Highly Selective Aldose Reductase Inhibitors. II. Optimization of the Aryl Part of 3-(Arylmethyl)-2,4,5-trioximidazolidine-1-acetic Acids

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Accumulation of intracellular sorbitol, the product of glucose reduction catalyzed by aldose reductase (AR) [EC 1.1.1.21], is thought to be the main culprit in the development of diabetic complications. A series of 3-arylalkyl-2,4,5-trioximidazolidine-1-acetic acids was prepared and tested for inhibitory activities towards AR and aldehyde reductase (ALR) [EC 1.1.1.2]. These derivatives showed strong inhibitory activity against AR without markedly inhibiting ALR. In particular, the compounds with 3-nitrophenyl, 4-chloro-3-nitrophenyl, and chloro-substituted benzothiazolyl groups as the aryl part showed powerful AR-inhibitory activity. The chloro-substituted benzothiazolyl compound showed an AR selectivity of more than 5000 fold.

Key words diabetes complication; aldose reductase inhibitor; aldose reductase; aldehyde reductase; selectivity; 2,4,5-trioximidazolidine-1-acetic acid

Recently, much attention has been paid to aldose reductase inhibitors (ARI) owing to their therapeutic potential for the amelioration of diabetic complications.1–4 In the course of our studies on 3-(arylalkyl)-2,4,5-trioximidazolidine-1-acetic acids, we found that some of the derivatives showed strong inhibitory activity against aldose reductase (AR) [EC 1.1.1.21] without markedly inhibiting aldehyde reductase (ALR) [EC 1.1.1.2].5 Though it is not clear how ARL works in diabetic patients, AR may be important in the reduction of many aldehydes and may have functions such as counteraction, excretion of drugs, synthesis of ascorbic acid, and metabolism of 4-hydroxybutyric acid.6) AR is present in mesangium cells or renal medulla in human kidney, where over 100 fold greater expression of ARL can be observed.7) This means that ARI in the kidney would be consumed by ARL before it can react with AR unless they have high selectivity for AR.8) To apply ARI for the treatment of diabetes complications clinically, long-term administration would be required.8 Thus, great case is needed to avoid adverse effects. Therefore, highly selective inhibition of AR seems to be a critical feature. Here, we describe a number of 3-(arylalkyl)-2,4,5-trioximidazolidine-1-acetic acids and their inhibitory activities against rat lens AR and rat kidney ARL. In particular, the compounds with 3-nitrophenyl (1a), 4-chloro-3-nitrophenyl (I), and chloro-substituted benzothiazolyl groups (4g and 4h) as the aryl part showed powerful AR-inhibitory activity. The chloro-substituted benzothiazolyl group 4h has an AR selectivity of more than 5000 fold.

Chemistry

All of the parabanic acid derivatives 1–4 described in this paper were synthesized in two steps. Thus, most of the esters 7 and 8 were obtained by alkylation of 59 and 6 with arylmethyl bromides (method A, Chart 1) or by the Mitsunobu reaction of 5 and 6 with arylmethyl alcohols (method B, Chart 1). In the case of the 2-(chloromethyl)benzothiazoles (23c–i, Chart 5) and 3,5-dinitrobenzyl chloride, they were converted to the corresponding bromides or iodides by treatment with NaBr or NaI in N,N-dimethylformamide (DMF) and condensed with 5 (method C, Chart 1).

The ethyl esters 7 could be hydrolyzed with concentrated HCl and AcOH according to the reported method5) (method D, Chart 2). On the other hand, when substrates on the aryl rings were susceptible to acid, benzyl esters 8 were adopted instead of the ethyl ester and the benzyl group was removed by hydrogenation with 10% palladium on charcoal under a hydrogen atmosphere (method E, Chart 2). Ethyl and benzyl 2,4,5-trioximidazolidine-1-acetates (5 and 6) were prepared by the treatment of the ureidoacetates 12 and 13 with oxalyl chloride, respectively (Chart 3).

Substituted arylmethyl halides or alcohols which were not commercially available were prepared as follows. Benzyl bromides 15, naphthylmethyl bromides 17 and

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benzothiazoylmethyl bromides 19 were prepared by bromination of the corresponding toluenes 14, methyl-naphthalenes 16, and 2-methylbenzothiazoles 18 with bromine or N-bromosuccinimide (NBS) in the presence of benzoyl peroxide ((BuO)2)9 in CCl4, as shown in Chart 4. Each of the brominated intermediates thus obtained was used in the next step after purification by column chromatography on silica gel and/or recrystallization.

The substituted (2-chloromethyl)benzothiazoles 23e—i (except for 23e) were prepared by condensation of the 2-aminothiophenols 22 with orthoethyl chloroacetate in EtOH under reflux, according to the reported procedure (Chart 5).9,10 The 2-aminothiophenols 22 were prepared from 2-aminobenzothiazoles 20 by using the reported procedure.9,11 The 6-nitrobenzothiazole 23e was obtained by nitration of the benzothiazole 23a.

Results and Discussion

The test compounds were evaluated for in vitro inhibitory activity against rat lens AR12 and rat kidney ALR13 in a spectrometric assay with Dl-glyceraldehyde as the substrate and NADPH as the cofactor. In vitro activity against AR was expressed as % inhibition at 1.0 × 10⁻⁷ M concentration of the test compounds. In vitro activity against ALR was expressed as % inhibition at 1.0 × 10⁻⁴ M concentration of the test compounds.

Substituted Benzy1 Derivatives In agreement with the previous study,5 all substituted benzyl derivatives showed moderate to strong AR-inhibitory activity and weak ALR-inhibitory activity. Though the introduction of electron-withdrawing groups on the aryl unit seems to increase the inhibitory activity against AR, a strongly electron-withdrawing group such as the 3,5-dinitro group caused loss of AR-inhibitory activity (Table 1, compound 1m). The 4-chloro-3-nitrobenzyl group was the optimum substituent in in vitro (Table 1, compound 1f). Hydrophobicity and steric effects of the substituent seemed to have no significant relationship with AR- and ALR-inhibitory activities.

Substituted Pyridyl and Naphthyl Derivatives Introduction of the 1-naphthyl group as the aryl moiety resulted in moderate inhibitory activity against AR, whereas the 2-naphthyl compound showed strong inhibitory activity (Table 2). The electronic factor appeared to be similar benzyl and naphthyl derivatives. Thus, introduction of an electron-withdrawing group onto the naphthyl ring enhanced AR-inhibitory activity compared with the non-substituted compound (Table 2, 2a vs. 2b). The compounds with pyridine unit as the aryl part showed no AR-inhibitory activity at 1 × 10⁻⁷ M concentration (Table 2, compounds 3).

Substituted Benzothiazyl Derivatives A number of ARIs having the benzothiazole unit have been reported.9,14,15 Therefore, the benzothiazole unit was introduced as the aryl part (Table 3). Though the benzothiazole derivatives seemed to have relatively strong ALR-inhibitory activity compared with the other parabanic acid derivatives, the degree of ALR inhibition is still weak compared with other ARIs. As discussed above, introduction of electron-withdrawing groups resulted in enhancement of AR activity. The chlorine atom was found to be optimum in benzothiazole derivatives.

In order to avoid adverse effects of ARI therapy, AR selectivity is one of the most important indices.14,16–18 We examined the AR selectivity of the parabanic acid derivatives with strong AR-inhibitory activity. As shown in Table 4, all the parabanic acid derivatives showed weak inhibition of ALR. In particular, the ratio of IC₅₀(ALR)/IC₅₀(AR) of II was more than 6250. There are many reports to show that ARIs which have so far been found also inhibit the closely related enzyme ALR.8,16,17 Since
Table 1. Physical and Biological Data for 3-Benzyl-2,4,5-trioxoimidazolidine-1-acetic Acids (1)

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
\text{No.} & \text{X} & \text{mp (°C)} & \text{Formula}\text{a)} & \% \text{inhibition for AR at } 1 \times 10^{-7} (\text{m}) & \% \text{inhibition for ALR at } 1 \times 10^{-4} (\text{m}) \\
\hline
1a & 3-\text{NO}_2 & 192-194 & C_{13}H_{10}N_3O_3 & 57.9 & 37.3 \\
1b & 2-\text{CF}_3 & 217-219 & C_{13}H_{10}F_3N_3O_3 & 8.0 & 25.4 \\
1c & 3-\text{CF}_3 & 190-192.5 & C_{13}H_{10}F_3N_3O_3 & 12.3 & 38.8 \\
1d & 4-\text{CF}_3 & 190-192 & C_{13}H_{10}F_3N_3O_3 & 6.6 & 27.1 \\
1e & 3-\text{CN} & 181-181.5 & C_{13}H_{10}N_3O_5 & 8.3 & 25.0 \\
1f & 4-\text{CN} & 175-176 & C_{13}H_{10}N_3O_5 & 0.8 & 12.7 \\
1g & 3-\text{COOH} & 244.5-245 & C_{13}H_{10}N_3O_5 & 8.9 & 77.2 \\
1h & 3-\text{COOMe} & 89-90 & C_{14}H_{12}N_2O_3 & 6.5 & 46.5 \\
1i & 3-\text{F} & 208.5-209 & C_{12}H_{10}F_2N_2O_3 & 10.6 & 62.6 \\
1j & 2-\text{Br} & 221-223 & C_{12}H_{10}Br_2N_2O_3 & 16.3 & 55.8 \\
1k & 3-\text{Br} & 202-202.5 & C_{12}H_{12}BrN_2O_3 & 17.0 & 48.8 \\
1l & 3-\text{NO}_2-4-\text{Cl} & 234.5-236 & C_{12}H_{12}ClN_2O_3 & 66.3 & 39.5 \\
1m & 3,5-(\text{NO}_2)_2 & 182.5-183 & C_{12}H_{12}N_2O_5 & 8.4 & 67.7 \\
\hline
\end{array}
\]

\text{a)} \text{Elemental analyses were within } \pm 0.4\% \text{ of the calculated values.}

Table 2. Physical and Biological Data for 3-Arylmethyl-2,4,5-trioxoimidazolidine-1-acetic Acids (2 and 3)

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\begin{array}{|c|c|c|c|c|c|}
\hline
\text{No.} & \text{Ar} & \text{mp (°C)} & \text{Formula}\text{a)} & \% \text{inhibition for AR at } 1 \times 10^{-7} (\text{m}) & \% \text{inhibition for ALR at } 1 \times 10^{-4} (\text{m}) \\
\hline
2a & \begin{array}{c}
\text{C}_{16}H_{12}N_2O_3
\end{array} & 192.5-194 & C_{14}H_{12}N_2O_3 & 42.4 & 59.0 \\
2b & \begin{array}{c}
\text{C}_{16}H_{11}BrN_2O_3
\end{array} & 205-210 & C_{16}H_{11}BrN_2O_3 & 60.2 & 47.0 \\
2c & \begin{array}{c}
\text{C}_{16}H_{12}N_2O_3
\end{array} & 225-228 & C_{16}H_{12}N_2O_3 & 22.5 & 57.4 \\
3a & \begin{array}{c}
\text{C}_{11}H_8N_3O_5
\end{array} & 251-253 & C_{11}H_8N_3O_5 & 4.7 & 24.4 \\
3b & \begin{array}{c}
\text{C}_{11}H_8N_3O_5
\end{array} & 251-252 & C_{11}H_8N_3O_5 & 4.1 & 67.5 \\
3c & \begin{array}{c}
\text{C}_{11}H_8N_3O_5
\end{array} & 251-253 & C_{11}H_8N_3O_5 & 0.0 & 0.0 \\
\hline
\end{array}
\]

\text{a)} \text{Elemental analyses were within } \pm 0.4\% \text{ of the calculated values.}

the primary sequences of AR and ALR are quite similar,\textsuperscript{19} this lack of specific inhibition is not surprising. Presumably the inhibitor binding sites of the two enzymes are structurally similar.\textsuperscript{19} The parabanic acid-type ARI inhibitors are the first to distinguish the binding sites of these enzymes.

**Conclusion**
Since the aryl moiety of ARI plays an important role, we synthesized and screened a number of ARI having the
parabanic acid core unit. We discovered that this is an effective core unit for AR-selective and strong inhibitory activity. On the other hand, 3-nitrophenyl (1a), 4-chloro-3-nitrophenyl (II), and benzothiazolyl groups (4g and 4h) were suitable as thearyl part. In particular, 1a and II have sufficiently strong in vitro activity against AR and extremely weak ALR inhibition. Compound 1a has been selected for clinical trials.

**Experimental**

Melt points (mp) were measured on a Yanaco MP-21 melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (1H-NMR) spectra were determined in chloroform-d or dimethyl-sulfoxide-d6 on a Bruker AM-400 (400 MHz) or a Bruker ARX-500 (500 MHz) spectrometer. Chemical shifts are reported in δ value from internal tetramethylsilane. Infrared (IR) spectra were recorded with a Hitachi 260-30 or a Horiba FT-200 spectrophotometer. Mass spectra (MS) were taken on a Hitachi M-80B mass spectrometer. Elementar analyses (C, H, N) were carried out on a Perkin-Elmer 240C or a Yanaco CHN Corder MT-5 elemental analyser. Thin-layer chromatography (TLC) analyses and chromatographic separations were performed with Silica gel 60 F254 plates (Merck Art 5715) and Silica gel 60 (Merck Art 7734, 70-230 mesh), respectively.

**Preparation of Arylaryl Bromides**

2-Bromomethylbenzothiazole (19a): A solution of 2-methylbenzothiazole (18a, 10.0 ml, 78.6 mmol) and NBS (17.7 g, 99.4 mmol) in CC14 (500 ml) was refluxed in the presence of a catalytic amount of benzoyl peroxide (1.00 g) for 3 h. The mixture was cooled to room temperature, and filtered through a Celite pad to remove the precipitate. The filtrate was dried over Na2SO4, and concentrated, then the residue was by column chromatography on silica gel to afford the bromide 19a (5.4 g, 30%). 1H-NMR (CDCl3) δ: 8.1 (s, 2H, CH2), 7.42 (dd, J = 8.0, 7.5 Hz, 2H, Ar-H), 7.50 (dd, J = 8.0, 7.5 Hz, 2H, Ar-H), 7.88 (d, J = 8.0 Hz, 2H, Ar-H), 8.02 (d, J = 8.0 Hz, 2H, Ar-H).

**Preparation of Arylaryl Chlorides**

6-Methylbenzothiazole (21c): 2-Amino-6-methylbenzothiazole (20c, 10.9 g, 66.2 mmol) was dissolved in warm 85% H3PO4 (200 ml). The resulting homogeneous solution was cooled to −10 to −5 °C and a solution of NaNO2 (23.0 g, 0.33 mol) in H2O (130 ml) was slowly added below the surface with stirring while the temperature was maintained below −4 °C. Then cold (0 °C) 50% H2SO4 (75 ml) was added dropwise with stirring and the whole was allowed to warm to room temperature. After gas evolution had ceased, the solution was diluted with ice-cold water, neutralized with Na2CO3, and extracted several times with CHCl3. The combined extracts were dried over Na2SO4, and concentrated. The crude solid was purified by column chromatography on silica gel (EtOAc: hexane = 20:1) to give 21c (2.98 g, 30%). 1H-NMR (CDCl3) δ: 2.51 (s, 3H, CH3), 7.33 (dd, J = 8.3, 1.1 Hz, 1H, Ar-H), 7.75 (d, J = 1.1 Hz, 1H, Ar-H), 8.01 (d, J = 8.3 Hz, 1H, Ar-H), 8.90 (s, 1H, Ar-H). IR (KBr) cm⁻¹: 1693, 1547, 1473, 1441, 1342, 903, 833.

2-Chloromethyl-6-methylbenzothiazole (23c): A mixture of H2NNH2, H2O (15 ml) and 6-methylbenzothiazole (21c, 4.52 g, 30.3 mmol) was stirred at ambient temperature for 24 h. It was then concentrated and H2O was gradually added. The pH of the solution was adjusted to about 2 by addition of concentrated HCl. The precipitated yellow solid was collected by filtration and dried in vacuo. The crude aminothiophenol 22c was used in the subsequent step without further purification.

A solution of 22c and orthoethyl chlorocarboxate (5.0 ml, 26.2 mmol) in EtOH (35 ml) was stirred at 60 °C (bath temperature) for 2 h, then poured into H2O and the mixture extracted 3 times with ether. The ether extracts were washed with 2 x HCl, H2O saturated NaHCO3, and brine. The extract was dried over Na2SO4, then concentrated and the residue was purified by column chromatography on silica gel (EtOAc: hexane = 10:1) to give 2-chloromethyl-6-methylbenzothiazole (23c) (2.23 g, 37%), mp 84–85 °C (dec.). 1H-NMR (CDCl3) δ: 2.49 (s, 3H, CH3), 4.92 (s, 2H, CH2), 7.31 (d, J = 8.2 Hz, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.70 (d, J = 8.2 Hz, 1H, Ar-H). IR (KBr) cm⁻¹: 3007, 1514, 1425, 1310, 1136, 806, 735, 625.

2-Chloromethyl-6-nitrobenzothiazole (23e): A mixture of 2-chloromethylbenzothiazole (23a, 14.3 g, 78.8 mmol) and concentrated HCl2O (40 ml) was treated dropwise with HNO3 (70%, 40 ml) at 0 °C. The mixture stirred for 4 h, then poured into ice-H2O and the whole was extracted several times with CHCl3. The combined extracts were dried over Na2SO4 and concentrated. The residual solid was purified by column chromatography on silica gel (AcOEt: hexane = 10:1) to give the nitro compound 23e (10.7 g, 60%), mp 87–88 °C (dec.). 1H-NMR (DMSO-d6) δ: 3.32 (s, 2H, CH2), 8.22 (d, J = 9.0 Hz, 1H, Ar-H), 8.38 (dd, J = 9.0, 2.2 Hz, 1H, Ar-H), 9.22 (d, J = 2.2 Hz, 1H, Ar-H). IR (KBr) cm⁻¹: 1508
Preparation of Benzyl 2,4,5-Trioxoimidazolidine-1-acetate (6) Benzyl Ureidoacetate (13): A solution of glycine benzyl ester p-TosOH salt (80.0 g, 0.24 mol) and urea (72.0 g, 1.20 mol) in H₂O (100 ml) was refluxed with AcOH (2 ml) and concentrated HCl (2 ml) for 5 h. The reaction mixture was crystallized by allowing it to stand at ambient temperature. Recrystallization from EtOH gave the urea 13 as white crystals (32.0 g, 64%) of [H-NMR (DMSO-d₆): δ: 4.80 (d, J = 7.0 Hz, 2H, NCH₂CO₂H), 5.11 (s, 2H, OCH₂), 5.26 to 5.91 (d, J = 7.0 Hz, 2H, NH and HNCO₂H). The solid was recrystallized from EtOH (100 ml) to give the ester 11 (2.23 g, 30%), mp 150—151 °C. [H-NMR (DMSO-d₆): δ: 1.22 (t, J = 7.1 Hz, 3H, CH₃), 4.19 (q, J = 7.1 Hz, 2H, OCH₂CO₂H), 4.49 (s, 2H, NCH₂CO₂H), 5.33 (s, 2H, CH₂Ar), 7.80 (d, J = 8.4 Hz, 1H, Ar-7), 8.35 (s, 1H, Ar-8), 8.40 (d, J = 8.4 Hz, 1H, Ar-9). IR (KBr cm⁻¹): 1742 (C=O). Compounds 7-11 were obtained as described above.}

Ethyl 3-(3-Arylmethyl-2,4,5-trioxoimidazolidine-1-acetate (7-11) Method A. Ethyl 3-(3-nitrobenzyl)-2,4,5-trioxoimidazolidine-1-acetate (7a): A solution of the parabanic acid 5 (10.0 g, 50 mmol) in DMF (50 ml) was added to a suspension of NaH (60 wt % in oil, 2.00 g, 50 mmol) in DMF (50 ml) while the temperature was maintained below 0°C over a period of 30 min. The mixture was stirred for 1 h, then a solution of 3-nitrobenzyl bromide (10.5 g, 50 mmol) in DMF (50 ml) was slowly added at 0°C. The whole was stirred for a further 2 h at 0°C and poured into ice-H₂O containing concentrated HCl (1 ml) to give a solid, which was collected by filtration and washed with H₂O and hexane. Recrystallization from EtOH gave the desired compound 7a (12.5 g, 75%) as white crystals, mp 124.5—125.5 °C. [H-NMR δ: 1.21 (t, J = 7.2 Hz, 3H, CH₃), 4.17 (q, J = 7.2 Hz, 2H, OCH₂), 4.41 (s, 2H, NCH₂CO₂H), 4.91 (s, 2H, CH₂Ar), 6.74—6.26 (m, 4H, Ar-H), 7.40 (d, J = 8.5 Hz, 1H, Ar-1), 8.35 (s, 1H, Ar-8), 8.40 (d, J = 8.4 Hz, 1H, Ar-9). IR (KBr cm⁻¹): 1740 (C=O), 1720 (C=O).]

Method B. Ethyl 3-(3-Pyridylmethyl)-2,4,5-trioxoimidazolidine-1-acetate (10b): A solution of diethyl azodicarboxylate (DEAD) (6.0 ml, 38.1 mmol) in THF (20 ml) was added dropwise to a solution of 3-pyridinemethanol (2.7 ml, 27.8 mmol), the parabanic acid 5 (5.00 g, 25.0 mmol), and PPh₃ (10.00 g, 38.1 mmol) in THF (40 ml) with vigorous stirring at 0°C. The mixture was stirred for 4.5 h, then poured into H₂O and ethereal solution of Na₂CO₃. The ethereal layer was washed several times with water, dried over Na₂SO₄, and evaporated. The residue was purified by column chromatography on silica gel to give a solid, which was recrystallized from EtOH-H₂O to give the desired compound 10b (4.22 g, 58%), mp 112—113 °C. [H-NMR δ: 1.20 (t, J = 7.0 Hz, 3H, CH₃), 4.16 (q, J = 7.0 Hz, 2H, OCH₂), 4.39 (s, 2H, NCH₂CO₂H), 4.79 (s, 2H, CH₂Ar), 7.38 (dd, J = 8.0, 4.5 Hz, 1H, Ar-1), 7.75 (ddd, J = 8.0, 2.0, 1.5 Hz, 1H, Ar-5), 8.29 (d, J = 8.5 Hz, 1H, Ar-3), 8.57 (d, J = 2.0 Hz, 1H, Ar-9), IR (KBr cm⁻¹): 1730 (C=O).]

Method C. Ethyl 3-(3[2-Trifluoromethoxybenzothiazol-2-yl)methyl]-2,4,5-trioxoimidazolidine-1-acetate (11): A solution of the parabanic acid 5 (3.53 g, 17.6 mmol) in DMF (50 ml) was added dropwise to a suspension of NaH (60 wt % in oil, 0.89 g, 22.3 mmol) in DMF (10 ml) over a period of 30 min while the temperature was maintained below 0°C. The mixture was stirred at the same temperature for an additional h. A solution of 2-bromomethyl-5-trifluoromethyl-1H-pyrazole (6.20 g, 33.0 mmol) prepared by the treatment of the corresponding chloro compound 23H (3.78 g, 17.5 mmol) with NaBr (2.26 g, 22.0 mmol) in DMF (40 ml), was then added dropwise to the reaction mixture at 0°C. After having been stirred for 2 h, the mixture was poured into ice-cooled 2N HCl with vigorous stirring. The precipitate was collected by filtration and washed well with H₂O and hexane. The solid was crystallized from EtOH (100 ml) to give the ester 11 (2.23 g, 30%), mp 150—151 °C. [H-NMR (DMSO-d₆): δ: 1.22 (t, J = 7.1 Hz, 3H, CH₃), 4.19 (q, J = 7.1 Hz, 2H, OCH₂), 4.49 (s, 2H, NCH₂CO₂H), 5.33 (s, 2H, CH₂Ar), 7.80 (d, J = 8.4 Hz, 1H, Ar-7), 8.35 (s, 1H, Ar-8), 8.40 (d, J = 8.4 Hz, 1H, Ar-9). IR (KBr cm⁻¹): 1742 (C=O).]
Ethyl 3-(1-Bromonaphthalen-2-ylmethyl)-2,4,5-trioximidazoline-1-acetate (9b): (Method A, 32%), mp 184.5–185.5°C. 1H-NMR (DMSO-d6): 1.21 (t, J = 7.0 Hz, 3H, CH3), 4.17 (q, J = 7.0 Hz, 2H, OCH2), 4.44 (2H, NCH2COO), 5.04 (2H, CH2Ar), 7.56 (d, J = 8.5 Hz, 1H, Ar-7), 7.64 (dd, J = 7.0, 7.0 Hz, 1H, Ar-1), 7.72 (dd, d, J = 8.5, 7.0 Hz, 1H, Ar-2), 7.95–8.05 (m, 2H, CHAr), 8.25 (d, J = 8.5 Hz, 1H, Ar-3). IR(KBr) cm⁻¹: 1734 (C = O).

Ethyl 3-(1-Naphthylmethyl)-2,4,5-trioximidazoline-1-acetate (9c): (Method A, 64%), mp 172–175°C. 1H-NMR (DMSO-d6): 1.19 (t, J = 7.0 Hz, 3H, CH3), 4.15 (q, J = 7.0 Hz, 2H, OCH2), 4.43 (2H, NCH2COO), 5.22 (2H, CH2Ar), 7.45–7.64 (m, 4H, Ar), 7.91 (d, J = 8.6 Hz, 1H, Ar-7), 8.19 (d, J = 8.6 Hz, 1H, Ar-8), 8.23 (d, J = 8.5 Hz, 1H, Ar-3). IR(KBr) cm⁻¹: 1734 (C = O).

Ethyl 3-(4-Pyridylmethyl)-2,4,5-trioximidazoline-1-acetate (10a): (Method B, 74%), mp 122–123°C. 1H-NMR (DMSO-d6): 1.21 (t, J = 7.0 Hz, 3H, CH3), 4.17 (q, J = 7.0 Hz, 2H, OCH2), 4.55 (2H, NCH2COO), 4.88 (2H, CH2Ar), 7.32 (dd, J = 7.5, 4.5 Hz, 1H, Ar-7), 7.46 (d, J = 7.5 Hz, 1H, Ar-7), 7.81 (dd, J = 7.5, 7.5 Hz, 1H, Ar-8), 8.50 (d, J = 4.5 Hz, 1H, Ar-3). IR(KBr) cm⁻¹: 1734 (C = O).

Ethyl 3-(4-Pyridylmethyl)-2,4,5-trioximidazoline-1-acetate (10c): (Method B, 58%), mp 92–94°C. 1H-NMR (DMSO-d6): 1.21 (t, J = 7.0 Hz, 3H, CH3), 4.17 (q, J = 7.0 Hz, 2H, OCH2), 4.82 (2H, NCH2COO), 4.80 (2H, CH2Ar), 7.37 (d, J = 5.5, 5.5 Hz, 2H, Ar), 7.46 (d, J = 5.5, 5.5 Hz, 2H, Ar). IR(KBr) cm⁻¹: 1740 (C = O).

Ethyl 3-(2-Benzothiazolylmethyl)-2,4,5-trioximidazoline-1-acetate (11a): (Method A, 52%), mp 151–153°C. 1H-NMR (DMSO-d6): 1.21 (t, J = 7.0 Hz, 3H, CH3), 4.17 (q, J = 7.0 Hz, 2H, OCH2), 4.47 (2H, NCH2COO), 5.25 (2H, CH2Ar), 7.46 (ddd, J = 7.7, 7.7, 1.2 Hz, 1H, Ar-7), 7.52 (ddd, J = 7.7, 7.7, 1.2 Hz, 1H, Ar-7), 7.99 (dd, J = 7.7, 1.2 Hz, 1H, Ar-8). IR(KBr) cm⁻¹: 1734 (C = O).

Ethyl 3-[6-Methoxybenzothiazol-2-ylmethyl]-2,4,5-trioximidazoline-1-acetate (11b): (Method A, 55%), mp 150–152°C. 1H-NMR (DMSO-d6): 1.21 (t, J = 7.1 Hz, 3H, CH3), 3.83 (2H, OCH2), 4.17 (q, J = 7.1 Hz, 2H, OCH2), 4.64 (2H, NCH2COO), 5.18 (2H, CH2Ar), 7.11 (dd, J = 9.9, 2.2 Hz, 1H, Ar), 7.68 (d, J = 2.6 Hz, 1H, Ar), 7.86 (d, J = 9.0 Hz, 1H, Ar), 7.96 (d, J = 9.0 Hz, 1H, Ar). IR(KBr) cm⁻¹: 1734 (C = O).

Ethyl 3-[6-Methoxybenzothiazol-2-ylmethyl]-2,4,5-trioximidazoline-1-acetate (11c): (Method C, 31%), mp 155–156°C. 1H-NMR (DMSO-d6): 1.21 (t, J = 7.1 Hz, 3H, CH3), 2.44 (3H, CH3), 4.18 (q, J = 7.1 Hz, 2H, OCH2), 4.47 (2H, NCH2COO), 5.21 (2H, CH2Ar), 7.34 (d, J = 8.2 Hz, 1H, Ar), 7.86 (d, J = 8.2 Hz, 1H, Ar), 7.96 (d, J = 9.1 Hz, 1H, Ar). IR(KBr) cm⁻¹: 1734 (C = O).
3-[6-Methylbenzothiazol-2-ylmethyl]-2,4,5-trioxoimidazolidine-1-acetic acid (4e) (Method D, 83%). \( \text{H-NMR (DMSO-d}_{6} \): 2.44 (s, 3H, CH_{3}), 4.35 (2H, NHC\textsubscript{2}OH), 7.11 (dd, \( J = 7.8, 1.9 \) Hz, 1H, Ar), 7.67 (d, \( J = 2.4 \) Hz, 1H, Ar), 7.87 (d, \( J = 8.9 \) Hz, 1H, Ar). IR (KBr cm\(^{-1}\)): 3000 (OH), 1738 (C=O). MS m/z: 333 (M\(^{+}\)).

3-[6-Fluorobenzothiazol-2-ylmethyl]-2,4,5-trioxoimidazolidine-1-acetic acid (4b) (Method D, 75%). \( \text{H-NMR (DMSO-d}_{6} \): 4.34 (s, 2H, NHC\textsubscript{2}OH), 5.22 (s, 2H, CH\textsubscript{2}Ar), 7.34—7.41 (m, 1H, Ar), 7.94 (s, 1H, Ar), 8.13 (d, \( J = 8.9 \) Hz, 1H, Ar). IR (KBr cm\(^{-1}\)): 3000 (OH), 1724 (C=O). MS m/z: 337 (M\(^{+}\)).

3-[6-Fluorobenzothiazol-2-ylmethyl]-2,4,5-trioxoimidazolidine-1-acetic acid (4c) (Method D, 40%). \( \text{H-NMR (DMSO-d}_{6} \): 4.36 (2H, NHC\textsubscript{2}OH), 5.34 (s, 2H, CH\textsubscript{2}Ar), 7.15 (d, \( J = 9.9 \) Hz, 1H, Ar), 8.34 (dd, \( J = 9.0, 2.3 \) Hz, 1H, Ar), 9.21 (d, \( J = 2.3 \) Hz, 1H, Ar), 13.47 (brs, 1H, COOH). IR (KBr cm\(^{-1}\)): 1743 (C=O), 1738 (C=O), 1524 (NO\textsubscript{2} (506 NO\textsubscript{2}).

3-[6-Chlorobenzothiazol-2-ylmethyl]-2,4,5-trioxoimidazolidine-1-acetic acid (4f) (Method D, 34%). \( \text{H-NMR (DMSO-d}_{6} \): 4.36 (s, 2H, NHC\textsubscript{2}OH), 5.25 (s, 2H, CH\textsubscript{2}Ar), 7.56 (d, \( J = 8.7 \) Hz, 1H, Ar), 7.99 (s, 1H, Ar), 8.06 (m, 2H, CH\textsubscript{2}Ar), 7.32 (s, 1H, Ar), 8.77 (s, 1H, Ar). IR (KBr cm\(^{-1}\)): 3000 (OH), 1746 (C=O), 1724 (C=O). MS m/z: 355 (M\(^{+}\), 337 (C=O), 353 (C=O)).

3-[6-Chlorobenzothiazol-2-ylmethyl]-2,4,5-trioxoimidazolidine-1-acetic acid (4g) (Method D, 52%). \( \text{H-NMR (DMSO-d}_{6} \): 4.36 (2H, NHC\textsubscript{2}OH), 5.26 (s, 2H, CH\textsubscript{2}Ar), 7.52 (dd, \( J = 8.6, 2.0 \) Hz, 1H, Ar), 8.10 (d, \( J = 2.0 \) Hz, 1H, Ar), 8.16 (d, \( J = 8.6 \) Hz, 1H, Ar), 13.45 (bs, 1H, COOH). IR (KBr cm\(^{-1}\)): 3006 (OH), 1782 (C=O), 1741 (C=O). MS m/z: 355 (M\(^{+}\), 337 (C=O), 353 (C=O)).

3-[6-Chlorobenzothiazol-2-ylmethyl]-2,4,5-trioxoimidazolidine-1-acetic acid (4h) (Method D, 59%). \( \text{H-NMR (DMSO-d}_{6} \): 4.35 (2H, NHC\textsubscript{2}OH), 5.27 (s, 2H, CH\textsubscript{2}Ar), 7.47 (dd, \( J = 8.0 \) Hz, 1H, Ar), 7.63 (d, \( J = 8.0 \) Hz, 1H, Ar), 8.11 (d, \( J = 8.0 \) Hz, 1H, Ar), 13.41 (bs, 1H, COOH). IR (KBr cm\(^{-1}\)): 2900 (OH), 1732 (C=O), 1741 (C=O). MS m/z: 355 (M\(^{+}\), 337 (C=O), 353 (C=O)).

3-[5-Trifluoromethylbenzothiazol-2-ylmethyl]-2,4,5-trioxoimidazolidine-1-acetic acid (4i) (Method D, 77%). \( \text{H-NMR (DMSO-d}_{6} \): 4.36 (s, 2H, NHC\textsubscript{2}OH), 5.32 (s, 2H, CH\textsubscript{2}Ar), 7.80 (d, \( J = 8.4 \) Hz, 1H, Ar), 8.37 (s, 1H, Ar), 8.39 (d, \( J = 8.4 \) Hz, 1H, Ar), 13.47 (brs, 1H, COOH). IR (KBr cm\(^{-1}\)): 2926 (OH), 1741 (C=O). MS m/z: 387 (M\(^{+}\)).

**Purification of Enzymes**

The procedures employed for isolation of rat lens AR and rat kidney AR were reported in the previous publication.  

**Enzyme Assay**  
*In vivo* AR and AR inhibition assays were conducted according to the method reported elsewhere.

**References and Notes**


