# Highly Selective Aldose Reductase Inhibitors. 1. 3-(Arylalkyl)-2,4,5-trioxoimidazolidine-1-acetic Acids

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*Received November 14, 1995*[⊗]

A series of 3-(arylalkyl)-2,4,5-trioxoimidazolidine-1-acetic acids (**1**) was prepared and tested for aldose reductase (AR) and aldehyde reductase (ALR) inhibitory activities. These compounds showed strong inhibitory activity against AR without significant inhibitory activity for ALR. The ratio of $IC_{50}$(ALR)/$IC_{50}$(AR) was >1000 in some compouds. On the basis of pharmacological tests such as the recovery of reduced motor nerve conduction velocity and toxicological profile, 3-(3-nitrobenzyl)-2,4,5-trioxoimidazolidine-1-acetic acid (NZ-314) was selected as the candidate for clinical development.

## Introduction

Recently, much attention has been paid to the aldose reductase inhibitor (ARI) due to its therapeutic potential for reduction of diabetic complications such as neuropathy, nephropathy, retinopathy, keratopathy, angiopathy, and cataract.[1−4] Although the mechanisms through which hyperglycemia causes tissue damage are a matter of controversy, accumulating experimental evidences indicate that increased aldose reductase (AR) (EC 1.1.1.21) activity initiates biochemical changes which result in the onset of secondary diabetic complications. AR is an enzyme in the polyol pathway that utilizes NADPH to catalyze the reduction of aldose to the sugar alcohol alditol. Many of aldose reductase inhibitors have also been observed to inhibit aldehyde reductase (ALR) (EC 1.1.1.2).[5] This suggests that both AR and ALR possess certain structural similarities capable of binding these ARIs.[6] Though it is not clear how ALR works in diabetic patients, ALR would be one of the important enzymes for reduction of many aldehydes and would have functions such as counteraction, excretion of drugs, synthesis of ascorbic acid, and metabolism of 4-hydroxylactic acid.[7] AR can exist only in Mesangium cells or renal medullas in human kidney where over 100-fold expression of ALR can be observed.[8] This means that ARI in kidney would be consumed by ALR before they can react with AR unless they have different affinity.[4] To apply ARI for the treatment of diabetes complications clinically, long term administration of a drug is required.[9] For such reasons, we must pay a lot of attention to adverse effects, and the drug that inhibits AR selectively is truly expected. Therefore, the selective inhibition against AR seems to be important to avoid undesired adverse effects. In fact, various potential ARIs were developed (Figure 1), but many of them were far from ideal. Here, we describe a number of 2,4,5-trioxoimidazolidine-1-acetic acids and their inhibitory activities against rat lens AR and rat kidney ALR. In particular, the unique parabanic acid derivatives **3** were novel AR inhibitors having a desired effect in recovery of motor nerve conduction velocity without significant inhibitory activity against ALR (Figure 2).

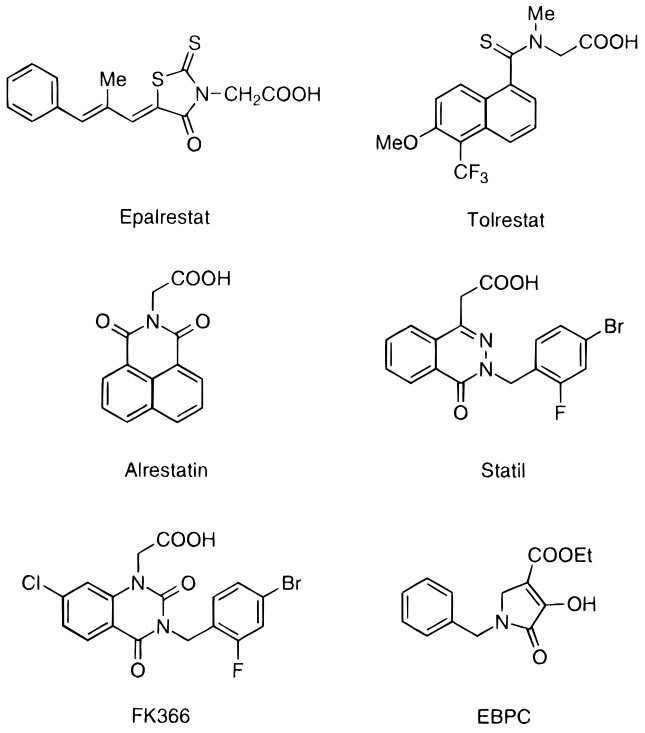
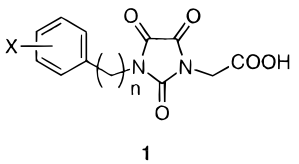



**Figure 1.**



1

**Figure 2.**

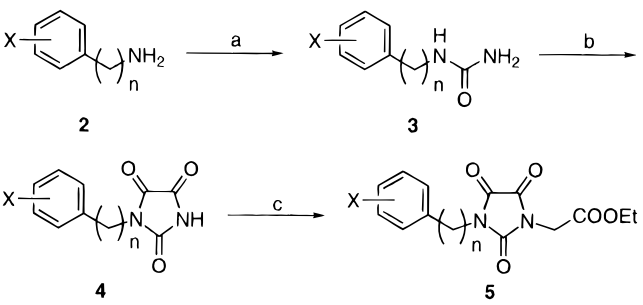
## Chemistry

All of the parabanic acid derivatives **1** in this paper were prepared according to method A or B. Method A was applicable to the synthesis of most 3-(arylmethyl)-2,4,5-trioxoimidazolidine-1-acetic acid derivatives **1**. Thus, condensation of the amines **2** with urea gave the alkylated ureas **3**, which were treated with oxalyl chloride to give 1-(arylmethyl)-2,4,5-imidazolidinetrione **4**. The acetates **5** obtained by the treatment of **4** with ethyl bromoacetate (**8**) were hydrolyzed in acidic condition to give the desired carboxylic acids **1** in good yields.

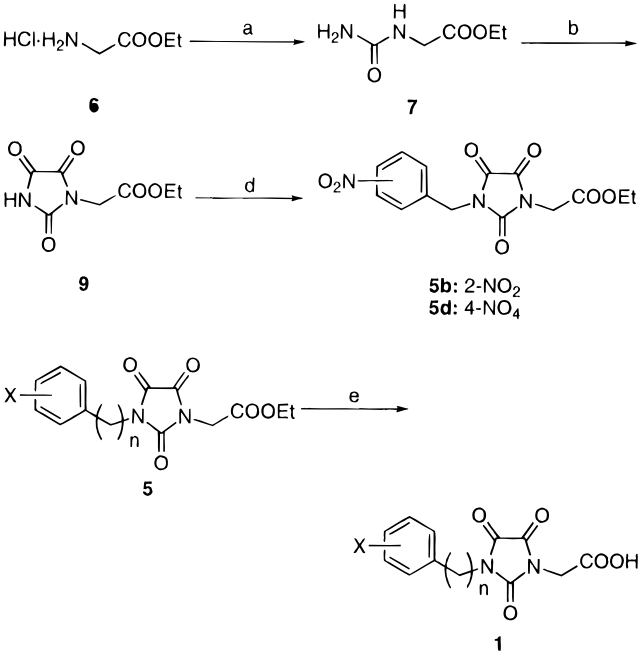
[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1996.

**Scheme 1**[a]

**Method A:**




**Method B:**




[a] Reagents: (a) urea, concentrated HCl, $H_2O$, reflux; (b) $(COCl)_2$, THF, 0 °C; (c) $BrCH_2CO_2Et$ (**8**), KOH−EtOH or $KHCO_3$−acetone; (d) 2- or 4-nitrobenzyl bromide, NaH, DMF; (e) concentrated HCl, AcOH, reflux.

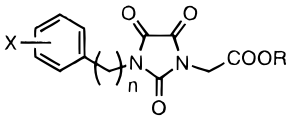
In the case of 2- and 4-nitrobenzyl derivatives **1b**,**d**, method B was adopted. Condensation of ethyl ureidoacetate (**7**) with oxalyl chloride gave ethyl 2,4,5-trioxoimidazolidine-1-acetate (**9**), which was then treated with 2- or 4-nitrobenzyl bromide to give the ester **5b** or **5d**, respectively. The acetates **5** were treated in the same manner as described above to give the acids **1**.

## Results and Discussion

Inhibitory activities of the parabanic acids **1** against rat lens AR[10] and rat kidney ALR[11] were evaluated in a spectrometric assay with DL-glyceraldehyde as the substrate and NADPH as the cofactor. *In vitro* AR inhibitory activity was expressed as the inhibition of the test compounds at $5.0 \times 10^{-7}$ M concentration. ALR inhibitory activity was also measured for the compounds with strong AR inhibitory activity.

Structure−activity relationship studies of the parabanic acid derivatives have suggested that acetic acid functionality is critical to the AR inhibitory activity (**5c** vs **1c**), possibly participating in a key interaction with

**Table 1.** Physical Data for Substituted Parabanic Acids **1**




| compd | X | *n* | R | method[a] | mp (°C) | formula[b] |
|---|---|---|---|---|---|---|
| **1a** | H | 1 | H | A | 207.5−209.5 | $C_{12}H_{10}N_2O_5$ |
| **5c** | 3-$NO_2$ | 1 | Et | A | 124.5−125.5 | $C_{14}H_{13}N_3O_7$ |
| **1b** | 2-$NO_2$ | 1 | H | B | 197−197.6 | $C_{12}H_9N_3O_7$ |
| **1c** | 3-$NO_2$ | 1 | H | A | 192−194 | $C_{12}H_9N_3O_7$ |
| **1d** | 4-$NO_2$ | 1 | H | B | 180−181 | $C_{12}H_9N_3O_7$ |
| **1e** | 2-Cl | 1 | H | A | 227−228 | $C_{12}H_9ClN_2O_5$ |
| **1f** | 3-Cl | 1 | H | A | 207−208 | $C_{12}H_9ClN_2O_5$ |
| **1g** | 4-Cl | 1 | H | A | 185−186 | $C_{12}H_9ClN_2O_5$ |
| **1h** | 3,4-$Cl_2$ | 1 | H | A | 196.5−197.5 | $C_{12}H_8Cl_2N_2O_5$ |
| **1i** | 2,4-$Cl_2$ | 1 | H | A | 230.5−232 | $C_{12}H_8Cl_2N_2O_5$ |
| **1j** | 2-MeO | 1 | H | A | 185−186 | $C_{13}H_{12}N_2O_6$ |
| **1k** | 3-MeO | 1 | H | A | 189−190 | $C_{13}H_{12}N_2O_6$ |
| **1l** | 3,4-$(MeO)_2$ | 1 | H | A | 163−164 | $C_{14}H_{14}N_2O_7$ |
| **1m** | 2-Me | 1 | H | A | 198−199 | $C_{13}H_{12}N_2O_5$ |
| **1n** | 4-Me | 1 | H | A | 192−193.5 | $C_{13}H_{12}N_2O_5$ |
| **1o** | 4-Br | 1 | H | A | 200.5−201.5 | $C_{12}H_9BrN_2O_5$ |
| **1p** | 4-F | 1 | H | A | 173−174 | $C_{12}H_9FN_2O_5$ |
| **1q** | H | 2 | H | A | 154.5−155.5 | $C_{13}H_{12}N_2O_5$ |
| **1r** | H | 3 | H | A | 145.5−146.5 | $C_{14}H_{14}N_2O_5$ |
| **1s** | H | 4 | H | A | 102−103 | $C_{15}H_{16}N_2O_5$ |
| **1t** | 2-Cl | 2 | H | A | 148−149 | $C_{13}H_{11}ClN_2O_5$ |
| **1u** | 3,4-$(MeO)_2$ | 2 | H | A | 141−142 | $C_{15}H_{16}N_2O_7$ |
| **1v** | 4-Me | 2 | H | A | 158−159 | $C_{14}H_{14}N_2O_5$ |
| **1w** | 3,4-$Cl_2$ | 2 | H | A | 136−138 | $C_{13}H_{10}Cl_2N_2O_5$ |
| **1x** | 3-$NO_2$ | 2 | H | A | 139−142 | $C_{13}H_{11}N_3O_7$ |
| **1y** | 4-Br | 2 | H | A | 172−173 | $C_{13}H_{11}BrN_2O_5$ |

[a] Synthetic method shown in Scheme 1. [b] Elemental analyses were within ±0.4% of the calculated values.

**Table 2.** Aldose Reductase Inhibitory Activity of **1** and **5**

| compd | % inhib for AR *in vitro*[a] at $5.0 \times 10^{-7}$ M | compd | % inhib for AR *in vitro*[a] at $5.0 \times 10^{-7}$ M |
|---|---|---|---|
| **1a** | 17 | **1m** | 41 |
| **5c** | 31 | **1n** | 30 |
| **1b** | 66 | **1o** | 65 |
| **1c** (NZ-314) | 75 | **1p** | 9 |
| **1d** | 26 | **1q** | 30 |
| **1e** | 32 | **1r** | 16 |
| **1f** | 31 | **1s** | 25 |
| **1g** | 56 | **1t** | 37 |
| **1h** | 77 | **1u** | 42 |
| **1i** | 68 | **1v** | 42 |
| **1j** | 16 | **1w** | 57 |
| **1k** | 44 | **1x** | 36 |
| **1l** | 29 | **1y** | 47 |

[a] Inhibition in a partially purified rat lens aldose reductase.

the binding site of AR enzyme.[12] Additionally, it was revealed that increase in the number of methylene groups between the aryl part and the parabanic acid unit resulted in a significant loss of AR inhibitory activity. Introduction of electron-withdrawing groups onto the aromatic moiety enhanced the inhibitory activity against AR. Because of the sequence homology[13−15] and the nonselectivity of inhibition for both AR and ALR,[4,16−19] it has been suggested that the inhibitor binding sites of both enzymes are structurally similar.[18] Therefore, compounds **1b**,**c**,**h**,**i** were assayed for their ALR inhibitory abilities. Surprisingly, all of these compounds showed extremely weak inhibitory activity against ALR as shown in Table 3. For example, the ratio of $IC_{50}$(ALR)/$IC_{50}$(AR) of compound **1c** was >1600,[20] whereas that of epalrestat was 71. Since the ratio of $IC_{50}$(ALR)/$IC_{50}$(AR) of EBPC was reported to be >4000[21] using rat lens AR and guinea pig kidney ALR, the

**Table 3.** Ratio of AR $IC_{50}$ and ALR $IC_{50}$ of Compounds **1**

| compd | $IC_{50}$ (M) AR[a] | $IC_{50}$ (M) ALR[b] | $IC_{50}$(ALR)/$IC_{50}$(AR) |
|---|---|---|---|
| **1c** | $1.3 \times 10^{-7}$ | $1.0 \times 10^{-4}$ | 769 |
| **1c** | $6.2 \times 10^{-8}$ | $>1.0 \times 10^{-4}$ | >1613 |
| **1h** | $5.0 \times 10^{-8}$ | $>1.0 \times 10^{-4}$ | >2000 |
| **1i** | $1.0 \times 10^{-7}$ | $4.6 \times 10^{-5}$ | 460 |
| epalrestat | $2.1 \times 10^{-8}$ | $1.5 \times 10^{-6}$ | 71 |
| EBPC | $1.1 \times 10^{-7}$ | $3.2 \times 10^{-5}$ | 291 |

[a] $IC_{50}$ value for rat lens AR. [b] $IC_{50}$ value for rat kidney ALR.

selectivity of EBPC was tested in the same conditions as that for compounds described above. As summarized in Table 3, the ratio of EBPC in enzyme selectivity was 291. From these results, it was found that the parabanic acid moiety was the essential unit for AR selective inhibitory activity.

## Conclusion

It was found that the parabanic acid moiety was essential for AR selectivity and strong AR inhibitory activity. In particular, **1c** (NZ-314) showed strong inhibitory activity against AR with extremely weak ALR inhibition *in vitro*. These compounds are the first examples of ARIs that have high AR selectivity. Studies on the effect of the aryl part for enzyme selectivity are in progress.

## Experimental Section

Melting points (mp) were measured by a Yamato MP-21 melting point apparatus and are uncorrected. Proton nuclear magnetic resonance ($^1$H NMR) spectra were determined in dimethyl sulfoxide-$d_6$ on a Bruker AM-400 (400 MHz) spectrometer. Chemical shifts are reported in $\delta$ value from internal tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; br s, broad singlet; and m, multiplet. Coupling constants are reported in hertz (Hz). Infrared (IR) spectra were recorded with a Hitachi 260-30 instrument. Mass spectra (MS) were taken on a Hitachi M-80B mass spectrometer. Elemental analyses (C, H, N) were carried out on a Perkin-Elmer 240C element analyzer. Thin-layer chromatography (TLC) analyses and chromatographic separations were performed with silica gel 60 $F_{254}$ plates (Merck Art. 5715) and silica gel 60 (Merck Art. 7734; 70−230 mesh), respectively. Visualization was accomplished with UV light and/or 10% phosphomolybdic acid in ethanol. Unless otherwise noted, all commercially available materials were used without further purification.

**General Procedure for the Preparation of Ethyl 3-(Arylalkyl)-2,4,5-trioxoimidazolidine-1-acetates 5 (Scheme 1). Method A: 1-(3-Nitrobenzyl)urea (3c, *n* = 1, X = 3-$NO_2$).** To a solution of 3-nitrobenzylamine hydrochloride (**2c**, $n = 1$, X = 3-$NO_2$; 47.2 g, 0.25 mol) and urea (60.0 g, 1.0 mol) in $H_2O$ (100 mL) was added concentrated HCl (2 mL, 32 mmol), and the mixture was refluxed for 3 h. The reaction mixture was crystallized by standing at room temperature. The precipitate was filtered and rinsed well with $H_2O$. Recrystallization from EtOH gave **3c** as white crystals (47.2 g, 97%): mp 186−187 °C; $^1$H NMR $\delta$ 4.30 (d, $J = 6.2$ Hz, 2H, $CH_2$), 5.66 (s, 2H, $NH_2$), 6.22 (t, $J = 6.2$ Hz, 1H, NH), 7.62 (dd, $J = 8.8$, 7.6 Hz, 1H, Ar-*H*), 7.71 (d, $J = 7.6$ Hz, 1H, Ar-*H*), 8.09 (d, $J = 8.8$ Hz, 1H, Ar-*H*), 8.10 (s, 1H, Ar-*H*); IR (KBr, $cm^{-1}$) 3469 ($NH_2$), 3319 ($NH_2$), 1652 (C=O), 1585 ($NO_2$), 1346 ($NO_2$).

**1-(3-Nitrobenzyl)imidazolidine-2,4,5-trione (4c, *n* = 1, X = 3-$NO_2$).** To a suspension of 1-(3-nitrobenzyl)urea (**3c**, $n = 1$, X = 3-$NO_2$; 19.5 g, 100 mmol) in THF (300 mL) was added oxalyl chloride (10.2 mL, 120 mmol) dropwise at 0 °C. The mixture was warmed to room temperature and stirred vigorously for 3 h. After removal of the precipitate by filtration, the filtrate was concentrated. The residual solid was dissolved in EtOAc and washed with $H_2O$ and brine. The organic layer was dried over anhydrous $Na_2SO_4$ and passed through a short silica gel pad. Evaporation of the solvent followed by recrystallization from hexane−EtOAc gave **4c** as yellow crystals (20.3 g, 82%): mp 161−163 °C; $^1$H NMR $\delta$ 4.78 (s, 2H, $CH_2$), 7.62−8.26 (m, 4H, Ar-*H*), 12.08 (s, 1H, NH); IR (KBr, $cm^{-1}$) 1738 (C=O), 1527 ($NO_2$), 1336 ($NO_2$); MS *m*/*z* 249 ($M^+$). Anal. ($C_{10}H_7N_3O_5$) C, H, N.

**Ethyl 3-(3-Nitrobenzyl)-2,4,5-trioxoimidazolidine-1-acetate (5c).** The parabanic acid **4c** ($n = 1$, X = $NO_2$; 24.9 g, 100 mmol) and ethyl bromoacetate (**8**; 17 mL, 154 mmol) were added to a solution of KOH (8.0 g, 120 mmol) in EtOH (600 mL). After being refluxed for 8 h, the mixture was allowed to cool to 0 °C and filtered. The solid was dissolved in EtOAc and washed with $H_2O$ and brine. The organic phase was dried over anhydrous $Na_2SO_4$ and passed through a short silica gel pad. After being condensed, the crude solid was recrystallized from hexane−EtOAc to give **5c** as yellow crystals (17.6 g, 53%): $^1$H NMR $\delta$ 1.21 (t, $J = 7.2$ Hz, 3H, $CH_3$), 4.17 (q, $J = 7.2$ Hz, 2H, $OCH_2$), 4.41 (s, 2H, $NCH_2CO_2$), 4.91 (s, 2H, $CH_2$-Ar), 7.64−8.26 (m, 4H, Ar-*H*); IR (KBr, $cm^{-1}$) 1740 (C=O), 1720 (C=O), 1525 ($NO_2$), 1350 ($NO_2$); MS *m*/*z* 335 ($M^+$).

**Method B: Ethyl Ureidoacetate (7).** A solution of glycine ethyl ester hydrochloride (**6**; 1000 g, 7.16 mol) and urea (750 g, 12.5 mol) in water (600 mL) was refluxed for 5 h. The reaction mixture was left standing at ambient temperature. The solid was filtered and rinsed well with $H_2O$. Recrystallization from $H_2O$ (600 mL) gave the urea **7** as white crystals (734 g, 70%): mp 139−140 °C; $^1$H NMR $\delta$ 1.19 (t, $J = 7.0$ Hz, 3H, $CH_3$), 3.73 (d, $J = 6.0$ Hz, 2H, $NCH_2CO_2$), 4.08 (q, $J = 7.0$ Hz, 2H, $OCH_2$), 5.67 (s, 2H, $NH_2$), 6.25 (t, $J = 6.0$ Hz, 1H, NH); IR (KBr, $cm^{-1}$) 3475 ($NH_2$), 3375 ($NH_2$), 1730 (C=O), 1645 (C=O).

**Ethyl 2,4,5-Trioxoimidazolidine-1-acetate (9).** To a suspension of the urea **7** (730 g, 5.0 mol) in THF (100 mL) was added oxalyl chloride (515 mL, 6.0 mol) dropwise at 0 °C. After vigorous stirring for 4 h at room temperature, the precipitate was collected by filtration. The filtrate was concentrated, and the residual solid was combined with the precipitate obtained above. The solid was dissolved in MeOH and filtered to remove the insoluble material. The filtrate was concentrated, and the residue was recrystallized from EtOAc to give **9** as white crystals (850 g, 85%): mp 154−156 °C; $^1$H NMR $\delta$ 1.21 (t, $J = 7.2$ Hz, 3H, $CH_3$), 4.16 (q, $J = 7.2$ Hz, 2H, $OCH_2$), 4.34 (s, 2H, $NCH_2CO_2$), 12.40 (s, 1H, NH); IR (KBr, $cm^{-1}$) 1740 (C=O).

**Ethyl 3-(2-Nitrobenzyl)-2,4,5-trioxoimidazolidine-1-acetate (5b).** A solution of the parabanic acid **9** (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) with the temperature maintained below 0 °C over a period of 30 min. After stirring for 1 h, a solution of 2-nitrobenzyl bromide (10.8 g, 50 mmol) in DMF (50 mL) was slowly added at 0 °C. The mixture was stirred for a further 2 h at 0 °C. The resulting mixture was poured into ice−$H_2O$ containing concentrated HCl (1 mL) to give a solid, which was filtered and washed well with $H_2O$ and hexane. Recrystallization from EtOH gave **5b** (12.5 g, 75%) as white crystals: mp 158.5−159.5 °C; $^1$H NMR $\delta$ 1.21 (t, $J = 7.1$ Hz, 3H, $CH_3$), 4.17 (q, $J = 7.0$ Hz, 2H, $OCH_2$), 4.43 (s, 2H, $NCH_2CO_2$), 5.11 (s, 2H, $CH_2$Ar), 7.61 (ddd, $J = 8.2$, 7.8, 1.3 Hz, 1H, Ar-*H*), 7.64 (dd, $J = 7.8$, 1.3 Hz, 1H, Ar-*H*), 7.74 (ddd, $J = 7.8$, 7.8, 1.2 Hz, 1H, Ar-*H*), 8.13 (dd, $J = 8.2$, 1.2 Hz, 1H, Ar-*H*); IR (KBr, $cm^{-1}$) 1738 (C=O), 1527 ($NO_2$), 1336 ($NO_2$); MS *m*/*z* 335 ($M^+$). Anal. ($C_{14}H_{13}N_3O_7$) C, H, N.

**Hydrolysis of Esters 5: 3-(3-Nitrobenzyl)-2,4,5-trioxoimidazolidine-1-acetic Acid (1c).** A mixture of ethyl 3-(3-nitrobenzyl)-2,4,5-trioxoimidazolidine-1-acetate (**5c**; 3.70 g, 11.0 mol), AcOH (10 mL), and concentrated HCl (5 mL) was refluxed for 2.5 h. The reaction mixture was condensed under reduced pressure to give a residue, which was refluxed again with AcOH (10 mL) and concentrated HCl (5 mL) for another 2 h. The solid obtained by condensation was dissolved in EtOAc, washed with $H_2O$, and extracted with 10% aqueous $Na_2CO_3$. The aqueous layer was washed with EtOAc and acidified with concentrated HCl. The precipitated solid was extracted with EtOAc, washed with $H_2O$ and brine, and dried over anhydrous $Na_2SO_4$. Concentration followed by

crystallization from $Et_2O$ gave a crude solid, which was recrystallized from hexane−EtOAc to give **1c** as yellow crystals (2.30 g, 68%): $^1$H NMR $\delta$ 4.30 (s, 2H, $NCH_2CO_2$), 4.91 (s, 2H, $CH_2Ar$), 7.65−8.25 (m, 4H, Ar-*H*), 13.42 (br s, 1H, COOH); IR (KBr, $cm^{-1}$) 1740 (C═O), 1720 (C═O), 1525 ($NO_2$), 1350 ($NO_2$); MS *m*/*z* 307 ($M^+$).

**Biological Methods: Enzyme Preparation.** Rat lens AR was prepared according to the procedure developed by Kador with some modifications.[10] Lens from male Wister rats were homogenized in 3 vol of 10 mM phosphate buffer (pH 7.0) containing 5 mM 2-mercaptoethanol and 1 mM EDTA at 0−4 °C with a tissue homogenizer set at a speed of 5−6 (Polytron, Kinematica Gmbh, CH-6010). The homogenate was centrifuged at 18000*g* for 20 min. Ammonium sulfate was added to the supernatant fraction to form a 30% saturated solution. This solution was stirred for 1 h, allowed to stand for 20 min, and then centrifuged at 18000*g* for 20 min. The recovered supernatant fraction was then sequentially fractionated with 80% saturated ammonium sulfate. The precipitate recovered from the 80% saturated ammonium sulfate solution was dissolved in the phosphate buffer described above and dialyzed for 24 h against 10 L of the same buffer changed twice. The dialyzed material was separated into 1.0-mL aliquots and stored at −40 °C.

Rat kidney ALR was prepared with the procedure developed by Takahashi with some modifications.[11] Kidneys from male Wister rats were homogenized with 10 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA in a homogenizer (Polytron, Kinematica Gmbh, CH-6010). The homogenate was centrifuged at 10000*g* for 20 min, and the supernatant (crude extract) was collected. The crude extract was subjected to fractionation with ammonium sulfate between 40% and 70% saturation. The precipitate was collected by centrifugation at 10000*g* for 10 min, dissolved in a minimal volume of 10 mM Tris-HCl (pH 7.4) containing 1 mM EDTA and 1 mM 2-mercaptoethanol, and dialyzed for 24 h against 10 L of the same buffer changed twice. The dialyzed material was applied to a column of DEAE-cellulose (DE-52; Whatman) equilibrated with the same Tris buffer. The column was developed with a linear gradient of 0 and 0.3 M NaCl in the same buffer. The major peak was separated from the minor peak and concentrated to 5 mL by Amicon ultrafiltration with a PM-10 membrane. This concentrated fraction was applied to a column of Sephadex G-100 equilibrated with a same Tris buffer, and the major peak was collected. This collected fraction was applied to a column of Blue Sepharose CL-6B, equilibrated with the same Tris buffer. The column was washed with the same buffer and then eluted with the Tris buffer containing 5 mM NADPH. The fractions with ALR activity were collected after dialysis of this elute and stored at −40 °C.

**Enzyme Assay.** Both reductases activities were assayed spectrophotometrically on a Hitachi U-3300 spectrophotometer by measuring the decrease in absorption of NADPH at 340 nm. AR activity was determined by the procedure developed by Kador[10] with some modifications and assayed in a reaction mixture containing 100 mM phosphate buffer (pH 6.3), 0.3 M ammonium sulfate, 1 mM EDTA, 0.2 mM NADPH, and 10 mM DL-glyceraldehyde in a final volume of 2.5 mL.

ALR activity was determined by the procedure developed by Takahashi[11] with some modifications and assayed in a reaction mixture containing 100 mM phosphate buffer (pH 6.0), 0.2 mM NADPH, and 8 mM *n*-octyl aldehyde in a final volume of 2.5 mL. The water-insoluble substrate such as *n*-octyl aldehyde was dispersed in 3-fold molar of Triton X-100. The test substrates were dissolved in propylene glycol.

One unit of activity is defined as the amount of enzyme that catalyzes the oxidation of 1 $\mu$mol of NADPH/min under the assay conditions.

**Supporting Information Available:** Melting point, $^1$H NMR, IR, and MS data for compounds **1** and **3**−**5** (14 pages). Ordering information is given on any current masthead page.

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JM9508393