Synthesis of 1,4-Dihydro-4-oxo-quinoline-3-carboxylic Acid Derivatives as Inhibitors of Rat Lens Aldose Reductase

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The title compounds were prepared according to Scheme 2 and tested as inhibitors of the aldose reductase of rat lens.

Synthese von Derivaten der 1,4-Dihydro-4-oxo-chinolin-3-carbonsäure als Hemmer der Aldosereduktase aus der Rattenaugenlinse

Die Titelverbindungen wurden nach Schema 2 hergestellt und als Inhibitoren der Aldosereduktase aus der Linse von Rattenaugen geprüft.

The sorbitol pathway is a minor pathway of glucose metabolism in most tissues, consisting of two dehydrogenases, aldose reductase (AR) and sorbitol dehydrogenase (SDG):

$$\text{D-Glucose} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{AR}} \text{Sorbitol} + \text{NADP}^+$$

$$\text{Sorbitol} + \text{NAD}^+ \xrightarrow{\text{SDG}} \text{D-Fructose} + \text{NADH} + \text{H}^+$$

In mammals aldose reductase has low substrate affinity to glucose, the conversion of glucose to sorbitol is only slightly catalyzed. However, in diabetes mellitus certain tissues are subjected to exposure to high glucose levels. In lens, nerve, kidney, retina, AR enzyme appears to be the key factor in initiating the process of diabetes-dependent pathological conditions[2-4] and is regarded to be responsible for the reduction of glucose to sorbitol[5]. As a result of faster rate of sorbitol formation than its conversion to fructose, and because also the polarity of sorbitol prevents it from exiting the cell, accumulation of sorbitol in diabetic subjects causes complications including those of a lenticular, retinal, neuronal, and renal nature. These complications are generally known as diabetic cataract, retinopathy, neuropathy, and nephropathy[2]. Elevated sorbitol in lens leads to cataract and loss of the lens transparency; in peripheral nerves it interferes with the normal nervous function. Aldose reductase enzyme is also implicated in causing vasculature basement membrane thickening similar to that observed in the retinal capillaries and certain kidney tissues[2].

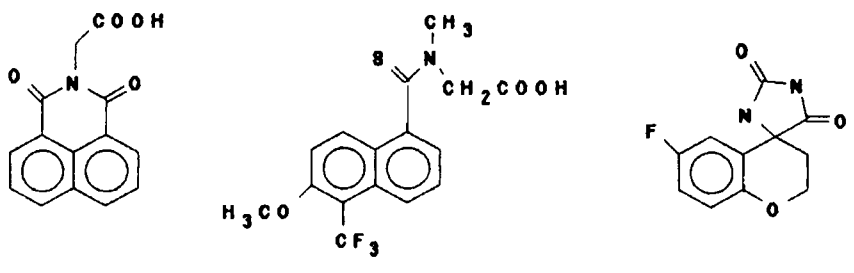
Aldose reductase enzyme inhibitors reduce tissue sorbitol accumulation in diabetic animals[6]. Studies have evidenced support for a major role for AR in the manifestation of various diabetic complications. Inhibitors of AR might be able to prevent, retard, or reverse such complications[7]. Although a number of inhibitors have been developed to the stage of clinical evaluation, some problems of lack of effect and adverse reactions have been encountered[8]. Alrestatin (AY-22,284) was the first inhibitor to be clinically investigated[9], followed by tolrestat[10] and sorbinil[11]. The latter has substantial beneficial effects in the treatment of diabetic symptoms, including changes in myoinositol, glutathione, ATP, and Na-K-ATPase[12]. Among the other compounds are flavonoids which are derived from many natural sources with exhibiting a broad scale of bioactivity[13], benzopyran-2-ones[14], tricylic arylacetic acids[15], substituted 2-oxoquinoline-1-acetic acid derivatives[16], and 2-(arylamino)-4-(3*H*)-quinazolinones[17].

In a study for seeking inhibitors of aldose reductase enzyme, we have reported the inhibitory pattern for AR enzyme, obtained by Computer Automated Structure Evaluation (CASE) Program[18]. The results of CASE led us to conclude a quinoline ring system to be mandatory.

As we know that several 2-oxoquinoline-1-acetic acids are potential inhibitors of AR enzyme[16], 1-4-dihydro-4-oxoquinoline-3-acetic acid derivatives were synthesized and determined for the ability of inhibiting AR. While it appeared that the effect of substituents on the phenyl ring was apparent among AR inhibitors[7], it was of interest to gain further insight into the structure-activity relationships (SAR) of this class of compounds.

Chemistry

1,4-Dihydro-4-oxoquinoline-2-carboxylic acids may be prepared by two routes: Reaction of an appropriate aniline



Alrestatin Tolrestat Sorbinil

Scheme 1

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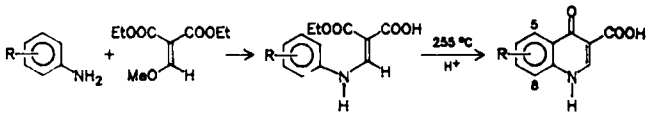
with diethyl oxalacetate[19] or with dimethyl acetylenedicarboxylate[20]. However, in our study, the 1,4-dihydro-4-oxoquinoline-3-carboxylic acids were prepared from the appropriate anilin and diethyl methoxymethylenemalonate. The resulting anilinomethylenemalonate was cyclized thermally[21].



Scheme 2

Results and Discussion

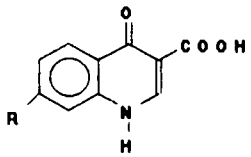
The inhibition was studied on *in vitro*. Dilutions were obtained from 10^{-4} M stock solution to obtain the concentration necessary to produce 50% inhibition. $Log(1/C_{50})$ values are shown in Table 1. Of the 12 derivatives, compounds **2**, **8**, **10**, and **11** produced the greatest inhibition of the AR enzyme. The inhibitory efficacies of the substituted compounds are higher than that of the corresponding non-substituted compound. The CH_2COOH-group produced the greatest effect, increasing the activity 10 times as compared to the non-substituted compound. This was followed by the COOH-substituent (3.5 times) and NO_2- and OH-substituents (2.8 times). Alkylation reduced the activity below that of the mentioned compounds. Halogenization of the phenyl ring led to derivatives 1.9 times more potent than the unsubstituted compound, bromide and chloride substituents were roughly equipotent. The result obtained by the esterified compound was as expected: in compound **12** a significant decrease was found.

It has been proposed that three regions might be responsible on the AR for the interaction with drugs: A substrate site, a nucleotide fold, and an inhibitor site[22]. In the model, a carbonyl group is the focus of the interaction leading to a tetrahedral intermediate with a tyrosine residue at the inhibitor site. On the other hand, an aromatic ring with the capability of H-bonding ability may also be required to afford aldose reductase inhibitory activity. We have shown[23] that the possible aldose reductase inhibitory pattern depends on a terminally polarized group on the side chain within a flexible moiety.

We postulated that this arrangements allowed the operation either by a mechanism similar to that observed for ternary complex formation between oxidized substrate and the host enzyme. In this an appropriately placed carboxylic group might be responsible for binding at the cationic arginine or histidine or possibly lysine residues located near the binding cleft of the AR[24], or the carboxyl group may be involved in an interaction with a probable tyrosine moiety at the substrate site[25]. The latter one has lesser possibility because a decrease of activity was obtained when the carboxyl group at C-7 position was converted to the ester. This is in contrast to the data obtained from 7-hydroxychromone-2-carboxylic acids[7]. It would appear that whenever the chain is flexible enough, a terminally polarized function might orient itself to cause maximum interaction with the aldose reductase inhibitory surface. However, the question then arises as to what is the function of the carboxyl group at C-3. Despite the discrepancy in the location of the carboxyl groups in the related compounds **10**,**11** in general, it is certain that C-3 and C-7 carboxyl groups do not interact at the same active site. Indeed, if the regioselectivity of the carboxyl groups located in opposite direction, had a function at the active site of the AR enzyme, then the inhibitory potency of the unsubstituted compound **1** should overcome the 'non-loss' in the activity potency regarding other derivatives. On the other hand, substituents on the phenyl ring may contribute to the inhibition of the enzyme by a combination of two factors. Such groups could bind to the enzyme by H-bonds; they could also produce a hydrophobic bond. Both cases may lead to a conformational change in the enzyme. However, it appears that phenyl rings possessing hydrophobic substituents are less potent than those possessing hydrophilic substituents. The OCH_3 derivative here is merely two times less potent than the phenolic analogue. Thus, the hydrogen bond donating ability of the hydroxyl and amino groups does seem to be of critical importance in the receptor interaction. The presence of halogens generally resulted in improved activity, most likely due to either their ability to act as hydrogen bonding acceptor or hydrophobic manners. In the present case, the results are so variable that it is difficult to predict the outcome of new substitution.

In fact, it seems that the optimal aldose reductase inhibitory activity in the quinoline derivatives depends on a flexible carboxyl group with a spatial arrangement to the carbonyl moiety. A flat area either with a capability of H-bonding or hydrophobic interaction can also play an important role in the interaction of inhibitors with AR.

Table 1: Physical Properties and Aldose Reductase Enzyme Inhibitory Activity of Substituted 1,4-Dihydro-4-oxoquinoline-3-carboxylic Acids



	R	Molecular formula M.P. °C	Elemental Analysis	Yield %	IC_{50}
1	H	$C_{10}H_7NO_3$ 227-230	63.5 3.70 7.4 63.4 3.74 7.0	88	34.1
2	OH	$C_{10}H_7NO_4$ >287	58.5 3.41 6.8 58.6 3.38 9.9	65	12.3
3	OCH_3	$C_{11}H_9NO_4$ 291 dec.	60.3 4.11 6.4 59.9 4.17 6.3	87	27.1
4	CH_3	$C_{11}H_9NO_3$ 237 dec.	65.0 4.43 6.9 64.9 4.51 6.9	54	31.7
5	C_2H_5	$C_{12}H_{11}NO_3$ 226 dec.	66.4 5.07 6.5 66.3 5.05 6.5	61	32.7
6	Br	$C_{10}H_6NO_3Br$ >296 dec.	44.6 2.29 5.2 44.7 2.35 5.2	33	15.3
7	Cl	$C_{10}H_6NO_3Cl$ >279 dec.	53.7 2.68 6.3 53.5 2.75 6.3	47	18.6
8	NO_2	$C_{10}H_6N_2O_5$ >286 dec.	51.3 2.56 12.0 51.6 2.39 11.9	89	12.7
9	NH_2	$C_{10}H_8N_2O_3$ >229 dec.	58.8 3.92 13.7 58.9 3.89 13.7	86	22.1
10	COOH	$C_{11}H_7NO_5$ >297 dec.	56.7 3.00 6.0 56.7 2.93 6.1	84	9.7
11	CH_2COOH	$C_{12}H_9NO_5$ >291 dec.	58.3 3.64 5.7 58.2 3.65 5.5	48	3.5
12	$CH_2COOC_2H_5$	$C_{14}H_{13}NO_5$ 191-193	61.1 4.73 5.1 60.9 4.71 5.0	17	21.2

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Experimental Part

Melting points: Thomas-Hoover, uncorrected.- IR spectra (KBr): Pye-Unicam SP1100.- Elemental analysis: Perkin-Elmer 240 Analyzer.

General Procedure

1,4-Dihydro-4-oxoquinoline-3-carboxylic acids were prepared by the modified method of *Price* and *Roberts*[21] (Scheme 2). The substituted aniline was condensed with diethyl methoxymethylenemalonate and heated to 100°C for 2 h. The resulting anilinomethylenemalonate was added carefully and slowly to boiling Dowtherm A within 10 min. The reaction mixture was heated at reflux for approximately 30 min depending on the substituents of aniline. The cyclized product was precipitated by cooling and recrystallized. Hydrolysis with N NaOH followed by acidification gave the 1,4-dihydro-4-oxoquinoline-3-carboxylic acids.

Pharmacological Evaluation

The aldose reductase enzyme was isolated and assayed in a modified version of *Cerelli et al.*[15], who obtained the enzyme from rabbit lens. 50 pooled lens which were obtained from 200-300 g albino rats, were thawed on ice and homogenized with 3 volume of distilled water, followed by centrifugation at 10 000 g for 20 min. Saturated ammonium sulfate was added to the supernatant to 40% saturation. The thick suspension was then allowed to stand with stirring for 15 min. The inert protein left in the supernatant was removed by increasing the ammonium sulfate concentration to 50% saturation, followed by the same procedure as above. The aldose reductase enzyme was precipitated from the 50% saturated solution by adding powdered ammonium sulfate to 75% saturation and was recovered by centrifugation at 10 000 g for 20 min. The protein concentration was measured by *Lowry's* method with bovine serum as the standard[26]. The aldose reductase enzyme activity of the freshly prepared supernatant was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm in a SP8-100 Pye-Unicam UV spectrophotometer attached to a recorder. D,L-Glyceraldehyde was used as substrate. The enzyme was dissolved in 10 ml of 0.05 M NaCl solution and 0.1 ml were added to a cuvette containing 0.2 ml phosphate buffer (0.067 M, pH 6.7), 0.5 ml NADPH (2×10^{-7} M, final concentration), 0.1 ml of the test drug (a 10^{-4} M solution was prepared in 5% DMSO. The stock solutions were diluted with distilled water to the desired concentrations), and 2.0 ml distilled water to obtain 2.9 ml solution. Baseline recording was obtained for 1 min. The reaction was started by addition of 0.1 ml D,L-glyceraldehyde (5×10^{-4} M, final concentration) and the decreasing of NADPH concentration was recorded at 340 nm for 10 min at 37°C. Readings were taken at 1 min intervals in the periods when the changes in absorbance were linear. The IC_{50} values were obtained by least-square regression line of the log dose-response curve.

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