compounds prepared (**4** and **16**) where active in the low micromolar range. Both have a chain length of five atoms between the aryl moiety and the carboxyl group. In the case of compound **4**, shortening the linker by one carbon (compound **3**) resulted in a 5-fold decrease in activity. However, in the case of compounds **6** and **8**, the shorter inhibitor having a 4-carbon linker was approximately twice as active as its homologue **8**. Nevertheless, this observation may not be significant since both compounds display only weak inhibitory activity. In addition to a 5-atom linker both active compounds (**4** and **16**) carry a free carboxyl group. In case of compound **16**, transformation of the carboxyl group in the corresponding methyl ester resulted in a reduction in activity by two orders of magnitude. The corresponding methyl ester of **4** was not prepared since the already one carbon shorter ester **2** was too insoluble to be assayed ap-

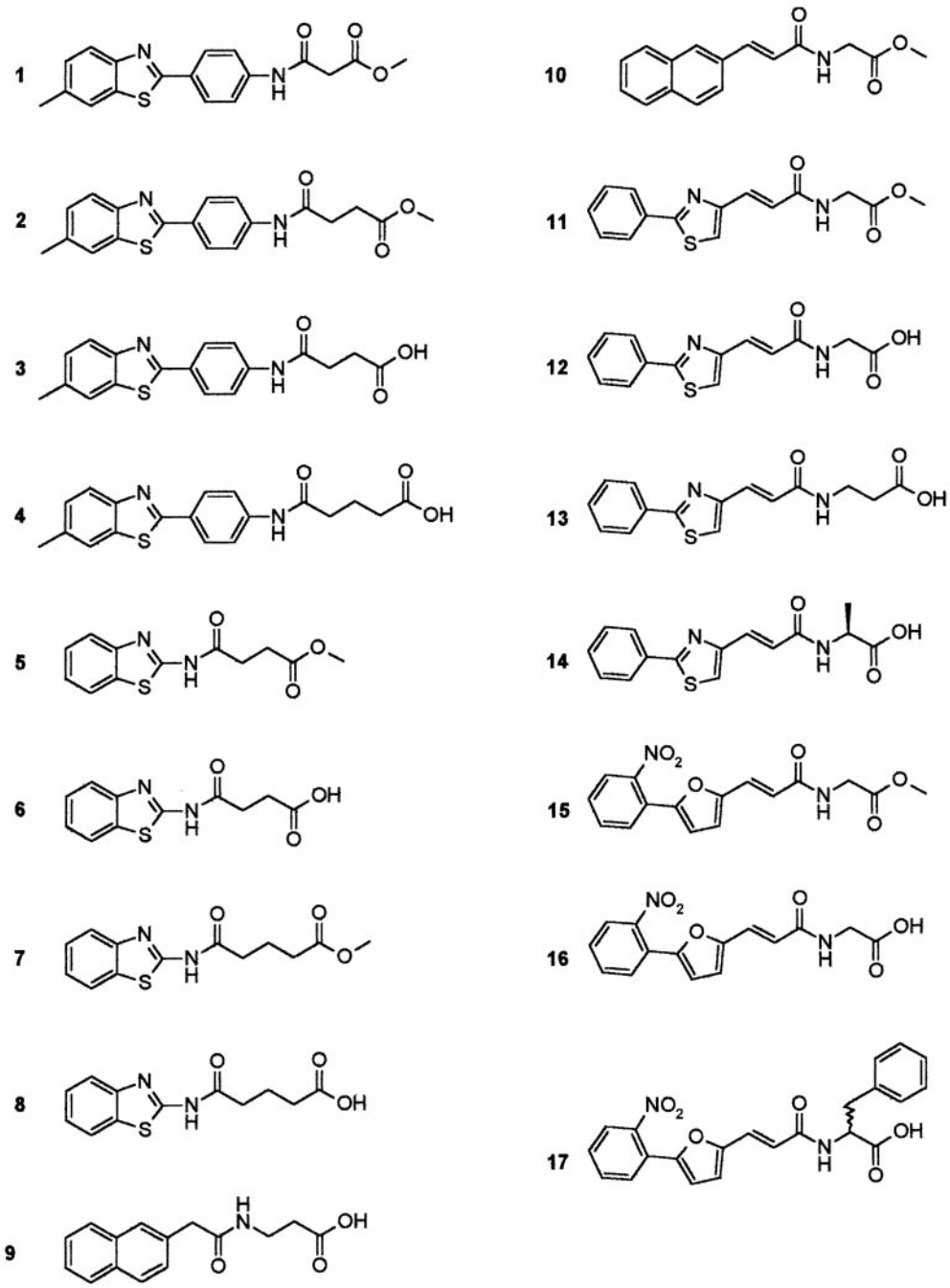

Figure 4 Structure of synthesized compounds.

propriately. In the cases of compounds **11** and **12**, there was no difference in activity between the free carboxyl derivative and its corresponding ester. Again, the apparent activity of these two compounds suggests that the interaction between the aryl and polar groups of these compounds and the enzyme are weak.

In this series, these observations suggest that an appropriate aryl moiety and a polar group, possibly a free carboxyl group, connected by a linker of five atoms seems to be the minimal structural requirement for aldose reductase inhibition while a double bond and an amide moiety are well tolerated in the linking structure.

Table 1 Inhibitory activity of compounds **2**–**18**

Compound	IC50 (μM)	Compound	IC50 (μM)
1	$> 10^a$	**11**	66
2	$> 10^a$	**12**	69
3	29	**13**	> 100
4	5.6	**14**	$> 10^a$
5	$> 10^a$	**15**	100
6	45	**16**	6.3
7	> 100	**17**	$> 10^a$
8	75	Ponalrestat[b]	0.02
9	> 100	Sorbinil[b]	0.07
10	$> 10^a$	Tolrestat[b]	0.01

[a]Solubility problems precluded assay at higher concentrations. [b]From Sato & Kador (1990)

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