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Original paper

Aldose reductase inhibition by 2,4-oxo and thioxo derivatives of 1,2,3,4-tetrahydroquinazoline

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Summary – Inhibitors of aldose reductase are believed to be useful for the treatment of diabetic complications. Original [(2,4-dioxo-1,2,3,4-tetrahydro)quinazolin-1-yl] acetic acids and their thioxo derivatives have been synthesized and examined for their ability to inhibit aldose reductase *in vitro* and *in vivo*. Most were active *in vitro* on rat lens aldose reductase in the 10^{-7} molar range. Compound $\mathbf{V_{16}}$, which has a (2'-fluoro-4'-bromo)benzyl substituent on nitrogen N-3 was found in hypergalactosemic rats to be a good inhibitor of galactitol accumulation in sciatic nerves and to prevent cataract formation.

Résumé – **L'inhibition de l'aldose réductase par des dérivés 2,4-oxo et thioxo de la 1,2,3,4-tétrahydro quinazoline.** *Les inhibiteurs de l'aldose réductase sont des candidats potentiels pour le traitement des complications diabétiques. Des dérivés originaux de l'acide [(2,4-dioxo ou thioxo 1,2,3,4-tétrahydro)quinazolin-1-yl] acétique ont été préparés et leur activité inhibitrice de l'aldose réductase a été étudiée* in vitro *et* in vivo. *La plupart de ces composés sont actifs* in vitro *à 10^{-7} mol sur l'aldose réductase du cristallin de rat. Le composé $\mathbf{V_{16}}$, qui possède un substituant (2'-fluoro-4'-bromo)benzyle sur l'azote en 3 de la quinazoline inhibe l'accumulation du galactitol dans le nerf sciatique et prévient la formation de cataracte chez le rat soumis à un régime enrichi en galactose.*

aldose reductase / diabetic complications / quinazoline

Introduction

The involvement of aldose reductase in diabetic complications such as peripheral neuropathy, nephropathy, retinopathy and cataract is now a generally well accepted hypothesis, reviewed by many authors [1–4]. Inhibition of aldose reductase seems a promising approach for the treatment of these complications, which are not prevented by control of blood sugar level [5].

Aldose reductase (E.C. 1.1.1.21) is the first enzyme of the "polyol pathway". It reduces glucose to sorbitol, which is then oxidized to fructose by sorbitol dehydrogenase. Aldose reductase has a low affinity for glucose and, under normoglycemic conditions, there is little flux through the polyol pathway. It is believed that under hyperglycemic conditions, as in diabetes, the polyol pathway becomes activated, leading to an excessive conversion of glucose to sorbitol, which accumulates in the cells [1]. Sorbitol accumulation is supposed to be responsible for altered cellular functions, ultimately leading to complications such as those observed in diabetes [4, 6, 7].

Aldose reductase inhibitors (Fig. 1) can be divided into three main classes according to their structure: a) flavonoids such as quercitrin **XIII** [8]; b) spirohydantoins or compounds containing related systems, among which the

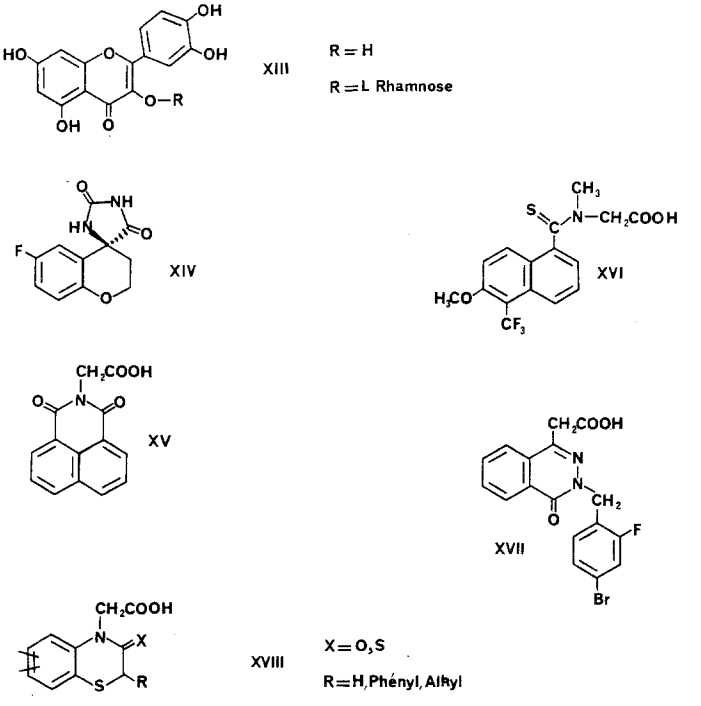
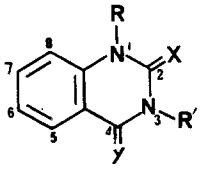


Fig. 1. Aldose reductase inhibitors.

most known is sorbinil **XIV** [5, 9]; c) substituted acetic acids, which can be exemplified by alrestatin **XV** [10], tolrestat **XVI** [11] and statil **XVII** [12].

Considerable efforts are now spent for the development of hydantoins [13] and acidic derivatives as aldose reductase inhibitors. Some hydantoins derivatives, such as dilantin, are centrally acting drugs and this could prove detrimental for the development of related molecules as antidiabetic drugs. In fact, according to Jaspan *et al.* [14], 8–10% of patients treated with sorbinil experienced dilantin hypersensitivity-like syndromes, principally rash and fever. For this reason, we have focussed our studies on acidic derivatives. We have previously found that *N*-acetic derivatives of (oxo or thioxo)-3-benzothiazine **XVIII** [15] are potent aldose reductase inhibitors. Comparison of their structure with alrestatin **XV** and statil **XVII** led us to consider the 2,4-dioxo quinazoline nucleus as a potentially interesting basic structural fragment (Fig. 2). We have thus synthesized and studied the aldose reductase activity of [(2,4-dioxo-1,2,3,4-tetrahydro-3-substituted) quinazolin-1-yl] acetic acids and their thioxo derivatives **XIX.**



XIX

X=O or S

Y=O or S

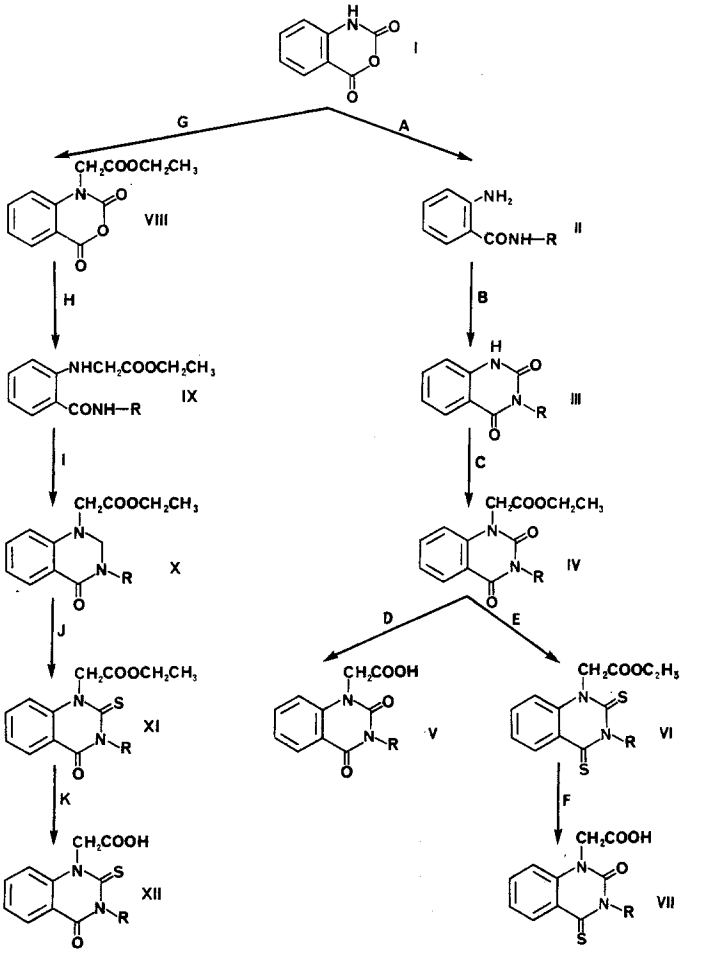
Fig. 2.

Chemistry

The desired quinazoline derivatives were synthesized through two different pathways according to the functional group borne by carbon C-2 (oxo or thioxo) (Scheme 1).

Derivatives of 2-oxo quinazolines **V** and **VII** were prepared by condensation of isatoic anhydride **I** with the appropriate primary amines [16]. Amides **II** were then cyclized with urea to yield 2,4-dioxo quinazolines **III** [17] which were substituted on nitrogen N-1 by treatment with ethyl bromoacetate in the presence of sodium hydride to give esters **IV.** Acids **V** were then obtained by alkaline hydrolysis, except for $\mathbf{V_5}$ which was directly prepared from ester $\mathbf{IV_4}$ by treatment with pyridine hydrochloride. Treatment of esters **IV** by phosphorous pentasulfide in refluxing xylene gave 2,4-dithioxo derivatives **VI** which were hydrolysed to compounds **VII** ([(2-oxo-4-thioxo-1,2,3,4-tetrahydro-3-substituted)quinazolin-1-yl] acetic acids) under mild alkaline conditions.

Derivatives of 2-thioxo quinazolines **XII** were obtained by alkylation of isatoic anhydride with ethyl bromoacetate in the presence of sodium hydride [18], condensation with the appropriate amines and cyclization with formaldehyde to give 4-oxo quinazolines **X** [19]. On treatment with molecular sulfur at 200°C, **X** yielded 4-oxo-2-thioxo quinazolines **XI** which were hydrolysed to acids **XII** under acidic conditions.



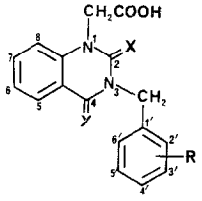
Scheme 1. Synthesis of quinazoline derivatives. **A** NH_2R, **B** NH_2CONH_2, **C** $BrCH_2COOCH_2CH_3$, **D** $NaOH/CH_3CH_2OH$, except for the acid $\mathbf{V_5}$ which was prepared from compound $\mathbf{IV_4}$ with pyridine hydrochloride, **E** P_2S_5, **F** $NaHCO_3/CH_3CH_2OH$. **G** $BrCH_2COOCH_2CH_3$, **H** NH_2R, **I** CH_2O, **J** molecular sulfur, **K** HCl/CH_3COOH.

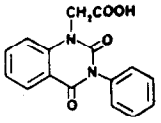
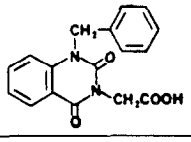
Pharmacology

Inhibition of aldose reductase was evaluated by *in vitro, ex vivo* and *in vivo* testing. *In vitro* inhibition was assayed by the method of Haymann and Kinoshita [20] with male Sprague–Dawley rat lens homogenates as source of aldose reductase and D,L-glyceraldehyde as substrate [21, 22]. For each compound, 3 concentrations (10^{-5} M, 10^{-7} M, 10^{-8} M) were tested and the results expressed as the percentage of inhibition of the enzyme (Table I). An *ex vivo* determination of rat lens aldose reductase inhibition was performed for some compounds by treating rats 100 mg/kg *p.o.*; they were killed after 2 h and lens aldose reductase activity was determined as previously described [21, 22]. The effect of compounds $\mathbf{V_1}$, $\mathbf{V_{10}}$, $\mathbf{V_{16}}$, $\mathbf{VII_{13}}$ and $\mathbf{XII_7}$ on the onset and the rate of development of cataract in hypergalactosemic rats was determined according to Simard–Duquesne and Peter [11, 22]. Compounds $\mathbf{V_1}$,

V_{10}, V_{16}, VII_6, VII_{13} and **XII_7** were also evaluated *in vivo* by their effect on the accumulation of galactitol in sciatic nerves of hypergalactosemic rats according to Petchey and Lloyd [24, 25] (Table II). Results were expressed as a percentage of inhibition of accumulation of galactitol in sciatic nerves of treated animals *versus* control animals, fed with a 45% galactose enriched diet but not treated with the inhibitor.

Table I. *In vitro* inhibition of aldose reductase by substituted quinazoline derivatives.



Compound No.	X	Y	R	Formula	mp (°C)	Method[b]	*In vitro*[a] (%)		
							10^{-5} M	10^{-7} M	10^{-8} M
V_1	O	O	H	$C_{17}H_{14}N_2O_4$	222–224	(1)	94	33	1
V_2	O	O	2′-F, 4′-F	$C_{17}H_{12}F_2N_2O_4$	191–194	(1)	96	63	8
V_3	O	O	4′-F	$C_{17}H_{13}FN_2O_4$	194–197	(1)	c	12	0
V_4	O	O	4′-OCH_3	$C_{18}H_{16}N_2O_5$	211–214	(1)	96	60	11
V_5	O	O	4′-OH	$C_{17}H_{14}N_2O_5$	290–295	(1)	c	12	0
VII_6	O	S	H	$C_{17}H_{14}N_2O_3S$	259–262	(2)	92	28	3
XII_7	S	O	H	$C_{17}H_{14}N_2O_3S$	234–237	(3)	96	38	12
V_{10}	O	O	3′,4′-OCH_2O	$C_{18}H_{14}N_2O_6$	201–204	(1)	95	80	17
VII_{11}	O	S	3′,4′-OCH_2O	$C_{18}H_{14}N_2O_5S$	235–240	(2)	c	c	c
V_{12}	O	O	2′-F	$C_{17}H_{13}FN_2O_4$	190–195	(1)	93	58	13
VII_{13}	O	S	2′-F	$C_{17}H_{13}FN_2O_3S$	195–200	(2)	94	74	13
XII_{14}	S	O	4′-OCH_3	$C_{18}H_{16}N_2O_4S$	210–211	(3)	98	85	13
V_{15}	O	O	3′-Cl, 4′-Cl	$C_{17}H_{12}Cl_2N_2O_4$	222–227	(1)	94	88	0
V_{16}	O	O	2′-F, 4′-Br	$C_{17}H_{12}BrFN_2O_4$	216–218	(1)	94	87	34
V_8				$C_{16}H_{12}N_2O_4$	235–236	(1)	27	0	0
V_9				$C_{17}H_{14}N_2O_4$	172–175	(1)	76	3	0

[a]Percentage of inhibition of enzymatic activity in rat lens homogenates as described under *Experimental protocols.*
[b]The three methods of synthesis are: (1): A, B, C, D; (2): A, B, C, E, F; (3): G, H, I, J, K.
[c]Insolubility of the compound under experimental conditions.
Basal enzymatic activity of rat lens homogenates: $4 \cdot 10^{-6}$ mol / min / g of protein.

Table II. *In vivo* inhibition of galactitol accumulation in sciatic nerves of hypergalactosemic rats.

No.	V_1	VII_6	XII_7	V_{10}	VII_{13}	V_{16}	**XVI** tolrestat	**XVII** statil
Dose (mg / kg *p.o.*)	100	100	100	100	30	30	30	30
Galactitol[a] (%)	−7 ± 6	−12 ± 4	−18 ± 8	−11 ± 6	−32[b] ± 6	−85[c,d] ± 5	−95 ± 5	−93 ± 3

[a]Diminution of galactitol concentration found in sciatic nerves of hypergalactosemic rats treated with inhibitors for 3 days as percentage of concentration of galactitol content in sciatic nerves of control hypergalactosemic rats.
[b]Statistically different from control hypergalactosemic: $P < 0.05$.
[c]Statistically different from control hypergalactosemic: $P < 0.001$.
[d]Not statistically different from tolrestat and statil (Student's *t*-test, $P = 0.05$).
Galactitol content in sciatic nerves of control hypergalactosemic rats: $23 \pm 3 \cdot 10^{-6}$ g / ml of homogenate.

Results and Discussion

In vitro inhibition of aldose reductase has been determined for 16 derivatives (Table I). Except for compounds V_8 and V_9, aldose reductase inhibition was found to be > 90% for 10^{-5} M inhibitor concentrations and ranged from 12–90% for 10^{-7} M. Most compounds retained a significant activity for 10^{-8} M concentrations (34% for V_{16}).

This confirmed the interest of (3-benzyl-2,4-dioxo quinazolin-1-yl) acetic acids as inhibitors of aldose reductase. Replacement of the benzyl moiety by phenyl and inversion of the position of benzyl and acetic acid groups yielded much less active compounds (V_8, V_9). The unsubstituted compound V_1 had a very promising activity (33% inhibition for 10^{-7} M); 2-thioxo XII_7, XII_{14}, or 4-thioxo VII_6, VII_{13} derivatives were found as, or just slightly more potent than the corresponding oxo compounds; this differed from results previously obtained in our laboratory in the 3-oxo benzothiazin series **XVIII** [15], in which replacement of oxygen on carbon C-3 by a sulfur atom led to substantial improvements. Substitutions on the benzyl moiety resulted in important variations of potency; 4′-hydroxy and 4′-fluoro substituents were clearly detrimental whereas 2′-fluoro, 4′-methoxy and 3′,4′-methylenedioxy had beneficial effects. Of all compounds tested, V_{16} (2′-fluoro-4′-bromo) was found the most potent (87% inhibition for 10^{-7} M, 34% inhibition for 10^{-8} M).

Unsubstituted compounds V_1 and XII_7 (for *in vivo* evaluation of 2-thioxo group influence) as well as the most active *in vitro* inhibitors (V_{10}, V_{16}, VII_6 and VII_{13}) were selected for testing their *in vivo* efficiency on galactitol accumulation in hypergalactosemic rat sciatic nerves according to Petchey and Lloyd [24, 25]. They were given *p.o.* (100 and 30 mg/kg) and statil and tolrestat were tested simultaneously for comparison (Table II). Significant activities were found for two tested quinazolines (V_{16}, VII_{13}), especially for V_{16} which efficiently prevented galactitol accumulation (−85% ± 5 inhibition for 30 mg/kg)− not significantly different from tolrestat (−95% ± 5) and statil (−93% ± 3).

Effects of compounds V_1, V_{10}, V_{16}, VII_{13} as well as XII_7 on the development of cataract in hypergalactosemic rats were tested as described by Simard−Duquesne and Peter [11, 22]. Among all the tested compounds given orally 30 mg/kg once a day *p.o.*, only V_{16} has been able to protect 88% of the animals after a 23 day treatment period. In the same conditions sorbinil (10 mg/kg, *p.o.*) and tolrestat (30 mg/kg, *p.o.*) gave full protection (data not shown). This led to suspect problems in distribution or duration of action. An *ex vivo* determination of aldose reductase activity in rat lens homogenates 3 h after *p.o.* treatment (100 mg/kg) by compounds V_2, V_4, V_{10}, V_{13} and V_{16} was therefore performed. V_2, V_4 and V_{10} were found inactive and treatment by V_{13} and V_{16} only slightly inhibited lens aldose reductase (respectively 20% and 27% inhibition *versus* 37% for statil) in spite of their *in vitro* activity, which confirmed the problems previously mentioned.

Conclusion

Quinazoline derivatives as those described here are therefore potent *in vitro* inhibitors of aldose reductase and are better than sorbinil. Some of them efficiently inhibit galactitol accumulation in sciatic nerves of hypergalactosemic rats. Under our experimental conditions, except for compound V_{16}, they fail to prevent lens cataract, suggesting problems of bioavailability, metabolism or distribution. Enhancement of *in vivo* activity should be obtained by changing route or rate of administration. However, the publication of a Japanese patent [26] during the course of our work, claiming some of the compounds described here, led us to abandon this series.

Experimental protocols

Chemistry

Reagents were commercially available and of synthetic grade unless otherwise stated. Melting points were determinated on a Köfler apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Perkin−Elmer R12A 60 MHz spectrometer, except for the final compounds which were recorded on a Bruker AC 200 MHz spectrometer, with $Si(CH_3)_4$ as the internal reference. The various splitting patterns were designated as follows : s : singlet ; d : doublet ; t : triplet ; q : quartet ; m : multiplet ; bs : broad signal. Chemical shifts were expressed in units (ppm). All final products were analyzed on a Carlo Erba C, H, N analyzer. Results obtained were within ± 0.4% of the theoretical values.

Method A

Example : 2-amino-N-(3′, 4′-dichlorobenzyl)benzamide ***II*****$_{15}$**. To a stirred solution of 20 g of isatoic anhydride in 50 ml of ethanol was slowly added 26 g of 3′, 4′-dichloro benzylamine [27] slowly to control the evolution of CO_2. The solution was carrefully heated to control the evolution of CO_2 and then refluxed for 2 h. The product crystallized directly on cooling and was filtered off and washed with water to give 27.6 g (77%) of II_{15}.

mp: 145°C ^{1}H NMR (DMSO−d6): 4.4 (d, 2H, CH_2); 6−7.7 (m, 9H, aromatics and NH_2); 8.7 (t, 1H, NH).

Method B

Example: 3-(3′,4′-dichlorobenzyl)-1,2,3,4-tetrahydro-2,4-dioxo quinazoline ***III*****$_{15}$**. A suspension of 27.6 g of II_{15} with 8.5 g of urea in 30 ml of *N*-methyl pyrrolidinone was stirred at 180°C for 12 h and cooled. The reaction mixture was poured into water and the solid was filtered off to give 27.9 g (93%) of III_{15}.

mp: 265°C ^{1}H NMR (DMSO−d6): 5.1 (s, 2H, CH_2); 7−8.1 (m, 7H, aromatics); 11−13 (bs, 1H, NH).

Method C

Example: ethyl [3-(3′,4′-dichlorobenzyl)-1,2,3,4-tetrahydro-2,4-dioxo-quinazolin-1-yl] acetate ***IV*****$_{15}$**. To a solution of 27.9 g of III_{15} in 220 ml of *N,N*-dimethyl formamide (DMF), 5 g of sodium hydride (NaH, 50% in mineral oil) was added by portion and the mixture was stirred for 1 h at 50°C. After cooling, 12.4 ml of ethyl bromoacetate was added dropwise. The suspension was stirred at 90°C during 7 h and cooled. After addition of water the compound was extracted ($CHCl_3$), washed with water and dried. Evaporation of the organic layer gave the product which was crystallized in ether to yield 24.4 g (69%) of IV_{15}.

mp: 120°C ^{1}H NMR (DMSO−d6): 1.2 (t, 3H, CH_3); 4.2 (q, 2H, OCH_2); 5.0 (s, 2H, CH_2CO); 5.12 (s, 2H, CH_2); 7−8.3 (m, 7H, aromatics).

Method D

Example 1: [3-(3′,4′-dichlorobenzyl)-1,2,3,4-tetrahydro-2,4-dioxo quinazolin-1-yl] acetic acid ***V*****$_{15}$**. To a stirred solution of 24.3 g of IV_{15} in 250 ml of ethanol was added a solution of 3.6 g of NaOH in 35 ml of water. After 3 h, ethanol was evaporated and the aqueous solution was acidified with HCl to yield the crude product which was recrystallized in acetonitrile to give 9.2 g (41%) of V_{15}.

mp: 222–227°C ^{1}H NMR (DMSO-d6): 4.89 (s, 2H, CH_2CO); 5.14 (s, 2H, CH_2); 7.33 (m, 2H, H_6 and $H_{6'}$); 7.42 (d, J_{7-8} = 8.5 Hz, 1H, H_8); 7.58 (d, $J_{2'-6'}$ = 2.3 Hz, 1H, $H_{2'}$); 7.58 (d, $J_{5'-6'}$ = 9.0 Hz, 1H, $H_{5'}$); 7.78 (ddd, J_{7-6} = 8.5 Hz, J_{7-5} = 1.6 Hz, J_{7-8} = 8.5 Hz, 1H, H_7); 8.09 (dd, J_{5-6} = 6.3 Hz, J_{5-7} = 1.6 Hz, 1H, H_5).
Anal. C, H, N. $C_{17}H_{12}Cl_2N_2O_4$.

Example 2: [3-(4'-hydroxybenzyl)-1,2,3,4-tetrahydro-2,4-dioxo quinazolin-1-yl] acetic acid ***V*****$_5$**. 4 g of **IV$_4$** was added to 60 g of pyridine hydrochloride at 190°C. The reaction mixture was stirred at 190°C during 5 h and then cooled. Addition of water gave the solid which was recrystallized in 2-methoxy ethanol to give 2.5 g (71.5%) of **V$_5$**.
mp: 290–295°C ^{1}H NMR (DMSO–d6): 4.88 (s, 2H, CH_2CO); 5.03 (s, 2H, CH_2); 6.68 (d, $J_{2'-3'}$ = $J_{5'-6'}$ = 8.3 Hz, 2H, $H_{2'}$ and $H_{6'}$); 7.18 (d, $J_{3'-2'}$ = $J_{5'-6'}$ = 8.3 Hz, 2H, $H_{3'}$ and $H_{5'}$); 7.32 (dd, J_{6-7} = 7.3 Hz, J_{6-5} = 7.6 Hz, 1H, H_6); 7.40 (d, J_{7-8} = 8.4 Hz, 1H, H_8); 7.76 (dd, J_{7-6} = 7.3 Hz, J_{7-8} = 8.4 Hz, 1H, H_7); 8.09 (d, J_{5-6} = 7.6 Hz, 1H, H_5).
Anal. C, H, N. $C_{17}H_{14}N_2O_5$.

Method E
Example: ethyl-[3-(3',4'-methylenedioxybenzyl)-1,2,3,4-tetrahydro 2,4-dithioxoquinazolin-1-yl] acetate ***VI$_{II}$***. A suspension of 14 g of **IV$_{10}$** with 30 g of phosphorus pentasulfide in 400 ml of xylene was refluxed. After 16 h, the xylene was evaporated. The crude product was dissolved in CH_2Cl_2 and separated by chromatography on silica gel using CH_2Cl_2 as eluant to give 13.5 g (65%) of **VI$_{11}$**.
mp: 170°C ^{1}H NMR (DMSO–d6): 1.36 (t, 3H, CH_3); 4.1 (q, 2H, OCH_2); 5.25 (s, 2H, CH_2CO); 5.78 (s, 2H, CH_2); 5.98 (s, 2H, OCH_2O); 6.78–8.75 (m, 7H, aromatics).

Method F
Example: [3-(3',4'-methylenedioxybenzyl)-1,2,3,4-tetrahydro-2-oxo-4-thioxoquinazolin-1-yl] acetic acid ***VII$_{II}$***. To a stirred solution of 6.5 g of **VI$_{11}$** in 400 ml of methanol and 400 ml of tetrahydrofuran was added a solution of 2.4 g of $KHCO_3$ in 20 ml of water. The reaction mixture was refluxed for 3 h. The product crystallized directly on cooling. It was filtered off to yield 3.7 g (66%) of **VII$_{11}$**.
mp: 235–240°C ^{1}H NMR (DMSO–d6): 4.96 (s, 2H, CH_2CO); 5.73 (s, 2H, CH_2); 5.97 (s, 2H, OCH_2O); 6.82 (s, 1H, $H_{2'}$); 6.86 (d, $J_{5'-6'}$ = 12 Hz, 2H, $H_{5'}$ and $H_{6'}$); 7.34 (dd, J_{5-6} = 7.9 Hz, J_{6-7} = 7.3 Hz, 1H, H_6); 7.45 (d, J_{8-7} = 7.5, 1H, H_8); 7.81 (dd, J_{7-6} = 7.3 Hz, J_{7-8} = 7.5 Hz, 1H, H_7); 8.59 (d, J_{5-6} = 7.9 Hz, 1H, H_5).
Anal. C, H, N. $C_{18}H_{14}N_2O_5S$.

Method G
Example: N-*(ethyl acetate) isatoic anhydride* ***VIII***. To a solution of 80 g of isatoic anhydride in 800 ml of *N,N*-dimethyl acetamide, 26 g of NaH (50% in mineral oil) was added by portion and the suspension was stirred for 1 h at room temperature, 65 ml of ethyl bromo acetate was added dropwise. After 12 h at room temperature the *N,N*-dimethyl acetamide was evaporated under vacuum and the crude product was diluted in water and extracted ($CHCl_3$). The organic layer was washed with water and dried. Evaporation of $CHCl_3$ gave the solid which was recrystallized in ethanol to yield 78 g (64%) of **VIII**.
mp: 154°C ^{1}H NMR ($CDCl_3$–DMSO–d6): 1.3 (t, 3H, CH_3); 4.3 (q, 2H, OCH_2); 4.9 (s, 2H, CH_2CO); 7.1–8.25 (aromatics).

Method H
Example: ethyl N-*[2-(4-methoxybenzylaminocarbonyl)phenyl]glycinate* ***IX$_{14}$***. To a stirred solution of 10 g of **VIII** in 50 ml of ethanol was added dropwise 7.5 g of methoxy benzylamine. The solution was heated slowly until reflux to control evolution of CO_2. After 2 h, the reaction mixture was cooled, poured into water and extracted (CH_2Cl_2). The organic layer was dried and evaporated. Addition of isopropyl ether gave 5.8 g (50%) of crystals **IX$_{14}$**.
mp: 100°C ^{1}H NMR (DMSO–d6): 1.18 (t, 3H, CH_3); 3.68 (s, 3H, OCH_3); 3.98 (d, 2H, CH_2CO); 4.13 (q, 2H, OCH_2); 4.38 (d, 2H, CH_2); 6.4–7.7 (m, 8H, aromatics); 8.15 (t, 1H, $NHCH_2$); 8.75 (t, 1H, NHCO).

Method I
Example: ethyl [3-(4'-methoxybenzyl)-1,2,3,4-tetrahydro-4-oxo quinazolin-1-yl] acetate ***X$_{14}$***. A solution of 5.8 g of **IX$_{14}$** with 4 ml of formaldehyde, and a few drops of HCl in 50 ml of ethanol was refluxed for 3 h and cooled. The solution was diluted with water and extracted (CH_2Cl_2). The organic layer was dried and evaporated. On cooling, white crystals appeared which were filtered off to give 4.7 g (81%) of **X$_{14}$**.

mp: 70°C ^{1}H NMR (DMSO–d6): 1.1 (t, 3H, CH_3); 3.65 (s, 3H, OCH_3); 4.02 (q, 2H, OCH_2); 4.18 (s, 2H, NCH_2N); 4.5 (s, 2H, CH_2CO); 4.55 (s, 2H, CH_2); 6.4–7.9 (m, 8H, aromatics).

Method J
Example: ethyl[3-(4'-methoxybenzyl)-1,2,3,4-tetrahydro-4-oxo-2-thioxoquinazolin-1-yl]acetate ***XI$_{14}$***. A mixture of 7.9 g of **X$_{14}$** with 8 g of sulfur was heated for half an hour at 180°C. After cooling, the methylene chloride soluble residue was separated by chromatography on silica gel using CH_2Cl_2 as eluant to give 2 g (35%) **XI$_{14}$**.
mp: 186°C ^{1}H NMR (DMSO–d6): 1.2 (t, 3H, CH_3); 3.7 (s, 3H, OCH_3); 4.2 (q, 2H, OCH_2); 5.58 (s, 2H, CH_2CO); 5.72 (s, 2H, CH_2); 6.75–8.28 (m, 8H, aromatics).

Method K
Example: [3-(4'-methoxybenzyl)-1,2,3,4-tetrahydro-4-oxo-2-thioxoquinazolin-1-yl] acetic acid ***XII$_{14}$***. A stirred solution of 2.2 g of **XI$_{14}$** in 20 ml of acetic acid was hydrolysed with a solution of 2 ml of HCl 37% in 10 ml of water. After 8 h under reflux the product crystallized directly on cooling. It was filtered off and recrystallized in methanol to give 1 g (50%) of **XII$_{14}$**.
mp: 210–211°C ^{1}H NMR (DMSO–d6): 3.70 (s, 3H, OCH_3); 5.55 (bs, changed into a singlet on heating at 330 K, 2H, CH_2–CO); 5.72 (s, 2H, CH_2); 6.85 (d, $J_{2'-3'}$ = $J_{6'-5'}$ = 8.6 Hz, 2H, $H_{2'}$ and $H_{6'}$); 7.28 (d, $J_{3'-2'}$ = $J_{5'-6'}$ = 8.6 Hz, 2H, $H_{3'}$ and $H_{5'}$); 7.46 (t, J_{6-7} = 7.7 Hz, J_{6-5} = 7.7 Hz, 1H, H_6); 7.60 (d, J_{7-8} = 8.7 Hz, 1H, H_8); 7.85 (ddd, J_{7-8} = 8.7 Hz, J_{7-6} = 7.6 Hz, J_{7-5} = 1.3 Hz, 1H, H_7); 8.15 (dd, J_{7-5} = 1.3 Hz, J_{5-6} = 7.7 Hz, 1H, H_5).
Anal. calcd. for $C_{18}H_{16}N_2O_4S$.
Sorbinil **XIV**, tolrestat **XVI** and statil **XVII** were synthesized in our laboratory as samples for pharmacological use [28–30].

Pharmacology

Aldose reductase assays
Male Sprague–Dawley (CD) rats Ch. River weighing 250–300 g were killed by ether; lenses were removed, homogenized with Daunce in 1 ml of iced phosphate buffer (pH 6.8) for two lenses and centrifugated 20 min at 27 000 *g* and 4°C. The supernatant was collected and stored at 4°C after addition of Li_2SO_4 (0.04 M). Activity was determined by an optical assay at 25°C, measuring continuously the disappearance of NADPH at 340 nm during 2 min, after addition of inhibitor in phosphate buffer (pH 6.8) and DL-glyceraldehyde as substrate (concentration in final solution :12.9· 10^{-3} M). NADPH disappearance rates were corrected by the blank rate without substrate and results were expressed as the percentage of inhibition (ratio 10^{-6} M NADPH consumed in the presence of the inhibitor *versus* 10^{-6} M NADPH consumed without inhibitor × 100).

Galactitol assays
Eight CD rats weighing 70–90 g were fed with a 45% galactose diet and simultaneously treated with an inhibitor (30 or 100 mg/kg *p.o.*, once a day) during 3 days. Then rats were killed, the sciatic nerves taken, homogenized and galactitol was quantified by HPLC according to Crabbe [24, 25] on a direct phase column by UV detection of 4-nitrobenzoate derivative.

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