director of this laboratory, for his support and encouragement. We thank Dr. M. Moriyama, Dr. M. Sotomura, and Mr. S. Ikeda for helpful discussions.

Registry No. 1, 70458-96-7; **3a**, 103240-23-9; **3b**, 103175-73-1; **3b'**, 103240-28-4; **3c**, 103240-24-0; **3d**, 103240-25-1; **3e**, 103258-03-3; **3f**, 103240-26-2; **3g**, 103240-27-3; **4a** (X = Br), 13190-11-9; **4a** (X = H), 4906-24-5; **4b** (X = Br), 598-31-2; **4c** (X = Br), 5469-26-1; **4d** (X = Br), 70-11-1; **4e** (X = Cl), 4091-39-8; **4f** (X = Br), 815-52-1; **4g** (X = H), 78-93-3; propylene oxide, 75-56-9.

Synthesis and Aldose Reductase Inhibitory Activity of Substituted 2-Oxoquinoline-1-acetic Acid Derivatives

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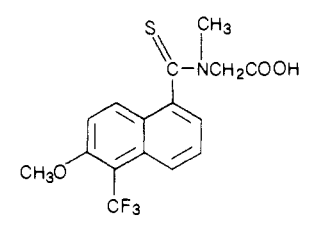
A number of 2-oxoquinoline-1-alkanoic acids that contain the *N*-acylglycine fragment found in several known inhibitors of aldose reductase were synthesized and tested in the rat lens assay. All of the target compounds were prepared by alkylation of the appropriate 2-oxoquinoline intermediates with a halo ester, followed by hydrolysis of the intermediate esters. In the rat lens assay, the 1-acetic acid derivatives **9a–e** display the highest level of aldose reductase inhibitor activity with IC_{50} values of 0.45–6.0 μM. Modification of the 1-acetic acid moiety by esterification, substitution of an α-methyl group, or insertion of an additonal methylene unit results in reduced inhibitory potency. Structure–activity data also suggests that both the benzene and 2-oxopyridine rings of **9a–e** contribute substantially toward activity and that inhibitory potency is influenced by aromatic ring substituents.

There is a growing body of evidence establishing a link between the formation and accumulation of sorbitol in ocular and nerve tissue and the development of several of the pathologies of chronic diabetes such as cataracts, retinophaty, and neuropathy.[1] Since sorbitol is formed in these tissues from glucose in a reaction mediated by the enzyme aldose reductase, one approach that has been explored to prevent or, at least, delay the onset of these diabetic pathologies has involved the development of inhibitors of aldose reductase. A large number of compounds of diverse structure have been found to inhibit this enzyme.



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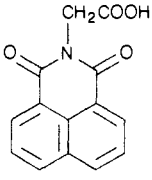

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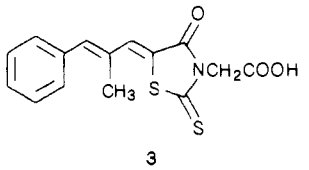

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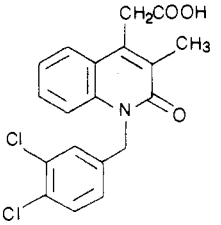

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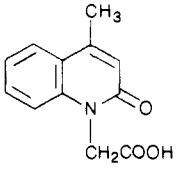

9a

Several of these inhibitors, including tolrestat (1), alrestatin (2), and ONO-2235 (3), contain an *N*-acylglycine fragment, which is believed to contribute significantly to the inhibitor–enzyme interaction.[2] This observation prompted us to synthesize and determine the aldose reductase inhibitory activity of a number of simple heterocyclic systems that contain the *N*-acylglycine fragment. One of these heterocycles, 4-methyl-2-oxoquinoline-1-acetic acid (**9a**), was found to display significant inhibitory acitivity in the rat lens assay with an IC_{50} value of 6 μM. This compound, in addition to being a derivative of **1–3**, is also related structurally to the known aldose reductase inhibitor ICI-105552 (**4**).[3] Herein we report the synthesis and enzyme inhibitory activity of **9a** as well as a number of other 2-oxoquinolines designed to explore structure–activity relationships among this class of aldose reductase inhibitors.

Chemistry

Several 2-oxoquinoline-1-acetic acids (**9a–e**) and -1-(2-propanoic acids) (**11a–c**) as well as a -1-(3-propanoic acid) (**13**) and 2-oxopyridine-1-acetic acid (**16**) were prepared for evaluation as inhibitors of aldose reductase (Table I). All of these compounds were synthesized by the general method reported by Potts of Yao for the preparation of 2-oxoquinoline-1-acetic acid.[4] Therefore, alkylation of the sodium salts of 2-oxoquinolines **7a–c** with ethyl bromoacetate provided the intermediate esters **8a–c**. Alkaline hydrolysis of the crude esters yielded the acetic derivatives **9a–c** upon neutralization (Scheme I). Similarly, the 1-(2-propanoic acid) **11a–c** and 1-(3-propanoic acid) **13** were prepared by alkylation of **7a–c** with ethyl 2-bromopropionate and ethyl 3-chloropropionate, respectively, and 2-oxopyridine-1-acetic acid (**16**) by alkylation of **14** with ethyl bromoacetate (Scheme I). The intermediate 2-oxoquinolines **7b–c** were prepared by converting quinolines **5b–c** to the corresponding *N*-oxides **6b–c** and then treating these *N*-oxides with acetic anhydride, followed by ammonium hydroxide as described by Pettit and co-workers

(1) Lipinski, C. A.; Hutson, N. J. *Annu. Rep. Med. Chem.* **1984**, *19*, 169.

(2) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. *J. Med. Chem.* **1985**, *28*, 841.

(3) Poulsom, R.; Heath, H. *Biochem. Pharmacol.* **1983**, *32*, 1495.

(4) Potts, K. T.; Yao, S. *J. Org. Chem.* **1979**, *44*, 977.

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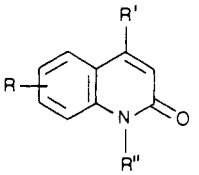
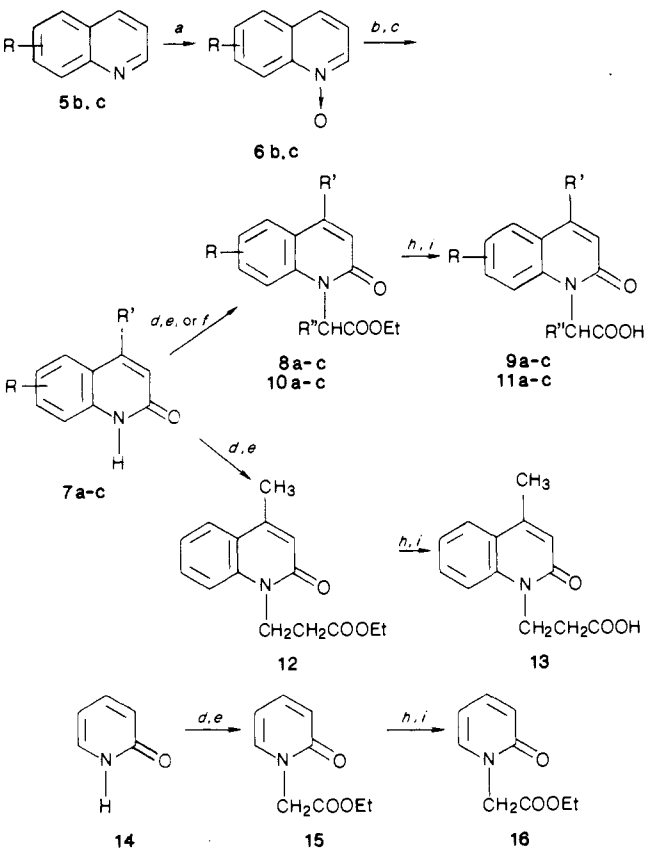
Table I. 2-Oxoquinolines



compd	R	R′	R″	mp, °C (lit. mp)	yield,[a] %	formula[b]	anal.[c]
7b	6-OCH_3	H	H	216–219 (215–217)[d]	69	$C_{10}H_9NO_2$	
7c	8-$OCOCH_3$	H	H	248–252 (251–254)[e]	67	$C_{11}H_9NO_3$	
7d	H	CH_3	CH_3	127–132 (131)[f]	74	$C_{11}H_{11}NO$	
8a	H	CH_3	$CH_2CO_2CH_2CH_3$	146–147	66	$C_{14}H_{15}NO_3$	C, H, N
9a	H	CH_3	CH_2CO_2H	227–230	77	$C_{12}H_{11}NO_3$	C, H, N
9b	6-OCH_3	H	CH_2CO_2H	244–249	59	$C_{12}H_{11}NO_4$	C, H, N
9c	8-OH	H	CH_2CO_2H	275–285	22	$C_{11}H_9NO_4 \cdot {}^1/_5CH_3CO_2H$	C, H, N
9d	6-OH	H	CH_2CO_2H	260–265	53	$C_{11}H_9NO_4$	C, H, N
9e	6-NO_2	CH_3	CH_2CO_2H	238–243	95	$C_{12}H_{10}N_2O_5$	C, H, N
11a	H	CH_3	$CH(CH_3)CO_2H$	158–160	66	$C_{13}H_{13}NO_3$	C, H, N
11b	6-OCH_3	H	$CH(CH_3)CO_2H$	140–143	41	$C_{13}H_{13}NO_4$	C, H, N
11c	8-OH	H	$CH(CH_3)CO_2H$	242–245	19	$C_{12}H_{11}NO_4$	C, H, N
13	H	CH_3	$CH_2CH_2CO_2H$	182–184	71	$C_{13}H_{13}NO_3$	C, H, N
16			2-oxopyridine-1-acetic acid	222–224 (217–218)[g]	50	$C_7H_7NO_3$	C, H, N

[a] These values represent the yield of analytically pure product obtained, and no attempt was made to optimize yields. All products were recrystallized from aqueous ethanol. [b] All products exhibited IR and ^1H NMR spectra consistent with assigned structures. [c] All products gave satisfactory C, H, N analyses. The presence of acetic acid in crystalline **9c** was confirmed by ^1H NMR. [d] Reference 13. [e] Reference 5. [f] Reference 6. [g] Reference 14.

Scheme I

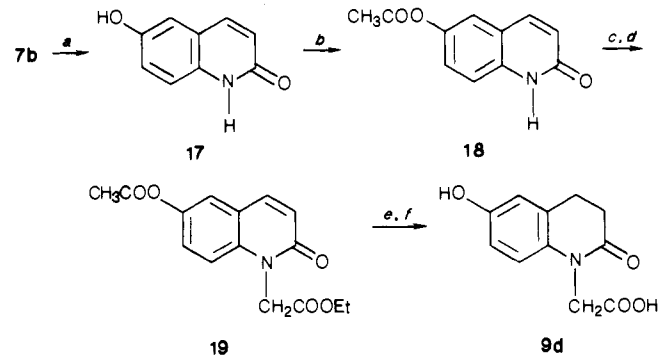


[a] H_2O_2, HOAc. [b] Ac_2O. [c] NH_4OH. [d] NaH, DMF. [e] $BrCH_2CO_2Et$. [f] $CH_3CHBrCO_2Et$. [g] $ClCH_2CH_2CO_2Et$. [h] NaOH, EtOH, H_2O. [i] HCl.

(**7a** was commercially available).[5]

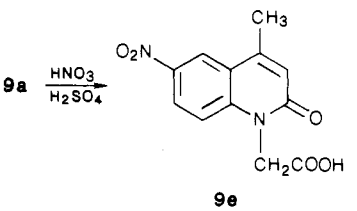
Attempts to prepare 6-hydroxy-2-oxoquinoline-1-acetic acid (**9d**) by demethylation of **9b** with 48% HBr resulted in both O- and N-demethylation. Therefore **9d** was synthesized from the intermediate 6-methoxy-2-oxoquinoline (**7b**) as outline in Scheme II. Demethylation of **7b** gave the intermediate phenol **17**, which was protected by acetylation. Subjection of **18** to the N-alkylation–hydrolysis procedure described above provided the desired 6-hydroxy-2-oxoquinoline-1-acetic acid (**9d**).

Scheme II



[a] 48% HBr. [b] Ac_2O. [c] NaH, DMF. [d] $BrCH_2CO_2Et$. [e] NaOH, EtOH, H_2O. [f] HCl.

Scheme III



The 6-nitro derivative **9e** was prepared directly from the acid **9a** by nitration (Scheme III). The position of nitration was confirmed by comparing the ^1H NMR spectrum of this compound with the spectrum of the known compound 6-nitro-1,4-dimethyl-2-oxoquinoline.[6]

Results and Discussion

The 2-oxoquinoline-1-acetic acids **9a–e**, -1-(2-propanoic acids) **11a–c**, -1-(3-propanoic acid) **13**, and 2-oxopyridine-1-acetic acid (**16**) were tested for their ability to

(5) Pettit, G. R.; Fleming, W. C.; Paull, K. D. *J. Org. Chem.* **1968**, *33*, 1089.

(6) Nabih, I.; Nasr, M. *J. Pharm. Sci.* **1966**, *55*, 736.

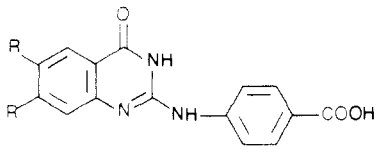
Table II. Aldose Reductase Inhibitory Activity of 2-Oxoquinolines

compd	R	R′	R″	% inhibn at 100 μM (SEM)[a]	IC_{50}, μM[b]
7a	H	CH_3	H	24.0 (8)	
7b	6-OCH_3	H	H	25.8 (9)	
7c	8-$OCOCH_3$	H	H	9.5 (5)	
7d	H	CH_3	CH_3	42.0 (9)	
8a	H	CH_3	$CH_2CO_2CH_2CH_3$	I[c]	
9a	H	CH_3	CH_2CO_2H		6.0
9b	6-OCH_3	H	CH_2CO_2H		0.91
9c	8-OH	H	CH_2CO_2H		3.3
9d	6-OH	H	CH_2CO_2H		2.4
9e	6-NO_2	CH_3	CH_2CO_2H		0.45
11a	H	CH_3	$CH(CH_3)CO_2H$		470.0
11b	6-OCH_3	H	$CH(CH_3)CO_2H$		590.0
11c	8-OH	H	$CH(CH_3)CO_2H$		33.0
13	H	CH_3	$CH_2CH_2CO_2H$		110.0
16			2-oxopyridine-1-acetic acid	33.0	
20			naphthyl-1-acetic acid		32
21			sorbinil		0.017
2			alrestatin		1.5[d]
3			ONO-2235		0.01[e]

[a] Percent inhibition produced at an inhibitor concentration of 100 μM, followed by the standard error of the mean (SEM). [b] IC_{50} values represent the concentration required to produce 50% inhibition. Values were determined from least-square analysis of log dose–response curves. Each log dose–response curve was generated with use of at least four concentrations of inhibitor, with four replicates at each concentration. The significance level for the least-squares fit was <0.01 in each case. [c] Compound **8a** was inactive, producing no measurable inhibition at a concentration of 100 μM. [d] Reference 12. [e] Reference 15.

inhibit crude aldose reductase obtained from rat lens as described earlier.[7] In addition to these compounds, several synthetic intermediates including the 2-oxoquinolines **7a–d** and the ester **8a**, as well as several standards such as naphthyl-1-acetic acid (**20**) and sorbinil (**21**), were also assayed. For compounds **9a–e**, **11a–c**, **13**, **20**, and **21**, IC_{50} values were determined by simple linear regression with use of the LINEFIT line-fitting program of Barlow.[8] The results of these evaluations are presented in Table II.

Examination of the aldose reductase inhibitory data reveals several general structure–activity trends. First, those derivatives that possess a 1-acetic acid moiety (**9a–e**) are significantly more potent than the 1-unsubstituted 2-oxoquinolines **7a–c** and the 1-methyl derivative **7d**. Also, all of the alkanoic acid derivatives (**9a–e**, **11a–c**, and **13**) are more potent as inhibitors of aldose reductase than the ester **8a**. From these data it is apparent that the 1-alkanoic acid fragment, and particularly the free carboxylic acid moiety, contributes significantly toward enzyme inhibitory acitivity. These results are consistent with our earlier studies with the 2-(arylamino)-4(3*H*)-quinazolinones (**22**) and the benzopyran-2-ones (**23**), where those derivatives with an approp iately positioned acidic moiety were found to be most active.[9,10]



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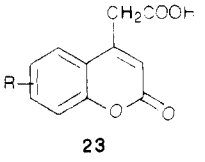
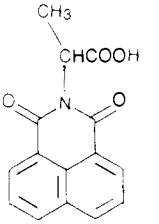


23

A second general structure–activity trend apparent from these data is that the 1-acetic acid derivatives **9a–c** are more potent inhibitors of aldose reductase than the corresponding -1-(2-propanoic acids) **11a–c** or the -1-(3-propanoic acid) **13**. This is perhaps best illustrated by comparing the inhibitory potencies of the 4-methyl-2-oxoquinoline-1-alkanoic acids **9a**, **11a**, and **13**. In this series, the acetic acid derivative **9a** is 78 times as potent as the 1-(2-propanoic acid) derivative **11a** and 18 times as potent as the 1-(3-propanoic acid) derivative **13**. Therefore, it appears that modification of the 1-acetic acid moiety by substitution of an α-methyl group or insertion of an additional methylene unit results in a decrease in the ability of these compounds to interact with aldose reductase. The effect of α-substitution on aldose reductase inhibitory activity in the 2-oxoquinoline-1-acetic acid series is consistent with structure–activity relationships reported earlier by Miller et al. with alrestatin.[11] They prepared the *R* and *S* enantiomers of 1,3-dioxo-1*H*-benz[*de*]isoquinoline-2(3*H*)-2-propionic acid (**24**) (α-methyl-substituted deriv-



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ative of alrestatin (**2**)) and found that, even though the *R* stereoisomer was more potent than the *S* stereoisomer, both isomers were substantially less potent than alrestatin. Furthermore, the decrease in inhibitory activity associated with insertion of an additional methylene unit between the heterocycle and the carboxylate moiety (**13**) was also noted in our earlier studies with the 2-oxobenzopyran-4-acetic acids (**23**).[10]

Kador and co-workers observed that addition of hydroxy groups to the aromatic rings of known aldose reductase inhibitors such as the flavonoids and 4-oxo-4*H*-chromenes results in an increase in inhibitory acitivity.[2,12] They

(7) Kador, P. F.; Sharpless, N. E. *Biophys. Chem.* **1978**, *8*, 81.
(8) Barlow, R. B. *Biodata Handling with Microcomputers*; Elsevier Science: Amsterdam, The Netherlands; p 76.
(9) DeRuiter, J.; Brubaker, A. N.; Millen, J.; Riley, T. N. *J. Med. Chem.* **1986**, *29*, 627.
(10) Brubaker, A. N.; DeRuiter, J.; Whitmer, W. L. *J. Med. Chem.* **1986**, *29*, 1094.
(11) Kador, P. F.; Goosey, J. D.; Sharpless, N. E.; Kolish, J.; Miller, D. D. *Eur. J. Med. Chem.—Chim. Ther.* **1981**, *16*, 293.
(12) Kador, P. F.; Sharpless, N. E. *Mol. Pharmacol.* **1983**, *24*, 521.
(13) Ishikawa, M.; Yamada, S.; Hotta, H.; Kaneko, C. *Chem. Pharm. Bull.* **1966**, *14*, 1102.
(14) Lawson, A.; Miles, D. H. *Chem. Ind.* **1958**, 461.

postulated that these hydroxyl groups may enhance inhibitory activity through direct interaction with a complimentary binding site present on the enzyme. These observations prompted us to prepare the 8- and 6-hydroxy-2-oxoquinoline-1-acetic acids, **9c** and **9d**. Both of these hydroxy derivatives were found to be approximately twice as potent as the unsubstituted derivative **9a**. However, the 6-hydroxy compound is significantly less active than the 6-methoxy (**9b**) and 6-nitro (**9e**) derivatives; these compounds are 2.7 and 5.3 times more active, respectively, than **9d**. Therefore, while addition of a ring hydroxy group at the 6-position does appear to enhance aldose reductase inhibitory activity, it does not do so to the same extent as substitution of a methoxy or nitro group at this position.

In an attempt to assess the contribution of the 2-oxoquinoline ring system toward aldose reductase inhibitory activity, both 2-oxopyridine-1-acetic acid (**16**) and naphthyl-1-acetic acid (**20**) were also evaluated. The pyridine derivative **16** was found to be substantially less potent than 2-oxoquinoline-1-acetic acids **9a–e**, producing only 33% inhibition at a concentration of 100 μM. These data demonstrate that the benzene ring of **9a–e** contributes significantly to inhibitory activity, perhaps through direct interaction with a complimentary binding site on the enzyme. Also, the observation that naphthyl-1-acetic acid (**20**) is 5–70 times less potent than **9a–e** suggests that the 2-oxopyridine ring present in these compounds may participate in the enzyme–inhibitor interaction.

In summary, the 2-oxoquinolines represent a new class of aldose reductase inhibitors with potencies comparable to that of alrestatin. In this series, optimal enzyme inhibitory activity is associated with those derivatives that possess a 1-acetic acid moiety, and modification of this moiety by esterification, substitution of an α-methyl group, or insertion of an additional methylene unit results in reduced activity. Also, our structure–activity data studies suggest that both the benzene ring and the 2-oxopyridine ring present in these compounds contribute to potency and that aromatic ring substituents significantly influence enzyme inhibitory activity. To better assess the contribution of ring substituents toward activity, however, it will be necessary to prepare and evaluate additional 2-oxoquinoline-1-acetic acid derivatives in which both the electronic nature and position of aromatic ring substituents are varied.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded with a Beckman 4230 spectrophotometer, and ^{1}H NMR spectra were recorded on a Varian T-60A spectrometer with tetramethylsilane as an internal standard. Enzyme reaction rates were determined with a Perkin-Elmer Hitachi 200 double-beam ultraviolet spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are within 0.4 of theoretical percentages. Common reagent grade chemicals were purchased from Aldrich Chemical Co. and were used as received. DL-Glyceraldehyde and NADPH (type I) were purchased from Sigma Chemical Co.

General Method for the Preparation of 2-Oxoquinolines 7b–c. A mixture of the quinoline **5b** or **5c** (70 mmol) and 30% H_2O_2 (15 mL) in glacial acetic acid (100 mL) was stirred at 80 °C for 18 h. The reaction mixture was cooled (ice bath) and neutralized with NH_4OH to give the *N*-oxide (**6b,c**) as a thick precipitate. The crude *N*-oxide was isolated by filtration and recrystallized from ethanol. A solution of each *N*-oxide (30 mmol) in acetic anhydride (30 mL) was stirred at 75 °C for 18 h and then poured over crushed ice (50 mL) and neutralized with NH_4OH. The resultant precipitate was isolated by filtration and recrystallized from ethanol to give the 2-oxoquinolines **7b–c** (see Table I for physical properties).

General Method for the Synthesis of 2-Oxoquinoline-1-alkanoic Acids 9–13 and 2-Oxopyridine-1-acetic Acid (16). Sodium hydride (50% oil dispersion, 1.3 g, 28 mmol) was added portionwise to a solution of 2-oxoquinoline (**7a–c**, 25 mmol) of 2-oxopyridine (**14**) in dry DMF (50 mL), and this mixture was stirred at 75–85 °C for 1.5 h. The reaction mixture was cooled to room temperature and the halo ester (30 mmol) added. After being stirred at 80 °C for 12–18 h, the reaction mixture was evaporated to dryness in vacuo, and the remaining solid was dissolved in a mixture of ethanol (150 mL) and H_2O (25 mL) containing NaOH (3.0 g, 78 mmol). This mixture was stirred at reflux for 2 h and then cooled to room temperature and evaporated to dryness. The remaining solid was dissolved in H_2O (100 mL), and this aqueous solution was washed with CH_2CL_2 (2 × 100 mL). Acidification of the aqueous solution with concentrated HCl (to pH 1) gave the free acid (**9–13, 16**), which was isolated by filtration and recrystallized from ethanol. The physical properties of these products are summarized in Table I.

6-Acetoxy-2-oxoquinoline (18). A mixture of **7b** (10.0 g, 57 mmol) in 48% HBr (75 mL) was stirred at reflux under nitrogen for 4 h and then at 80 °C for 14 h. The reaction was evaporated to dryness, and the resultant brown solid was dissolved in 1 N NaOH (100 mL) and washed with CH_2Cl_2 (2 × 100 mL). Acidification of the basic aqueous solution with concentrated HCl (pH 1) yielded the phenol **17** (6.3 g, 69%) as a fine yellow precipitate, and this was isolated by filtration, washed with H_2O (50 mL), and dried in vacuo. The dry phenol (6.0 g, 37 mmol) in acetic anhydride (50 mL) was stirred at reflux for 2 h and then at 60 °C for 16 h. A tan precipitate formed upon cooling, and this was isolated by filtration and recrystallized from ethanol to give **18** (6.3 g, 84%) as tan needles.

6-Nitro-2-oxoquinoline-1-acetic Acid (9e). Cold HNO_3 (1.7 mL) was added dropwise, over a period of 1 h, to a cold (ice bath) solution of **9a** (1.26 g, 5.8 mmol) in concentrated sulfuric acid (3.5 mL). After the addition was complete, the mixture was stirred with cooling (ice bath) for 14 h. Crushed ice (25 mL) was then added to the reaction mixture resulting in the formation of a thick yellow precipitate. The precipitate was isolated by filtration, washed with H_2O (3 × 20 mL), and recrystallized from aqueous ethanol to give **9e** (1.48 g, 97%) as off-white needles.

Enzyme Assay. Frozen rat eyes were purchased from Charles River Breeding Labs., Inc., MA, and the lenses were dissected from the partially thawed eyes, and were then kept at −6 °C until used for the assay. Crude enzyme supernatant was prepared by homogenizing 50 lenses in distilled H_2O (10 mL) and then centrifuging the crude homogenate at 10 000 rpm for 10 min while an ambient temperature of 0–5 °C was maintained.

Aldose reductase activity of the freshly prepared supernatant was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm in a Perkin-Elmer Hitachi 200 double-beam spectrometer. The reaction mixture contained 0.1 M phosphate buffer, pH 6.2; 0.104 mM NADPH (Sigma type I); 10 mM DL-glyceraldehyde; and 0.2 mL of the enzyme supernatant, in a total volume of 2 mL. The reference blank contained all of the above reagents except glyceraldehyde, to correct for nonspecific reduction of NADPH. The reaction was initiated by the addition of substrate, and it was monitored spectrophotometrically for 5 min. Enzyme activity was adjusted by diluting the supernatant with distilled H_2O so that 0.2 mL of the enzyme supernatant gave an average reaction rate for the control sample of 0.055 ± 0.0005 absorbance units/5 min. The effects of inhibitors on the enzyme activity were determined by including in the reaction mixture 0.2 mL of an aqueous solution of each inhibitor solution at the desired concentration in the reaction mixture as well as in the reference blanks to correct for absorbance of the inhibitor. The percent inhibition of each compound was calculated by comparing the reaction rate of the solution containing both substrate and inhibitor with that of control solutions containing only the substrate. Inhibitor IC_{50} values were obtained for compounds **9a–e**, **11a–c**, **13**, **20**, and **21** from least-squares analysis

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of the log dose-response curves with use of the Linefit program by R. B. Barlow, ElsevierBiosoft, Cambridge, UK.[8]

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Registry No. **5b**, 5263-87-6; **5c**, 2598-29-0; **6b**, 6563-13-9; **6c**, 103368-17-8; **7a**, 607-66-9; **7b**, 13676-00-1; **7c**, 15450-72-3; **8a**, 103368-18-9; **8b**, 103368-19-0; **8c**, 103368-20-3; **9a**, 103368-21-4; **9b**, 103368-22-5; **9c**, 103368-23-6; **9d**, 103368-24-7; **9e**, 103368-25-8; **11a**, 103368-26-9; **11b**, 103368-27-0; **11c**, 103368-28-1; **12**, 103368-29-2; **13**, 75371-21-0; **17**, 19315-93-6; **18**, 85770-30-5; $BrCH_2CO_2Et$, 105-36-2; $CH_3CH(Br)CO_2Et$, 535-11-5; $ClCH_2CH_2CO_2Et$, 623-71-2; aldose reductase, 9028-31-3.

Investigation of 4-(3-Hydroxyphenyl)-4-methylpipecolic Acid as a Conformationally Restricted Mimic of the Tyrosyl Residue of Leucine-Enkephalinamide[1]

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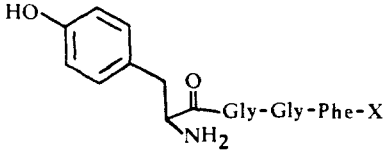
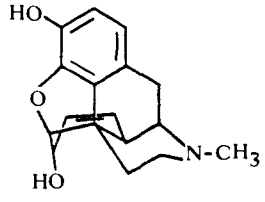
Analogues of leucine-enkephalinamide containing N-terminal *cis*- or *trans*-4-(3-hydroxyphenyl)-4-methylpipecolic acid were prepared to examine the conformational requirements of the N-terminal tyrosyl residue in opioid activity. The diastereomeric amino acids were prepared and purified by semipreparative HPLC before incorporation into the peptide. Spectroscopic analysis based on proton nuclear Overhauser enhanced differential spectroscopy (NOEDS) allowed assignment of the cis and trans stereochemistry. Despite spatial analogy between the trans isomer **5** and leucine-enkephalinamide, it possessed neither opioid agonist nor antagonist activity in the guinea pig ileal longitudinal muscle (GPI) or mouse vas deferens (MVD) preparations. Possible explanations for this inactivity are discussed.

Soon after characterization[2,3] of the enkephalins **1**, it was proposed[3,4] that the tyramine moiety was the key pharmacophore for both the opioid peptides and alkaloid opiates [e.g., morphine (**2**)]. This hypothesis was recently



1, X = Leu, Met



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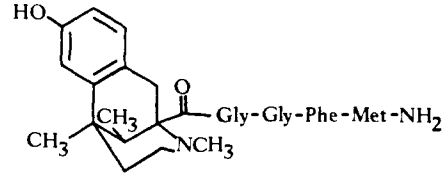
tested by the preparation of a hybrid opioid peptide **3** in which the tyrosyl residue was replaced by (-)-metazocine.[5] The absence of significant in vitro opioid agonist or antagonist activity with this analogue suggested that the peptidergic and alkaloid opioids differ in their recognition of opioid receptors, a concept that was reinforced by the characterization of multiple opioid receptors.[6,7] The tertiary amino terminus and conformational restriction of the phenolic moiety in **3** were considered among the possibilities contributing to the absence of receptor recognition for this ligand. Indeed, the more flexible aminotetralin analogue **4** was reported to be biologically active.[8]



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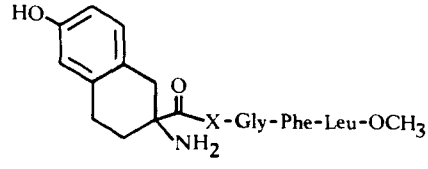
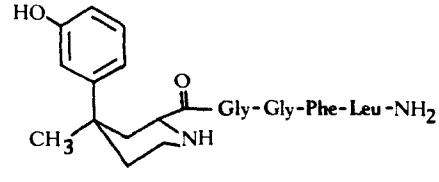


4, X = Gly, D-Ala

This report describes an effort to investigate further the conformational requirements for opioid receptor recognition of the tyrosyl side chain through the synthesis and in vitro biological evaluation of enkephalin analogue **5**.



trans-**5**

The use of C-4-substituted pipecolic acids allows predictable conformational restriction of the aromatic residue.[9] Thus, the aryl and amino moieties can be spatially superimposed upon those of the tyrosine residue in leu-

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