[Chem. Pharm. Bull. 32(3)1032—1039(1984)]

Inhibition of Aldose Reductases from Rabbit Lens by Oxazole Derivatives

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(Received June 3, 1983)

Twenty kinds of oxazole derivatives having various substituents at the C-2 and C-5 positions were synthesized and tested *in vitro* for inhibition of rabbit lens aldose reductase (Ia and Ib), the enzyme that initiates cataract formation in diabetes. Compounds possessing bulky groups at C-2 and C-5 of the oxazole skeleton were found to be potent inhibitors. Benzyl 5-phenyl-2-oxazolecarbamate (**12**) inhibited aldose reductases Ia and Ib by 50% at about 15 μM. *N*-Phenyl-*N'*-(5-phenyl-2-oxazolyl)urea also exhibited inhibitory activity comparable to that of compound **12**. The structure–inhibitory activity relationships are discussed.

Keywords——aldose reductase; aldose reductase inhibitor; oxazole derivative; rabbit lens; structure–inhibitory activity relationship

Large amounts of sorbitol are known to accumulate in the lens,[1] peripheral nerves[2] and renal papillae[3] of diabetic animals and humans. Elevated sorbitol levels cause loss of osmotic integrity and subsequent cellular damage. Many investigations in animals and humans have suggested that the accumulation of excessive sorbitol formed from glucose by aldose reductase may trigger cataract formations,[4] neuropathy[5] and retinopathy[6] in diabetes.

Recently, some aldose reductase inhibitors have been found to be useful for preventing or treating chronic complications due to diabetes. Many heterocyclic compounds, *i.e.*, imidazolidine-2,4-diones,[7] thiazolidine-2,4-diones,[8] oxazolidine-2,4-diones[9] and rhodanines[10] are known to be aldose reductase inhibitors. Based on the structural analogy to the above compounds, various oxazole derivatives (Chart 1) were synthesized, and screened for inhibitory activity against aldose reductase. In this paper, we describe the results of an inhibitory activity screening test of 20 kinds of oxazole derivatives and the effect of structural alteration on the inhibitory activity.

Experimental

Materials——Aldose reductases Ia and Ib from rabbit lens were prepared by the method described in the previous paper.[11] Briefly, a 25—55% ammonium sulfate fraction was subjected to gel filtration on Sephadex G-200, followed by three column chromatographic steps, *i.e.*, affinity chromatography using Mātrex gel orange A, gel filtration on Sephadex G-100 and chromatofocusing using polybuffer exchanger 94 and polybuffer 74. Aldose reductases Ia and Ib were purified about 800-fold and 1100-fold, respectively. The purified enzymes were each homogeneous on polyacrylamide gel electrophoresis. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Oriental Yeast Co.

Determination of Melting Points, Infrared (IR) Spectra and Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectra——All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Jasco IRA-1 spectrophotometer. ^{1}H-NMR spectra were determined on a

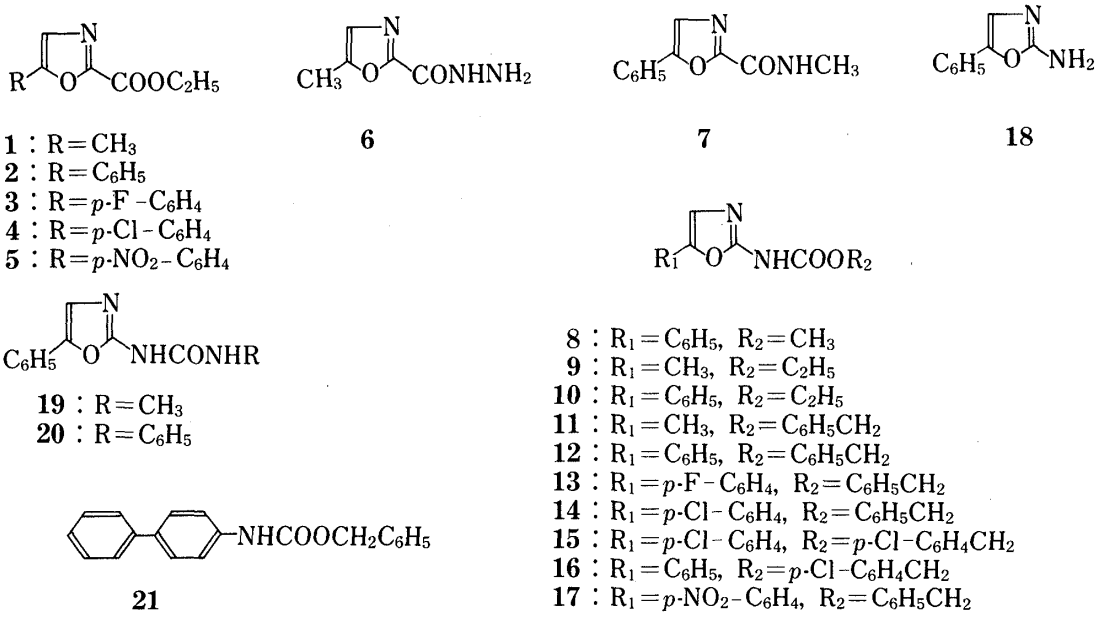


Chart 1

Hitachi Perkin Elmer R-40 spectrometer in $CDCl_3$ or dimethylsulfoxide (DMSO)-d_6 using tetramethylsilane as an internal standard.

Preparation of Oxazole Derivatives——Among the oxazole derivatives (Chart 1), 2-carboxylates (**1**—**5**), 2-carbohydrazide (**6**), 2-carbamates (**11**—**14** and **17**) and 2-amino (**18**) derivatives were prepared by the methods reported previously.[12]

Synthesis of *N*-Methyl-5-phenyl-2-oxazolecarboxamide (7)——A solution of ethyl 5-phenyl-2-oxazolecarboxylate (**2**) (0.22 g) in ethanol (10 ml) was added to a saturated solution of methylamine in ethanol (20 ml). The mixture was allowed to stand for 1 h, then the solvent was evaporated off. The residue was recrystallized from benzene–hexane to give **7** (0.17 g, 85%) as colorless plates, mp 117—118 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420 (NH), 1680 (CO). ^{1}H-NMR ($CDCl_3$) δ: 3.04 (3H, d, J=6 Hz, CH_3), 7.15 (1H, d, J=6 Hz, NH), 7.35 (1H, s, C_4–H), 7.38—7.79 (5H, m, Ar-H). *Anal.* Calcd for $C_{11}H_{10}N_2O_2$: C, 65.33; H, 4.98; N, 13.86. Found: C, 65.48; H, 4.91; N, 13.85.

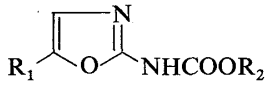
Synthesis of 2-Oxazolecarbamates (8—10, 15 and 16)——A solution of $NaNO_2$ (0.06 mol) in water was added dropwise to a stirred mixture of a 2-oxazolecarbohydrazide (0.05 mol), conc. HCl (6 ml), AcOH (50 ml) and benzene (100 ml) at 0—5 °C. The stirring was continued for 30 min. The reaction mixture was diluted with water, extracted with benzene, and dried. An alcohol (0.15 mol) was added to the above solution, and the mixture was refluxed for 5 h. After removal of the solvent, the excess alcohol was removed by steam distillation. The remaining material was extracted with $CHCl_3$ and the extract was dried and concentrated. The residue was recrystallized. Physical and spectral data for these compounds are listed in Table I.

Synthesis of 2-Oxazolylureas (19 and 20)——A mixture of 2-amino-5-phenyl-oxazole (**18**) (2 mol), an isocyanate (4 mmol) and benzene (50 ml) was refluxed for 2 h. After removal of the solvent the residue was recrystallized. Physical and spectral data for these compounds are listed in Table II.

Synthesis of Benzyl 4-Biphenylcarbamate (21)——A solution of 4-biphenylcarbonyl chloride (2.2 g) in benzene was slowly added to a stirred solution of sodium azide (2 g) in water (7 ml) and acetone (10 ml) with ice cooling. The stirring was continued for 1 h, then the reaction mixture was diluted with water, extracted with benzene and dried. Benzyl alcohol (3.3 g) was added to the above solution, and the whole was concentrated to *ca.* 5 ml, then refluxed for 3 h. After removal of excess benzyl alcohol by steam distillation, the residue was recrystallized from $CHCl_3$ to give **21** (2.1 g, 70%) as colorless needles, mp 148—150 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420 (NH), 1725 (CO). ^{1}H-NMR ($CDCl_3$) δ: 5.19 (2H, s, CH_2), 6.78 (1H, br, NH), 7.20—7.58 (14H, m, Ar-H). *Anal.* Calcd for $C_{20}H_{17}NO_2$: C, 79.18; H, 5.65; N, 4.62. Found: C, 79.46; H, 5.48; N, 4.52.

Assay of Aldose Reductase Activity——Assays were performed at 25 °C in 0.1 M sodium phosphate buffer (pH 6.2) containing 0.3 M ammonium sulfate, 10 mM DL-glyceraldehyde, 0.15 mM NADPH and an appropriate amount of enzyme in a total volume of 3.0 ml. The effects of the oxazole derivatives on the enzyme activity were determined by adding 4 μl of test compound solution to the reaction mixture. The appropriate blanks to correct for nonspecific oxidation of NADPH and absorption of the compounds tested were prepared. The reaction was initiated by the addition of enzyme, and the rate of NADPH oxidation was followed by recording the decrease in absorbance at 340 nm on a Union High-Sens SM-401 spectrophotometer equipped with a temperature-controlled cuvette chamber and a National X-Y recorder.

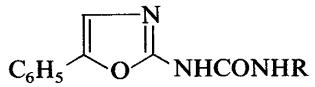
TABLE I. Physical and Spectral Data for 2-Oxazolecarbamates



Compd. No.	R_1	R_2	Yield (%)	mp (°C)	Recrystn. solvent	IR[a] ν_{max} (cm^{-1})	^{1}H-NMR[b] (ppm) (J=Hz)	Formula	Analysis (%) Calcd (Found)		
									C	H	N
8	C_6H_5	CH_3	28	162—164	$CHCl_3$–hexane	3420 (NH) 1750 (CO)	3.92 (3H, s, CH_3) 7.22 (1H, s, C_4–H) 10.80 (1H, br, NH)	$C_{11}H_{10}N_2O_3$	60.54 (60.58	4.62 4.36	12.84 12.88)
9	CH_3	C_2H_5	73	106—107	Et_2O	3420 (NH) 1735 (CO)	1.34 (3H, t, 7.5, $CH_2C\underline{H}_3$) 4.25 (2H, q, 7.5, $C\underline{H}_2CH_3$) 2.32 (3H, s, CH_3) 6.52 (1H, s, C_4–H) 10.68 (1H, br, NH)	$C_7H_{10}N_2O_3$	49.40 (49.23	5.92 5.95	16.46 16.32)
10	C_6H_5	C_2H_5	56	152—154	EtOH–H_2O	3430 (NH) 1745 (CO)	1.42 (3H, t, 7, $CH_2C\underline{H}_3$) 4.34 (2H, q, 7, $C\underline{H}_2CH_3$) 7.15 (1H, s, C_4–H) 10.56 (1H, br, NH)	$C_{12}H_{12}N_2O_3$	62.06 (61.83	5.21 5.08	12.06 12.28)
15	p-Cl–C_6H_4	p-Cl–$C_6H_4CH_2$	12	206—208	AcOEt	3425 (NH) 1750 (CO)	5.21 (2H, s, $C\underline{H}_2C_6H_4$) 7.45 (1H, s, C_4–H) 11.28 (1H, br, NH)	$C_{17}H_{12}Cl_2N_2O_3$	56.29 (56.57	3.32 3.07	7.70 7.76)
16	C_6H_5	p-Cl–$C_6H_4CH_2$	21	206—208	DMSO–EtOH	3425 (NH) 1750 (CO)	5.21 (2H, s, $C\underline{H}_2C_6H_4$) 7.46 (1H, s, C_4–H) 11.20 (1H, br, NH)	$C_{17}H_{13}ClN_2O_3$	62.11 (61.93	3.99 3.99	8.52 8.43)

a) Measured in $CHCl_3$ (**8**—**10**) or in KBr (**15** and **16**).
b) Measured in $CDCl_3$ (**8**—**10**) or in DMSO-d_6 (**15** and **16**).

TABLE II. Physical and Spectral Data for 2-Oxazolylureas



Compd. No.	R	Yield (%)	mp (°C)	Recrystn. solvent	IR $\nu_{max}^{CHCl_3}$ (cm^{-1})	^{1}H-NMR (ppm in DMSO-d_6) (J=Hz)	Formula	Analysis (%) Calcd (Found)		
								C	H	N
19	CH_3	71	203—205	Benzene	3420 (NH) 1690 (CO)	2.77 (3H, d, 6, $NHCH_3$) 7.50 (1H, s, C_4–H) 7.98 (1H, d, 6, $NHCH_3$) 10.66 (1H, br, NHCO)	$C_{11}H_{11}N_3O_2$	60.82 (61.04	5.10 5.08	19.35 19.50)
20	C_6H_5	88	196—198	DMSO–H_2O	3430 (NH) 1690 (CO)	7.60 (1H, s, C_4–H) 10.21 (1H, br, NH) 11.00 (1H, br, NH)	$C_{16}H_{13}N_3O_2$	68.80 (68.82	4.69 4.70	15.05 14.88)

Preparation of Test Compound Solutions——Because of the poor water solubility of the oxazole derivatives, compounds were dissolved in DMSO or in ethanol. DMSO or ethanol at 0.15% in the final assay solution did not inhibit the activity of rabbit lens aldose reductase by more than 5%.

Determination of IC_{50}——The concentration of inhibitor giving 50% inhibition of enzyme activity (IC_{50}) was estimated from the least-squares regression line of the log dose-response plot.

Results and Discussion

In recent years, many heterocyclic compounds have been found to act as aldose reductase inhibitors.[7-10] However, oxazole derivatives have not yet been tested for inhibitory activity against aldose reductase. We synthesized various oxazole derivatives, and examined the inhibitory activity of these compounds by the use of homogeneously purified rabbit lens aldose reductases Ia and Ib.

The percentages of inhibition of aldose reductases Ia and Ib by oxazole derivatives at 5×10^{-4} to 5×10^{-6} M are summarized in Table III. The concentrations giving 50% inhibition of enzyme activity (IC_{50}) of oxazole derivatives (**2**, **7**, **12**—**15**, **17** and **20**) having relatively strong inhibitory activity are shown in Table IV.

Among the compounds tested, the compounds (**1**, **6**, **9** and **11**) having a methyl substituent at C-5 of the oxazole skeleton were ineffective or only slightly effective at 10^{-4} M on both aldose reductase Ia and Ib activities. It can be seen from the difference in the inhibitory effects of 5-phenyloxazole derivatives (**2**, **10** and **12**) and the corresponding 5-methyloxazole derivatives (**1**, **9** and **11**) that the introduction of a phenyl group at C-5 remarkably increased the inhibitory activity of the oxazole derivatives. It appears that a bulky group at C-5 is necessary for potent inhibitory activity of oxazole derivatives. The introduction of a substituent such as F, Cl or NO_2, on the benzene ring at C-5 had no effect or even caused a significant decrease of the inhibitory effect in the case of 5-phenyl-2-oxazolecarboxylates (**3**—**5**). However, in the case of benzyl 5-phenyl-2-oxazolecarbamates (**13**, **14** and **17**), the inhibitory activity was not decreased or was only slightly decreased by the introduction of a substituent. These findings suggest that the alteration of the inhibitory activity of oxazole derivatives owing to change in the electron density of the benzene ring at C-5 may be dependent on the substituent at the C-2 position.

The inhibitory activity of oxazole derivatives was indeed affected by the nature of the substituent at the C-2 position of the oxazole skeleton. Compound **18**, with a small and non-carbonyl group (*i.e.* an amino group) at the C-2 position, had scarcely any inhibitory effect. Among 5-phenyloxazole derivatives, the ethyl ester (**2**) and methylamide (**7**) derivatives strongly inhibited the enzyme activities as compared with the methyl or ethyl carbamate (**8** and **10**) and methylurea (**19**) derivatives. These findings suggest that a carbonyl group may be necessary for the interaction of the inhibitor and the enzyme molecule, and that the degree of inhibitory activity of oxazole derivatives may be influenced by the distance between the oxazole nucleus and the carbonyl group in the substituent at C-2. In 5-phenyl-2-oxazolecarbamates derivatives, compound **12** having a benzyl carbamate moiety showed very strong inhibitory activity as compared with the methyl or ethyl carbamate derivative (**8** or **10**). The IC_{50} value of compound **12** was about 15 μM for aldose reductases Ia and Ib. *N*-Phenyl-*N'*-(5-phenyl-2-oxazolyl)urea (**20**) also exhibited very strong inhibitory activity as compared with *N*-methyl-*N'*-(5-phenyl-2-oxazolyl)urea (**19**). The IC_{50} value of compound **20** was 12 μM for aldose reductase Ia and 23 μM for aldose reductase Ib. This finding suggests that, even if the carbonyl moiety in the substituent at C-2 is not adjacent to the oxazole nucleus, the presence of a bulky moiety such as a phenyl or benzyl group in the substituent at C-2 greatly enhances the inhibitory activity of oxazole derivatives. On the other hand, benzyl 4-biphenylcarbamate (**21**) in which the oxazole nucleus is replaced by a benzene nucleus, did not completely inhibit aldose reductases Ia and Ib at the concentrations tested. Therefore, it is thought that the

TABLE III. Inhibition of Rabbit Lens Aldose Reductases by Oxazole Derivatives

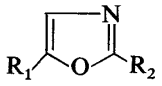
Compd. No.	 R_1	R_2	Inhibition (%)									
			Aldose reductase Ia					Aldose reductase Ib				
			5×10^{-4}	1×10^{-4}	5×10^{-5}	1×10^{-5}	5×10^{-6}(M)	5×10^{-4}	1×10^{-4}	5×10^{-5}	1×10^{-5}	5×10^{-6}(M)
1	CH_3–	$-COOC_2H_5$	19	0	0			27	0	0		
2	C_6H_5–	$-COOC_2H_5$		73	57	21			77	59	19	
3	*p*-F–C_6H_4–	$-COOC_2H_5$		25	16	1			28	19	0	
4	*p*-Cl–C_6H_4–	$-COOC_2H_5$		12	2	0			13	1	0	
5	*p*-NO_2–C_6H_4–	$-COOC_2H_5$		0	0	0			0	0	0	
6	CH_3–	$-CONHNH_2$	32	11	1			26	2	0		
7	C_6H_5–	$-CONHCH_3$		54	34	2			53	37	2	
8	C_6H_5–	$-NHCOOCH_3$		29	17	0			33	20	0	
9	CH_3	$-NHCOOC_2H_5$	6	0	0			8	0	0		
10	C_6H_5–	$-NHCOOC_2H_5$		28	0	0			41	0	0	
11	CH_3–	$-NHCOOCH_2C_6H_5$		18	6	0			19	3	0	
12	C_6H_5–	$-NHCOOCH_2C_6H_5$		83	70	40	28		94	85	41	22
13	*p*-F–C_6H_4–	$-NHCOOCH_2C_6H_5$			82	41	23			68	38	26
14	*p*-Cl–C_6H_4–	$-NHCOOCH_2C_6H_5$			44	9	0			32	4	0
15	*p*-Cl–C_6H_4–	$-NHCOOCH_2C_6H_4Cl$-*p*			47	5	0			49	7	0
16	C_6H_5–	$-NHCOOCH_2C_6H_4Cl$-*p*			8	0	0			9	0	0
17	*p*-NO_2–C_6H_4–	$-NHCOOCH_2C_6H_5$			52	25	14			48	19	7
18	C_6H_5–	$-NH_2$	31	0	0			39	0	0		
19	C_6H_5–	$-NHCONHCH_3$		2	0	0			7	0	0	
20	C_6H_5–	$-NHCONHC_6H_5$			99	37	2			93	10	0
21	C_6H_5–C_6H_4–	$-NHCOOCH_2C_6H_5$		0	0	0			0	0	0	

Table IV. IC_{50} Values of Oxazole Derivatives for Aldose Reductase

Compd. No.	IC_{50} (10^{-6} M)	
	Aldose reductase Ia	Aldose reductase Ib
2	35.8	34.1
7	86.0	87.7
12	15.6	15.4
13	14.1	19.1
14	63.6	163.9
15	57.6	55.2
17	44.5	54.2
20	12.0	23.1

Table V. Inhibitory Effects of Oxazole Derivatives on Adenine Nucleotide-Requiring Enzymes

Enzymes	Inhibition (%)			
	2	**12**	**13**	**20**
Hexokinase	0	4	0	3
20β-Hydroxysteroid dehydrogenase	0	1	0	0
Sorbitol dehydrogenase	1	0	7	2
Glucose 6-phosphate dehydrogenase	0	6	4	0
Lactate dehydrogenase	8	13	16	15
Aldose reductase Ia	37	56	63	93
Aldose reductase Ib	39	63	53	57

Assays were carried out in a reaction system with or without an inhibitor at 2×10^{-5} M. Inhibition is expressed as a percentage obtained by comparison with the uninhibited control.

inhibitory activity of oxazole derivatives is inherent in the oxazole structure.

To study the specificity of the inhibition of aldose reductase by compounds **2**, **12**, **13** and **20**, the inhibitory effects of these compounds on a number of adenine nucleotide-requiring enzymes (hexokinase, 20β-hydroxysteroid dehydrogenase, sorbitol dehydrogenase, glucose 6-phosphate dehydrogenase and lactate dehydrogenase) were investigated. The results are summarized in Table V. These enzymes were all unaffected or were only slightly inhibited by 2×10^{-5} M of **2**, **12**, **13** or **20**.

On the basis of the results of this study, although the inhibitory activity of the oxazole derivatives synthesized in this study was not necessarily stronger than that of other heterocyclic compounds reported already, it has become apparent that some oxazole derivatives can act as a specific inhibitors of aldose reductase. Further search may reveal more potent oxazole derivatives which could completely inhibit aldose reductase at low concentrations.

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