Synthesis and Aldose Reductase Inhibitory Activity of Triazine Derivatives Possessing Acetic Acid Group

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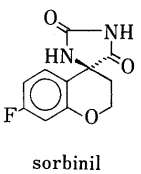
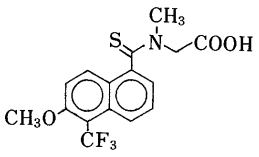
N-Acetic acid derivatives of 6-aryl-pyrazolo-triazin-4-ones were synthesized for evaluation as new aldose reductase inhibitors. The intrinsic activity of each compound was assessed by measuring the inhibition of enzymatic activity in an isolated pig lens enzyme preparation. All the prepared compounds exhibited a significant *in vitro* aldose reductase inhibitory effect (10^{-6} M $\leqslant IC_{50} \leqslant 10^{-4}$ M). Furthermore, biological activity (log $1/IC_{50}$) for most of the data sets could be correlated directly to electronic and steric parameters. Finally, spatial configuration of the most active derivative 6c ($IC_{50} = 2 \times 10^{-6}$ M) was compared with that of tolrestat and with pharmacophor requirements of the aldose reductase inhibitor site using a molecular modeling system.

Keywords substituted triazinone; triazine derivative; pyrazolotriazine; triazinylacetic acid; aldose reductase inhibitor; structure-activity relationship

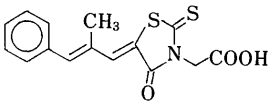
Chronic diabetes leads to long-term complications which include neuropathy, nephropathy, retinopathy and cataracts. It has been suggested that aldose reductase which catalyzes the reduction of glucose to sorbitol may be implicated in the pathogenesis of these complications.[1-3] Therefore, inhibition of the enzyme activity may provide a pharmacological approach to the treatment of these diabetic disorders. Efforts in this area have led to the discovery of a large number of compounds that inhibit lens aldose reductase *in vitro* as well as potent orally active derivatives.[4-7] There are, to date, two major structural classes of aldose reductase inhibitors, spirohydantoins, including the first of its class sorbinil, and carboxylic acids such as tolrestat, epalrestat and ponalrestat.



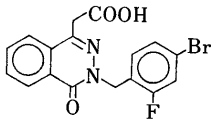
sorbinil



tolrestat



epalrestat



ponalrestat

In the last few years many compounds bearing an acetic acid moiety have been found of interest as new classes of aldose reductase inhibitors, *viz.* hydroxybutenolides, pyrimidine and benzothiazine derivatives.[8-10] These observations prompted us to introduce a carboxylic acid function in the 3-position of new 6-aryl-pyrazolo[2,3-*d*][1,2,4]triazin-4(3*H*)-ones the synthesis of which was reported in a previous paper.[11] All the prepared compounds were evaluated *in vitro* as inhibitors of aldose reductase obtained from pig lens.

Chemistry The *N*-substituted pyrazolotriazine derivatives **3** and **6** were synthesized from 6-aryl-pyrazolo[2,3-*d*][1,2,4]triazin-4(3*H*)-ones **1** and **4** as shown in Chart 1. The previously described preparation of compounds **1** involved the formation of carboxylic acid hydrazides *via* the appropriate aroylacrylates, followed by condensation with an orthoester.[11] On continued heating in dimethylformamide (DMF) for 60 h, compounds **1** were dehydrogenated in the 4a and 5-positions and led to pyrazolotriazinones **4**. Nucleophilic substitution of the hydrogen atom attached in the 3-position of the pyrazolotriazine ring could be obtained by reaction of ω-bromoalkylethyl carboxylates on **1** and **4** in acetone containing 1.5 eq of potassium carbonate. Base-catalyzed hydrolysis of the resulting esters **2** and **5**, followed by acidification to pH 1 with 60% aqueous sulfuric acid, afforded the expected products **3a—d** and **6a—i** (Table I).

Spectral data of *N*-substituted pyrazolotriazinones **3a—d** and **6a—i** are summarized in Table II. Their infrared (IR) spectra showed a large band, about 3500—3450 cm^{-1}, attributable to the OH carboxylic function. The carbonyles of the acidic group and of the triazinone ring appeared at about 1730 and 1660 cm^{-1}, respectively. Furthermore, the acidic proton of the carboxylic function was not apparent on any of the proton nuclear magnetic resonance (^{1}H-NMR) spectra (compounds **3a**, **c**, **6d**, **e**, **g**, **h**). Unambiguous assignment of the carbon of the carboxylic group, however, was supported by its own carbon-13 nuclear magnetic resonance (^{13}C-NMR) chemical shift near

TABLE I. Physical Constants of Pyrazolotriazine Derivatives **3** and **6**

Compd. No.	R^1	R^2	*n*	Yield (%)	mp (°C)	Formula
3a	H	H	1	70	254	$C_{13}H_{12}N_4O_3$
3b	Cl	H	1	64	175	$C_{13}H_{11}ClN_4O_3$
3c	OCH_3	H	1	75	248	$C_{14}H_{14}N_4O_4$
3d	H	C_6H_5	1	65	265	$C_{19}H_{16}N_4O_3$
6a	H	H	1	90	277	$C_{13}H_{10}N_4O_3$
6b	Cl	H	1	73	245	$C_{13}H_9ClN_4O_3$
6c	OCH_3	H	1	90	163	$C_{14}H_{12}N_4O_4$
6d	H	C_6H_5	1	98	277	$C_{19}H_{14}N_4O_3$
6e	Cl	C_6H_5	1	86	270	$C_{19}H_{13}ClN_4O_3$
6f	OCH_3	C_6H_5	1	98	275	$C_{20}H_{16}N_4O_4$
6g	H	H	2	40	195	$C_{14}H_{12}N_4O_3$
6h	H	H	3	40	152	$C_{15}H_{14}N_4O_3$
6i	H	H	4	42	205	$C_{16}H_{16}N_4O_3$

TABLE II. Spectral Data for Pyrazolotriazine Derivatives **3** and **6**

Compd. No.	IR (KBr) ν (cm^{-1})	^{1}H-NMR Chemical shift (δ) (in DMSO-d_6)
3a	3460, 1720, 1660	3.7 (m, 2H, CH_2), 4.5 (s, 2H, NCH_2), 4.9 (m, 1H, $C_{3a}H$), 7.5 (m, 3H, Ar), 7.9 (m, 2H, Ar), 9.2 (s, 1H, C_7H).
3b	3400, 1730, 1670	3.5 (m, 2H, CH_2), 4.5 (s, 2H, NCH_2), 5.0 (m, 1H, $C_{3a}H$), 7.4 (d, 2H, Ar), 7.7 (d, 2H, Ar), 8.9 (s, 1H, C_7H), 12.2 (br s, 1H, OH).
3c	3500, 1710, 1670	3.5 (m, 2H, CH_2), 3.9 (s, 3H, OCH_3), 4.3 (m, 1H, $C_{3a}H$), 4.8 (s, 2H, NCH_2), 7.1 (d, 2H, Ar), 8.0 (d, 2H, Ar), 9.2 (s, 1H, C_7H).
3d	3450, 1720, 1655	3.3 (m, 2H, CH_2), 4.5 (m, 1H, $C_{3a}H$), 4.8 (s, 2H, NCH_2), 7.7 (m, 10H, 2Ar), 13.2 (br s, 1H, OH).
6a	3500, 1720, 1670	4.7 (s, 2H, NCH_2), 7.5 (s, 1H, C_3H), 7.7 (m, 5H, Ar), 9.1 (s, 1H, C_7H), 13.2 (br s, 1H, OH).
6b	3480, 1720, 1670	4.2 (s, 2H, NCH_2), 7.2 (d, 3H, Ar+C_3H), 7.6 (d, 2H, Ar), 8.6 (s, 1H, C_7H), 12.1 (br s, 1H, OH).
6c	3500, 1730, 1670	3.8 (s, 3H, OCH_3), 4.8 (s, 2H, NCH_2), 7.1 (d, 2H, Ar), 7.8 (s, 1H, C_3H), 8.0 (d, 2H, Ar), 9.1 (s, 1H, C_7H), 11.6 (br s, 1H, OH).
6d	3440, 1740, 1660	4.8 (s, 2H, NCH_2), 7.5 (m, 5H, Ar), 7.9 (s, 1H, C_3H), 8.0 (m, 5H, Ar).
6e	3440, 1750, 1660	4.8 (s, 2H, NCH_2), 7.5 (d, 2H, Ar), 7.7 (s, 1H, C_3H), 7.9 (m, 7H, Ar).
6f	3460, 1740, 1670	3.8 (s, 3H, OCH_3), 4.9 (s, 2H, NCH_2), 7.0 (d, 2H, Ar), 7.7 (s, 1H, C_3H), 7.9 (m, 7H, Ar), 12.8 (br s, 1H, OH).
6g	3500, 1720, 1640	3.5 (t, 2H, CH_2CO), 4.8 (t, 2H, NCH_2), 7.5 (m, 3H, Ar), 7.7 (s, 1H, C_3H), 8.0 (m, 2H, Ar), 9.0 (s, 1H, C_7H).
6h	3500, 1690, 1630	1.9 (m, 4H, CH_2+CH_2CO), 3.6 (m, 2H, NCH_2), 7.2 (m, 3H, Ar), 7.3 (s, 1H, C_3H), 7.7 (m, 2H, Ar), 9.0 (s, 1H, C_7H).
6i	3480, 1700, 1660	1.7 (m, 4H, $2CH_2$), 2.3 (m, 2H, CH_2CO), 4.0 (m, 2H, NCH_2), 7.5 (m, 3H, Ar), 7.7 (s, 1H, C_3H), 8.1 (m, 2H, Ar), 9.1 (s, 1H, C_7H), 14.2 (br s, 1H, OH).

DMSO-d_6, deuterio-dimethyl sulfoxide.

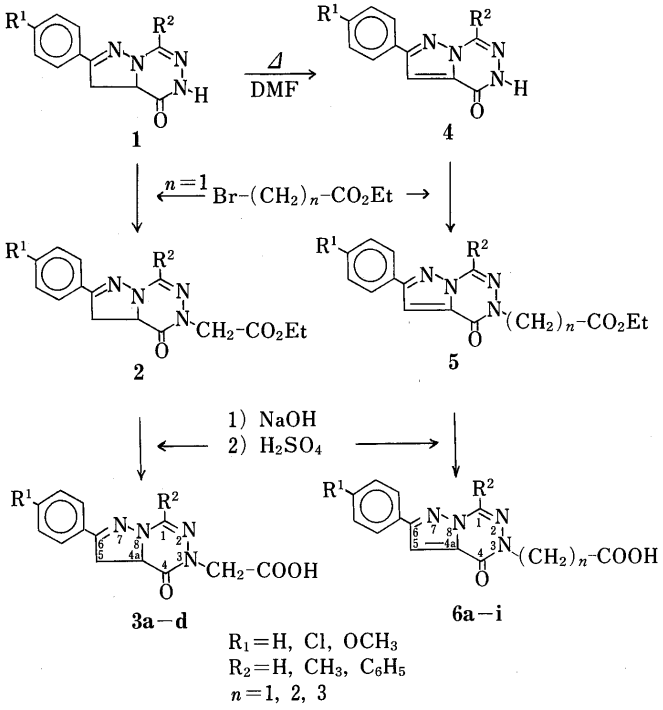


Chart 1

169 ppm for all synthesized derivatives. As for the carbonyle of the triazinone ring, it appeared on ^{13}C-NMR spectra near 155 ppm.

Biological Results and Discussion

All the *N*-substituted pyrazolotriazine derivatives **3a—d** and **6a—i** were tested *in vitro* for their ability to inhibit aldose reductase obtained from pig lens. The inhibitory IC_{50} values are shown in Table III and are included between 10^{-4} and 10^{-6} M for most of the products.

Dehydrogenation of the pyrazolo ring in the 4a and 5-positions resulted generally in an increase of the aldose reductase inhibitory activity. Compounds **3a—c** were effectively less active than derivatives **6a—c**. Addition of a second phenyl nucleus on the pyrazolotriazine heterocycle provided less active products (**6d—f**). Substitution of the aromatic ring in the 6-position of the fused pyrazolotriazine system with either electron-withdrawing group (**3b**, **6e**) or electron-donating group (**3c**, **6c**, **f**) significantly increased the activity. Nevertheless, compounds with a methoxy substituent (**3c**, **6c**, **f**) displayed more potent activity than triazinones with a chlorine atom (**3b**, **6b**, **e**). Triazine derivative **6c** was the most active compound of the series with an IC_{50} value of 2×10^{-6} M. Finally, the lengthening of the carboxylic acid side-chain considerably decreased the activity (**6g—i**), showing the necessity of an acetic acid moiety for potent activity.

So, with a view to understanding the real significance of the R^1 substituent contribution, we have attempted to correlate the observed aldose reductase inhibitory activity (log $1/IC_{50}$) with Hammett's constant (σ_p) as electronic parameter and with Taft's steric factor (E_s). These last values have been taken from the compilation by Hansch and Leo.[12]

TABLE III. Aldose Reductase Inhibitory Activity, Lipophilicity, Values Calculated and Found for log $1/IC_{50}$ of Pyrazolotriazine Derivatives **3** and **6**

Compd. No.	IC_{50} ($\times10^{-5}$ M)	log k_w	log $1/IC_{50}$		
			Found	Calcd Eq. 1	Calcd Eq. 2
3a	6.6	1.46	4.18	4.69	4.46
3b	1.5	1.70	4.82	4.90	4.90
3c	0.5	1.60	5.30	5.43	5.43
3d	1.4	2.00	4.85	4.69	4.46
6a	0.4	1.66	5.40	4.69	—
6b	0.8	2.66	5.10	4.90	4.90
6c	0.2	1.82	5.70	5.43	5.43
6d	4.5	3.48	4.34	4.69	4.46
6e	1.6	4.00	4.79	4.90	4.90
6f	0.5	3.60	5.30	5.43	5.43
6g	>10	ND	—	—	—
6h	>10	ND	—	—	—
6i	>10	ND	—	—	—

ND, not determined.

In a first step, derivatives **3** were considered separately. It was well known that biological activity of chiral compounds such as **3** was dependent on their absolute configuration, contrary to derivatives **6** which are not chiral; but no valid relationship was found with triazinones **3**. The result of regression analysis with only the derivatives **6** showed that aldose reductase inhibitory activity was modestly correlated with Hammett's constant ($r=0.531$).

It was thus of interest to see if dihydro compounds **3a—d** and the dehydro compounds **6a—f** could be accommodated in the same model as was done by Giraldez *et al.* for two different chemical series of compounds.[13] Regression analysis with electronic and steric parameters resulted in the following equation where the standard error of estimate is given in parentheses for the fitted values:

$$\log 1/IC_{50} = -1.552(\pm 0.684)\sigma_p - 0.585(\pm 0.326)E_s + 4.692(\pm 0.196) \quad (1)$$

In the above regression Eq. 1, the number of compounds was $n=10$, the squared correlation coefficient was $r^2=0.472$ ($r=0.687$), the residual standard deviation was $s=0.392$ and $F=3.134$ ($p=0.106$).

Only the observed aldose reductase inhibitory activity of derivative **6a** was fundamentally different from the calculated value, resulting in non-significance of the Eq. 1 ($p=0.106$). This result might suggest that in **6a** either factors other than those taken into account in establishing Eq. 1 notably influenced activity or that **6a** did not interact with the inhibitor binding site of the enzyme in precisely the same manner as other triazine derivatives. A new Eq. 2 excluding compound **6a** was then recalculated:

$$\log 1/IC_{50} = -1.807(\pm 0.463)\sigma_p - 0.889(\pm 0.238)E_s + 4.457(\pm 0.151) \quad (2)$$

$n=9$, $r=0.882$, $r^2=0.778$, $s=0.261$, $F=10.495$, $p=0.011$ The data used in the analysis and log $1/IC_{50}$ values recalculated from Eq. 2 are represented graphically in Fig. 1. Equations 1 and 2 led us to the conclusion that structural factors such as stereoelectronic and steric features played a determining role in this series of derivatives for a potent aldose reductase inhibitory activity.

In addition, the importance of the R^1 substituent and of the acetic acid side chain could be demonstrated by comparison of the most active triazine derivative **6c** with the pharmacophor model of Kador *et al.*[14,15] (Fig. 2) using Molecular Modeling Software Alchemy II (Tripos Associates, St. Louis, MO, U.S.A.). Pharmacophor requirements (d_1, d_2, d_3, d_4) of the aldose reductase inhibitor site seemed effectively to be respected in the case of **6c**.

This compound thus included a triazinone ring as a lipophilic region separated by 3.6 Å from a group susceptible to nucleophilic attack, as represented by the carboxylic function. A secondary lipophilic ring constituted by the pyrazolo nucleus located 5.2 Å from the carboxylic function could enhance hydrophobic binding to the aldose reductase inhibitor site. In addition, the location both of the carbonyl function in the 4-position and the methoxy group 2.6 and 8.6 Å, respectively, from the center of the triazinone ring could also enhance binding to this site. Superimposition of **6c** with tolrestat (Fig. 3) finally confirmed the necessity of having the benzene ring, the carboxylate anion and another hydrophobic moiety in an appropriate spatial alignment. The presence of a carbonyl group and of a hydroxy or a methoxy substituent also seemed important to increase aldose reductase inhibitory activity.

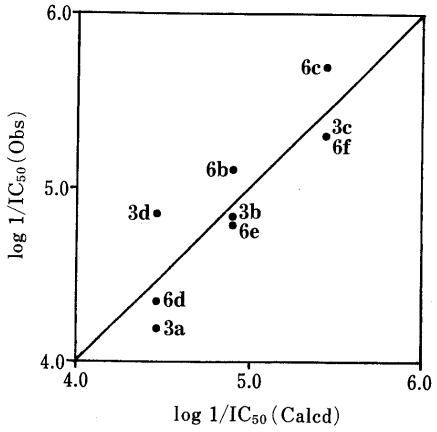


Fig. 1. Plot of Observed *versus* Calculated Aldose Reductase Inhibitory Potency Ratios (from Eq. 2) of Pyrazolotriazinone Derivatives

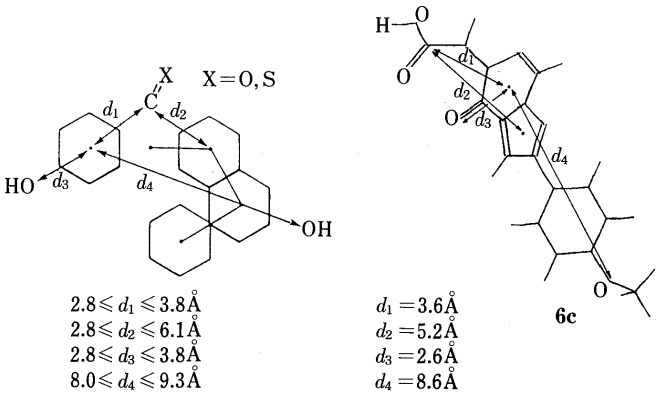


Fig. 2. Comparison between Pyrazolotriazinone **6c** and Pharmacophor Model of Aldose Reductase Inhibitors Proposed by Kador

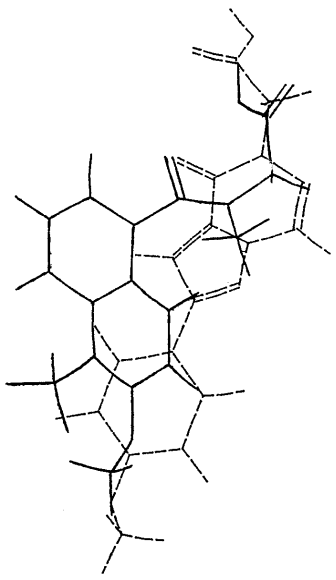


Fig. 3. Superimposition of Tolrestat (Continuous Line) and Pyrazolotriazine **6c** (Dotted Line)

Experimental

Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on a Beckman 4240 spectrophotometer. The ^{1}H-NMR spectra and the ^{13}C-NMR spectra were recorded, respectively, on a Varian EM 360 A spectrometer and on a JEOL FX 60 spectrometer. Resonance positions are given on the δ scale (parts per million) relative to internal tetramethylsilane. The NMR signals were designated as follows: s, singlet; d, doublet; br s, broad singlet; m, multiplet. Elemental analyses (C, H, N, Cl, within $\pm$0.4% of the theoretical values) were performed at the Service Central d'Analyses, Centre National de la Recherche Scientifique, 69390 Vernaison, France.

6-Aryl-pyrazolo[2,3-*d*][1,2,4]triazin-3-yl Alkylethyl Carboxylates (2 and 5) A suspension of the appropriate triazinone **1** or **4** (0.004 mol), potassium carbonate (0.83 g, 0.006 mol) and ω-bromoalkyl ethyl carboxylate (0.004 mol) in acetone (100 ml) was refluxed with stirring for 4 h. Then, the hot mixture was filtered off to eliminate mineral products. The filtrate was cooled and the precipitate which was formed, was collected by filtration and dried. The crude esters **2** and **5** were used without further purification.

6-Aryl-pyrazolo[2,3-*d*][1,2,4]triazin-3-yl Alkanoic Acids (3 and 6) The above ester (0.002 mol) was suspended in ethanol (100 ml) and sodium hydroxide (0.4 g, 0.01 mol) was added. The reaction mixture was refluxed for 2 h and then ethanol was removed. The residue was dissolved in water (20 ml) and acidified to pH = 1 with an aqueous solution of 60% sulfuric acid. The precipitate was filtered off, washed with water and dried. Acids **3** and **6** were recrystallized from a mixture of ethanol/water 50 : 50.

Biological Study The aldose reductase inhibiting activity was evaluated *in vitro* according to the technique adapted from Varma and Kinoshita.[16] The experiments carried out with the enzyme extracted from pig lens and DL glyceraldehyde substrate in buffer medium at pH = 6.2 together with reduced nicotinamide adenine dinucleotide phosphate (NADPH) + H^+. Each assay, repeated 5 times, was performed with the supernatant of lens homogenates at a suitable dilution and after a 15 min preincubation with several effector concentrations.

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