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STRUCTURE-ACTIVITY STUDIES OF ALDOSE REDUCTASE INHIBITORS CONTAINING THE 4-OXO-4*H*-CHROMEN RING SYSTEM

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Various 4-oxo-4*H*-chromen-2-carboxylic acids and their derivatives were screened for aldose reductase inhibitory activity. Their inhibitory response along with that of several flavonoids has been correlated with simple Hückel molecular orbital calculations. From these results a possible mode of action is postulated.

1. Introduction

The sorbitol pathway of glucose metabolism in the crystalline lens contains two enzymes, aldose reductase (alditol: NADP oxidoreductase, EC 1.1.1.21) and sorbitol dehydrogenase (L-iditol: NAD oxidoreductase, EC 1.1.1.14). Aldose reductase along with the coenzyme NADPH catalyzes the reduction of aldose to alditol [1, 2]. This production of sugar alcohols has been implicated in the pathogenesis of sugar cataracts [3, 4]. Since aldose reductase appears to trigger the events that lead to sugar cataracts, the development of potent and selective inhibitors of this enzyme to delay or prevent the cataractous process is highly desirable.

Several compounds of diverse structure including the acids tetramethylene glutaric acid (TMG) and 1,3-dioxo-1*H*-benz[de]-isoquinoline-2(3H)-acetic acid (AY 22-284) have been reported to inhibit *in vitro* rat aldose reductase [5, 6]. Recently flavonoids have been reported to be potent inhibitors of this enzyme [7, 8]. Of this series quercitrin (2-(3,4-dihydroxyphenyl)-3-0-rhamnosyl-5,7-dihydroxy-4-oxo-4*H*-chromen, *22*) appeared to be the most promising flavonoid inhibitor. Solubility problems, however, were encountered with many of these flavonoids. Here we report the screening of several chromone-2-carboxylic acids and their derivatives as potential *in vitro* inhibitors of aldose reductase. The apparently more water soluble compounds contain the 4-oxo-4*H*-chromen ring system of flavonoids. Furthermore, we report the results of simple Hückel molecular orbital calculations on these molecules and several flavonoids. That the Hückel molecular orbital method is more than adequate for such relationships has been carefully pointed out by Streitwieser [11, pp. 6, 7]. It is not the exact calculations from the first principles (ab initio) which define the relationships, but rather the insights and concepts for facilitating what at best can only be semi-empirical correlations. Even so, as a practical matter, while it would have been esthetically and personally satisfactory to have used CNDO, the size and make-up of many of these molecules precluded its use. The available program in CNDO is dimensioned to 35 atoms and/or 80 orbitals; many of these compounds are larger, especially in regard to numbers of orbitals. From these results a possible mechanism of inhibition is presented.

2. Results and discussion

As seen in table 1 aldose reductase activity is retained in the chromone-2-carboxylic acids surveyed. This activity, however, is generally less than that of the flavonoids presented in table 2. The addition of an electron donating 6- or 7-methyl, 6-chloro or 6-phenoxy substituent did not essentially alter the activity of the

Table 1

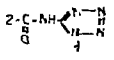
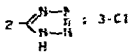
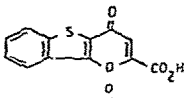
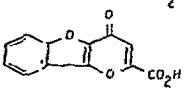
Compd. no.	Substituents	% Inhibition 10^{-4} M	10^{-5} M	10^{-6} M	HOMO	LEMO	qO=	pC=O	qC=
1	2-CO_2H	66	22	0	0.9158	−0.1854	1.5512	0.7072	0.7311
2	6-CH_3; 2-CO_2H	74	22	4	0.7274	−0.1865	1.5393	0.7226	0.7339
3	6-Cl; 2-CO_2H	74	23	0	0.8249	−0.1851	1.5367	0.7076	0.7304
4	6-OPh; 2-CO_2H	77	31	0	0.6560	−0.1857	1.5380	0.7242	0.7195
5	7-CH_3; 2-CO_2H	68	20	1	0.8093	−0.1995	1.5502	0.7150	0.7373
6	7-OH; 2-CO_2H	95	79	33	0.8100	−0.1905	1.5435	0.7120	0.7356
7	7-OH; 2-CO_2Et	95	77	31	0.8100	−0.1905	1.5435	0.7120	0.7356
8		80	38	6	0.5882	−0.2271	1.5455	0.7197	0.7340
9		94	77	34	0.7422	−0.3357	1.5689	0.7138	0.7370
10		87	66	22	0.2149	−0.2345	1.5429	0.7326	0.7256
11		69	29	7	0.7036	−0.2274	1.5650	0.7270	0.7311

Table 2

Compd. no.	Substituents	% Inhibition 10^{-4} M	10^{-5} M	10^{-6} M	HOMO	LEMO	qO=	pC=O	qC=
12	6-CO_2H	14	0	0	0.7338	−0.3404	1.5695	0.7027	0.7435
13	5, 7-diOH	80 [a]	68	12	0.7100	−0.3588	1.5816	0.6919	0.7511
14	5, 7-diOH; 3′-OH	98 [a]	90	46	0.6710	−0.3662	1.5840	0.6896	0.7526
15	5, 7-diOH; 3′-OCH_3	85 [a]	65	28	0.6956	−0.3592	1.5822	0.6913	0.7517
16	3, 5, 7-triOH; 3′-OH	87 [a]	62	0	0.5784	−0.3694	1.5814	0.6909	0.7553
17	3, 5, 7-triOH; 3′-OCH_3	90 [a]	50	0	0.5985	−0.3629	1.5794	0.6928	0.7545
18	3, 7-diOH; 2′, 3′-diOH	100 [a]	83	50	0.5739	−0.3610	1.5764	0.6951	0.7538
19	5, 7-diOH; 2′, 3′-diOH	90 [a]	85	45	0.6513	−0.3662	1.5840	0.6897	0.7530
20	3, 5, 7-triOH; 2′, 3′-diOH	100 [a]	83	60	0.5730	−0.3603	1.5790	0.6930	0.7546
21	3, 5, 7-triOH; 1′, 3′-diOH	100 [a]	75	0	0.5573	−0.3752	1.5843	0.6882	0.7585
22	3-O-rhamnosyl; 5, 7-diOH; 3′4′diOH	100 [a]	94	68	0.5683	−0.3694	1.5814	0.6910	0.7566
23	3, 5, 7-triCH; 2′, 3′, 4′-triOH	100 [a]	55	32	0.5603	−0.3695	1.5814	0.6910	0.7561

[a] Ref. [9].

parent acid *1*. Inhibition, however, was greatly increased by the addition of a 7-hydroxyl group. Furthermore, conversion of the 7-hydroxyl acid *6* to the ethyl ester *7* did not alter the inhibitory activity thus indicating that an acidic hydrogen may not be necessary. Conversion of the acid *1* to the N-tetrazol-5-yl amide *8* resulted in a compound of slightly greater inhibitory activity. Replacing the carboxylic acid of *1* with a tetrazole ring and adding a 3-chloro substituent resulted in a compound of activity comparable to compound *7*. The 1*H*-tetrazoles have been reported to generally show acidity comparable with carboxylic acids [9]. Replacing the chromone structure with either a 4-oxo-4*H*-[1]benzothieno[2,3-b]pyran or 4-oxo-4*H*-[1]benzofuro[3,2-b]pyran ring resulted in compounds which retained activity. Of the two, the sulfur analog is the more potent.

The 6-carboxyflavone *12* (1-phenyl-4-oxo-4*H*-chromen-6-carboxylic acid) was only weakly active at a concentration of 1×10^{-4} *M* and inactive at 1×10^{-5} *M*. This compound appeared to have little affinity for the aldose reductase enzyme since addition of 1×10^{-5} *M* *12* did not alter the inhibition produced by 1×10^{-6} *M* quercitrin (*22*). Similarly 3-carboxycoumarin (2-oxo-2*H*-chromen-3-carboxylic acid) was inactive at 1×10^{-5} *M*.

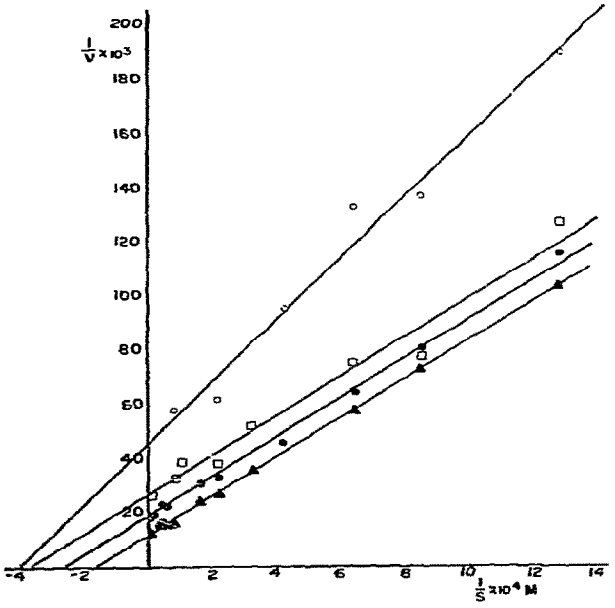


Fig. 1. Lineweaver-Burke plot of control (▲), 7-hydroxy-4-oxo-4*H*-chromen-6-carboxylic acid, *7* (1×10^{-6} *M* ●, 1×10^{-5} *M* ○) and quercitrin, *22* (1×10^{-6} *M* □). The substrate is glyceraldehyde and the velocity units are changes in OD_{340}/5 min.

Kinetic studies were conducted with 7-hydroxy-4-oxo-4*H*-chromen-2-carboxylic acid (*6*), one of the most potent compounds of the series surveyed and quercitrin (*22*) in order to determine if both exhibited a similar inhibition of aldose reductase. As seen in fig. 1 both compounds at a concentration of 1×10^{-6} *M* exhibited uncompetitive inhibition with the substrate glyceraldehyde. This was verified by a Hanes-Woolf plot of S/V versus S. Increasing the concentration of inhibitor, however, decreased the effect of the inhibitor on Km causing a convergence of the Km's in the reciprocal plot to show noncompetitive inhibition. Similar results were obtained with tetramethylene glutaric acid [5]. Quercitrin has previously been reported to be a noncompetitive inhibitor of aldose reductase [8].

In order to determine if these inhibitors compete with the coenzyme, NADPH, for aldose reductase, kinetic studies were also conducted in which the concentration of glyceraldehyde (1.5 m*M*) and 7-hydroxy-4-oxo-4*H*-chromen-2-carboxylic acid (*6*) (1×10^{-5} *M*) remained constant while the concentration of NADPH (0.624–0.00106 m*M*) was varied. No change in the percent inhibition of aldose reductase was observed thus indicating no competition between NADPH and the chromone *6* for a similar binding site on aldose reductase.

In order to correlate the wide variety of structures eliciting inhibitory reponse (e.g. *6*, *7*, *9* and *22*) quantum mechanical calculations were investigated. These calculations were carried out using the simple Hückel method, which is a π electron method only. This rapid and computationally convenient method was utilized because each of these compounds contains a similar ring system and ionic charges appeared not to affect inhibitory activity (e.g. *6* and *7*). The data so obtained were the eigenvalues, i.e. energies of the molecular orbitals and eigenvectors, i.e. sets of coefficients of the molecular orbitals from which additional molecular parameters were easily calculated.

For the chromones, the π system consists of an aromatic ring conjugated to a carbonyl-vinyl-ether system, connected to the ring at the ortho positions. The

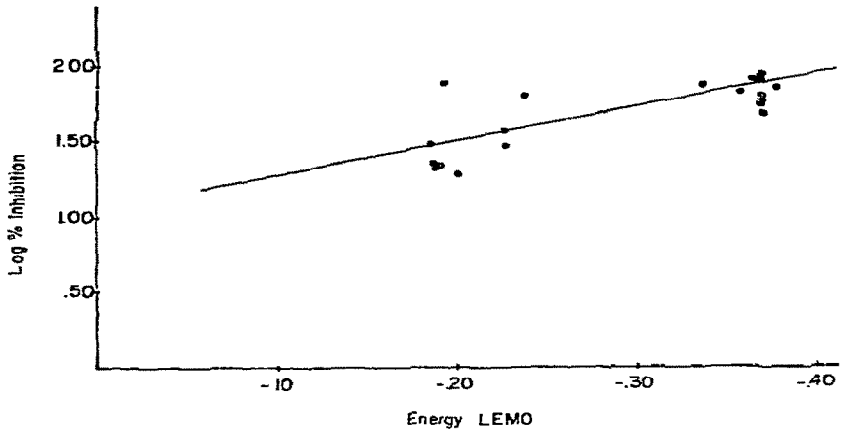


Fig. 2. Relationship between the log % inhibition and the energy of LEMO.

parameters were those suggested by Foenzler and Martin [10], Streitwieser [11], or Evleth and Cox [12]. Since these molecules are non-planar, the affected bond integrals were corrected by the scheme proposed by Roberts [13]. The values for the highest occupied molecular orbital (HOMO), lowest empty molecular orbital (LEMO), charge on the carbonyl carbon (qC=), charge on the carbonyl oxygen (qO=) and bond order i.e. a value associated with the binding power of the π bond between the carbonyl oxygen and carbon (pC=O) are presented in tables 1 and 2.

As seen in fig. 2, a significant correlation ($r = -0.785$, $\rho = -0.49$ to -0.91) was observed between the energy of the lowest empty molecular orbital (LEMO) and the log % inhibition of compounds *1–23* at concentrations of 1×10^{-5} *M*. (The rho (ρ) values are the range of the 95% confidence belts for the regressions with the indicated values of the correlation coefficients. If these ranges do not include the value $\rho = 0$, the correlation is significant at the 95% level [14].) Similarly, significant correlations were obtained for the log % inhibition of compounds *1–23* versus charge on the carbonyl carbon ($r = 0.658$, $\rho = 0.22–0.86$), charge on the carbonyl oxygen ($r = 0.693$, $\rho = 0.34–0.87$) and bond order between the carbonyl oxygen and carbon ($r = 0.589$, $\rho = 0.18–0.85$). Calculations of the frontier molecular orbital (C_i^2), which using the isolated molecular orbital approximation identifies the most reactive position of the molecule, indicated a site of attack on the carbon of the carbonyl group of these molecules. The only exception was the sulfur analog *10* in which the carbon of the carboxyl group was predicted to be the most reactive ($C_i^2 = 0.1847$). Its reactivity, however, is only slightly greater than that of the carbonyl carbon ($C_i^2 = 0.1813$) indicating an only slightly greater probability of interaction. This discrepancy may be due to inherent errors in the estimation of the d-orbital contributions of the sulfur atom [11].

The preferred correlation of the activity with the energy of the lowest empty molecular orbital (LEMO), together with the observation that the orbital having the maximum value of C_i^2 is that of the carbonyl carbon, indicates that the fundamental (or initiating) phenomenon is a nucleophilic attack at the carbonyl carbon [15]. This is usually effected by entities such as Y^- or Y: (radical indiced reactions of carbonyl compounds are rare and in a biological environment would be expected to be even more so.)

From these results a possible mechanism for the inhibition of aldose reductase by these various 4-oxo-4*H*-chromens may be postulated. Since classical uncompetitive inhibitors react with the enzyme-substrate complex to yield an inactive enzyme-substrate inhibitor complex, these compounds must be interacting with the enzyme at a site different from that of the substrate. At this inhibitor site the enzyme possesses a lone pair of electrons which is available to attack the carbonyl group of these inhibitors. This may then effectively change the enzyme by possibly altering its conformation, by tying up the lone pair of electrons

needed to reduce the substrate, or by some other mode which then renders the enzyme unable to react with its normal substrate.

3. Experimental

Compounds *1* and *22* in tables 1 and 2 were obtained from the Aldrich Chemical Company and K and K Laboratories, Inc. respectively. Both were recrystallized before use. Compounds *3* and *4* were supplied by Dr. Donald T. Witiak, College of Pharmacy, The Ohio State University, Columbus, Ohio. Compounds *10* and *11* were supplied by Dr. John B. Wright of the Upjohn Company, Kalamazoo, Michigan. Compounds *2*, *5–9* and *12* were obtained from Dr. G.P. Ellis, Department of Chemistry, University of Wales Institute of Science and Technology, Cardiff, U.K.

Sprague-Dawley rats weighing 125–150 g were suffocated with carbon dioxide gas. After removal of the eyes the lens were carefully dissected and homogenized in cold distilled water (0.4 ml/lens). The homogenate was centrifuged at 10,000 rpm for 10 min.

Aldose reductase activity of the supernatant was assayed spectrophotometrically determining the decrease in NADPH concentration at 340 nm in a Guilford 2400-2 automated compensating double beam spectrophotometer. The reaction mixture contained 0.1 *M* phosphate buffer, pH 6.2; 0.104 m*M* NADPH (Sigma type I); 1.5 m*M* *DL*-glyceraldehyde (Sigma) and 0.10 ml of the above supernatant in a total volume of 1.00 ml. The reference blank contained all of the above compounds except the glyceraldehyde substrate. Appropriate blanks to correct for the nonspecific reduction of NADPH and any absorbance of the inhibitor were used. The percent inhibition of each of the compounds was calculated by comparing the reaction rate of the solution containing substrate and compound at a given concentration with that of the control solution containing only substrate. The average reaction rate for the control solution was 0.025 ± 0.0019 mmol NADPH oxidized per 5 min.

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