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# Synthesis and aldose reductase inhibitory activity of a new series of benzo[*h*]cinnolinone derivatives

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Dedicated to Professor Pietro Pratesi

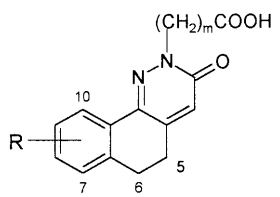
**Abstract**

Following our previous studies on pyridazinone carboxylic acids as potent and selective aldose reductase (ALR2) inhibitors, a new series of benzo[*h*]cinnolinone carboxylic acids, variously substituted at the positions 4, 7–10 and differently modified both at the central ring and at the acidic side chain, were synthesized and tested as inhibitors of ALR2. Comparison with previously synthesized compounds allows us to define more precisely structure–activity relationships for this class of compounds. In fact, in addition to the importance of the acidic side chain, their properties are highly influenced by the substituents present on the benzo[*h*]cinnolinone nucleous, with potency ranging from that of Sorbinil to very weakly active compounds. 

*Keywords:* Aldose reductase; Benzo[*h*]cinnolinone carboxylic acids; Diabetic complications; Sorbinil

## 1. Introduction

Aldose reductase (alditol:$NADP^+$ oxidoreductase, EC 1.1.1.21, ALR2) is an enzyme that catalyzes the conversion of glucose to sorbitol, which is in turn converted to fructose by sorbitol dehydrogenase. The increased glucose flux through this metabolic pathway has been linked to the development of late-onset diabetic complications such as neuropathy, nephropathy, retinopathy and cataract. Inhibitors of ALR2 thus seem to have the potential to prevent or treat diabetic complications, without the need of a strict control of glycemia [1].




**(A)**

I: R = H; m = 1

In a previous paper [2] we reported that pyridazinone carboxylic acids of general formula (A) are selective inhibitors of ALR2, with a potency comparable to Sorbinil.

According to these studies, the substituents present on the benzene ring, limited to 8,9-$(OCH_3)_2$ and 7,9-$(CH_3)_2$ were found to influence the activity in different ways depending on the acidic side chain. In fact, while elongation of the latter to the propionic homolog always led to less active derivatives, the corresponding butanoic analogs showed different properties.

In this study we extend the SAR for this class of compounds by the synthesis of numerous derivatives, differently substituted at the phenyl ring, carrying either acetic or longer acidic chains. Moreover, the 5,6-unsaturated substrate as well as compounds having an additional substituent at position 4 were evaluated as ARIs.

## 2. Chemistry

The target compounds **1**–**26** (see Table 1) were prepared from the corresponding benzocinnolinones **29**–**42** by treatment with the required ethyl bromoesters; the

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compounds thus obtained were easily converted into the corresponding carboxylic acids by alkaline hydrolysis followed by acidification (Scheme 1). The cyano derivative **27** (see Table 1) was obtained from **43** [2] by condensing with chloroacetonitrile and subsequently converted to the corresponding tetrazole **28** (see Table 1) according to literature methods (Scheme 1). The 8-methansulfonamido derivative **16** was obtained from the 8-amino-5,6-dihydrobenzo[*h*]cinnolin-3(*2H*)one [3] which was transformed into the corresponding ethyl ester, then condensed with methanesulfonyl chloride according to normal procedures and finally hydrolized.

Compound **20** was prepared from **41**, in turn obtained by transesterification, of the corresponding ethyl ester [2], by treatment with benzyl bromoacetate followed by catalytic reduction.

The still unknown intermediate methoxy- and benzyloxy-5,6-dihydrobenzo[*h*]cinnolin-3(2*H*)-ones (**29**–**36**) were prepared according to a general procedure previously reported [2] starting from the appropriate tetralones **44**–**51** (Scheme 2). However, while the 7-, 8- and 9-methoxysubstituted-4,4a-dihydroderivatives (**58**–**60**) were easily dehydrogenated using *m*-nitrobenzenesulfonate in alkaline medium, the low yield obtained in the case of 7-benzyloxy-5,6-dihydrobenzocinnolinone (**61**) suggested to use DDQ in xylene for the remaining 8- and 9-benzyloxyderivatives (**62, 63**). Moreover, it should be noted that in the case of the 8-substituted tetralones **47** and **51,** the reaction with glyoxylic acid in alkaline medium yielded the corresponding α-hydroxy-2-tetraloneacetic acids, which allowed the synthesis of 10-substituted compounds **32**, **36** by direct reaction with hydrazine hydrate in acetic acid (see Scheme 2).

## 3. Results and discussion

The ALR2 inhibition data reported in Table 2 clearly show that the activity of this class is influenced both by the nature and by the position of the substituents on the benzo[*h*]cinnolinone nucleous. Their potency ranges from values comparable to that of Sorbinil to very weak derivatives.

The presence of substituents at position 7 and 8 of the model **I** led to compounds (**8**, **9**, **13**) provided with similar activity (for **I** [2], $IC_{50} = 12.6$ μM). Insertion of a double bond at position 5–6 of **I** (compound **19**) still retained good properties. On the contrary, substituents at position 9 and 10 (**10**, **11**, **17**) as well as at the 4 position of the pyridazinone ring (**20**, **21**) lowered the activity. Replacement of the carboxylic function with a tetrazole (**28**) caused a marked decrease in potency while the cyano derivative (**27**) was inactive.

Among the butanoic homologs, the most potent was the 9-methoxyderivative (**5**; $IC_{50} = 4.79$ μM). All the other compounds were less active than **I**, the 8-aminosubstituted (**12**) being almost inactive. A further increase by a C atom of the side chain in compounds **1**, **3**, **5** to give **2**, **4**, **6**, respectively, did not significantly alter their profile.

Finally, insertion of an insaturation into the acidic chain (**22**–**24**) gave potent compounds, **24** being the most interesting term of this new series. On the contrary, branched chains proved to be detrimental (**25**, **26**).

## 4. Experimental

### 4.1. Chemistry

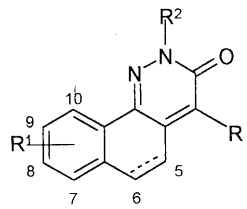
Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. Elemental analyses for the tested compounds were within $+/-$ 0.4% of the theoretical values. $^1$H NMR spectra were recorded on a Bruker AC200 spectrometer; chemical shifts are reported as $\delta$ (ppm) relative to tetramethylsilane as internal standard. DMSO-$d_6$ was used as solvent unless otherwise noted. TLC on silica gel plates was used to check product purity. Silica gel 60 (Merck, 70–230 mesh) was used for column chromatography. Tolrestat was synthesized according to a procedure reported in the literature [4]. Sorbinil was kindly provided by Pfizer. 8-Methoxytetralone (**47**) was synthesized from 8-hydroxytetralone [5,6] as described by Bilger et al. [7]. 5-, 6- and 7-Phenylmethoxytetralones (**48**–**50**) as well as 8-phenylmethoxytetralone (**51**) were synthesized according to previously reported methods [8,9]. Benzo[*h*]cinnolin-3(2*H*)-one and 4-aminobenzo[*h*]cinnolin-3(2*H*)-one were synthesized from 5,6-dihydrobenzo[*h*]cinnolin-3(2*H*)-one [10]. 8-Amino (**37**), 8- and 9-Acetylamino-5,6-dihydrobenzo[*h*]cinnolin-3(*2H*)-one (**38**,**39**) were synthesized as described in Ref. [3].

#### 4.1.1. Benzo[h]cinnolin-3(2H)-one-2-aliphatic acids (1–26) and compound 27

*4.1.1.1. General method.* (a) A mixture of the required pyridazinone (**29**–**42**) (0.01 mol), the appropriate ethyl bromoester (0.02 mol) and potassium carbonate (0.02 mol) in acetone (40 ml) was refluxed overnight (in the case of **20** benzyl ethylbromoacetate was used). After cooling, the inorganic salts were filtered off, the solvent evaporated to give the esters, which were purified by flash chromatography. Compound **27** was prepared in an analogous way, using chloroacetonitrile.

(b) A mixture of the appropriate ester (0.01 mol) and sodium hydroxide (0.04 mol) in 95% ethanol (40 ml) was stirred at room temperature (r.t.) for 2 h. After evaporation of the solvent, the residue was acidified with hydrochloric acid in diethyl ether, diluted with

Table 1
Physico-chemical properties of compounds **1**–**28**




| Compd | R | $R^1$ | $R^2$ | % Yield [a] | Formula | $^1$H NMR |
|---|---|---|---|---|---|---|
| **1** | H | 7-$OCH_3$ | –$(CH_2)_3$–COOH | 77 [a] | $C_{17}H_{18}N_2O_4$ | 2.0 (2H, m), 2.3 (2H, m), 2.8 (4H, s), 3.8 (3H, s), 4.2 (2H, t), 6.9 (1H, s), 7.0 (1H, d), 7.3 (1H, t), 7.6 (1H, d), 12.2 (1H, s). |
| **2** | H | 7-$OCH_3$ | –$(CH_2)_4$–COOH | 70 | $C_{18}H_{20}N_2O_4$ | 1.8 (4H, m), 2.3 (2H, t), 2.8 (4H, s), 3.8 (3H, s), 4.1 (2H, t), 6.9 (1H, s), 7.0 (1H, d), 7.3 (1H, t), 7.6 (1H, d), 12.2 (1H, s). |
| **3** | H | 8-$OCH_3$ | –$(CH_2)_3$–COOH | 77 | $C_{17}H_{18}N_2O_4$ | 2.0 (2H, m), 2.3 (2H, t), 2.8 (4H, s), 3.8 (3H, s), 4.2 (2H, t), 6.8 (1H, s), 7.0 (2H, d), 7.9 (1H, d), 12.2 (1H, s). |
| **4** | H | 8-$OCH_3$ | –$(CH_2)_4$–COOH | 69 | $C_{18}H_{20}N_2O_4$ | 1.6 (2H,m), 1.8 (2H, m), 2.3 (2H, t), 2.8 (4H, s), 3.8 (3H, s), 4.1 (2H, t), 6.8 (1H, s), 6.9 (2H, d), 7.9 (1H, d). |
| **5** | H | 9-$OCH_3$ | –$(CH_2)_3$–COOH | 66 | $C_{17}H_{18}N_2O_4$ | ($CDCl_3$): 2.0 (2H, m), 2.3 (2H, t), 2.8 (4H, s), 3.8 (3H, s), 4.3 (2H, t), 6.8 (2H, m), 7.1 (1H, d), 7.5 (1H, s). |
| **6** | H | 9-$OCH_3$ | –$(CH_2)_4$–COOH | 70 | $C_{18}H_{20}N_2O_4$ | ($CDCl_3$): 1.6 (2H, m), 1.8 (2H, m), 2.3 (2H, t), 2.8 (4H, s), 3.8 (3H, s), 4.1 (2H, t), 6.80 (2H, m), 7.10 (1H, d), 7.50 (1H, s). |
| **7** | H | 10-$OCH_3$ | –$(CH_2)_3$–COOH | 68 | $C_{17}H_{18}N_2O_4$ | ($CDCl_3$): 2.2 (2H, m), 2.4 (2H, m), 2.8 (4H, m), 3.9 (3H, s), 4.2 (2H, m), 6.7 (1H, s), 6.8 (2H, m), 7.2 (1H, m). |
| **8** | H | 7-$OCH_2$–$C_6H_5$ | –$CH_2$–COOH | 75 | $C_{21}H_{18}N_2O_4$ | 2.9 (4H, m), 4.8 (2H, s), 5.2 (2H, s), 6.9 (1H, s), 7.4 (8H, m), 13.1 (1H, s). |
| **9** | H | 8-$OCH_2$–$C_6H_5$ | –$CH_2$–COOH | 70 | $C_{21}H_{18}N_2O_4$ | 2.9 (4H, s), 4.8 (2H, s), 5.2 (2H, s), 6.9 (1H, s), 7.0 (2H, m), 7.4 (5H, m), 7.8 (1H, m), 13.0 (1H, s). |
| **10** | H | 9-$OCH_2$–$C_6H_5$ | –$CH_2$–COOH | 69 | $C_{21}H_{18}N_2O_4$ | 2.8 (4H, s), 4.8 (2H, s), 5.2 (2H, s), 6.9 (1H, s), 7.0 (1H, dd), 7.4 (7H, m), 13.0 (1H, s). |
| **11** | H | 10-$OCH_2$–$C_6H_5$ | –$CH_2$–COOH | 70 | $C_{21}H_{18}N_2O_4$ | 2.8 (4H, m), 4.7 (2H, s), 5.2 (3H, s), 6.9 (1H, s), 7.0 (1H, d), 7.2 (1H, d), 7.3 (4H, m), 7.6 (2H, m), 13.1 (1H, s). |
| **12** | H | 8-$NH_2$ | –$(CH_2)_3$–COOH | 72 | $C_{16}H_{17}N_3O_3$ | 2.3 (2H, m), 2.7 (4H, m), 4.1 (2H, m), 5.5 (2H, s), 6.4 (1H, s), 6.6 (1H, d), 6.8 (1H, s), 7.6 (1H, d). |
| **13** | H | 8-$NHCOCH_3$ | –$CH_2$–COOH | 77 | $C_{16}H_{15}N_3O_4$ | 2.1 (3H, s), 2.8 (4H, s), 4.8 (2H, s), 6.9 (1H, s), 7.5 (1H, d), 7.7 (1H, s), 7.9 (1H, d), 10.1 (1H, s), 13.0 (1H, s). |

Table 1 (*Continued*)

| Compd | R | $R^1$ | $R^2$ | % Yield [a] | Formula | $^1$H NMR |
|---|---|---|---|---|---|---|
| **14** | H | 8-$NHCOCH_3$ | –$(CH_2)_2$–COOH | 40 | $C_{17}H_{17}N_3O_4$ | 2.1 (3H, s), 2.8 (4H, m), 3.3 (4H, s), 4.3 (2H, t), 6.9 (1H, s), 7.5 (1H, d), 7.7 (1H, s), 7.9 (1H, d), 10.1 (1H, s). |
| **15** | H | 8-$NHCOCH_3$ | –$(CH_2)_3$–COOH | 72 | $C_{18}H_{19}N_3O_4$ | 2.1 (4H, m), 2.3 (2H, t), 2.8 (3H, s), 4.1 (2H, t), 6.4 (1H, s), 6.9 (1H, s), 7.5 (1H, d), 7.7 (1H, s), 7.9 (1H, d), 10.2 (1H, s). |
| **16** | H | 8-$NHSO_2CH_3$ | –$(CH_2)_3$–COOH | 40 | $C_{17}H_{19}N_3O_5S$ | ($CD_3OD$): 2.1 (2H, m), 2.5 (2H, t), 2.9 (4H, s), 3.1 (3H, s), 4.3 (2H, t), 6.8 (1H, s), 7.2 (2H, dd), 8.1 (1H, d). |
| **17** | H | 9-$NHCOCH_3$ | –$CH_2$–COOH | 77 | $C_{16}H_{15}N_3O_4$ | ($CD_3OD$): 2.2 (3H, s), 3.0 (4H, s), 5.0 (2H, s), 6.9 (1H, s), 7.3 (1H, d), 7.6 (1H, dd), 8.2 (1H, s). |
| **18** | H | 9-$NHCOCH_3$ | –$(CH_2)_3$–COOH | 72 | $C_{18}H_{19}N_3O_4$ | ($CD_3OD$):1.3 (2H, m), 2.1 (3H, m), 2.4 (2H, t), 2.9 (4H, s), 4.3 (2H, t), 6.8 (1H, s), 7.2 (1H, d), 7.6 (1H, dd), 8.2 (1H, s). |
| **19** | H | H | –$CH_2$–COOH 5,6-double bond | 74 | $C_{14}H_{10}N_2O_3$ | 4.8 (2H, s); 7.2 (1H, s); 7.4 (AB system, 2H), 7.6 (2H, m), 7.8 (1H, m); 8.5 (1H, m). |
| **20** | –COO–$(CH_2)_3$–$CH_3$ | H | –$CH_2$–COOH | 71 | $C_{19}H_{20}N_2O_5$ | 0.9 (3H, t), 1.3 (2H, m), 1.5 (2H, m), 2.7 (4H, m), 4.4 (2H, t), 4.8 (2H, s), 7.4 (3H, m), 8.0 (1H, m). |
| **21** | –$NH_2$ | H | –$CH_2$–COOH 5,6-double bond | 74 | $C_{14}H_{11}N_3O_3$ | 4.8 (2H, s); 7.3 (2H, s, exch. with $D_2O$); 7.5 (2H, AB system); 7.6 (2H, m); 7.7 (1H, m); 8.6 (1H, m). |
| **22** | H | 7-$OCH_3$ | –$CH_2$–CH=CH–COOH | 77 | $C_{17}H_{16}N_2O_4$ | 2.8 (4H, s), 3.8 (3H, s), 4.9 (2H, d), 5.7 (1H, d), 7.0 (2H, m), 7.3 (1H, q), 7.7 (2H, m). |
| **23** | H | 9-$OCH_3$ | –$CH_2$–CH=CH–COOH | 77 | $C_{17}H_{16}N_2O_4$ | 2.8 (4H, s), 3.8 (3H, s), 5.0 (2H, d), 5.8 (1H, d), 6.8 (1H, s), 6.9 (1H, dd), 7.1 (2H, m), 7.6 (1H, s). |
| **24** | H | 7-$OCH_3$ | –CH=CH–$CH_2$–COOH | 71 | $C_{17}H_{16}N_2O_4$ | ($CDCl_3$): 1.3 (3H, t), 2.8 (4H, m), 3.3 (2H, d), 3.9 (3H, s), 4.2 (2H, q), 6.5 (1H, m), 6.80 (1H, s), 7.0 (1H, d), 7.3 (1H, t), 7.7 (2H, m). |
| **25** | H | 7-$OCH_3$ | –$C(CH_3)_2$–COOH | 77 | $C_{17}H_{18}N_2O_4$ | 1.8 (6H, d), 2.8 (4H, s), 3.8 (3H, s), 6.9 (1H, s), 7.1 (1H, d), 7.9 (1H, d), 8.3 (1H, d). |
| **26** | H | 9-$OCH_3$ | –$CH(CH_2CH_3)$–COOH | 75 | $C_{17}H_{18}N_2O_4$ | 0.9 (3H, t), 2.2 (2H, q), 2.8 (4H, s), 3.8 (3H, s), 5.3 (1H, m), 6.9 (2H, m), 7.2 (1H, d), 7.4 (1H, s). |
| **27** | H | H | –$CH_2$–CN | 75 | $C_{14}H_{11}N_3O$ | ($CDCl_3$): 2.9 (4H, s), 5.1 (2H, s), 6.8 (1H, s), 7.2 (1H, s), 7.4 (2H, m), 8.1 (1H, m). |
| **28** | H | H | –$CH_2$–tetrazole | 76 | $C_{14}H_{12}N_6O$ | 2.9 (4H, s), 3.9 (1H, br. s, exch. with $D_2O$), 5.5 (2H, s), 6.9 (1H, s), 7.4 (3H, m), 7.9 (1H, m). |

[a] Yields are based on the 2-unsubstituted pyridazinones.

water and extracted with dichloromethane (3 × 10 ml). After drying ($Na_2SO_4$) and evaporation of the solvent, the residue was purified by trituration with diethyl ether or crystallized from acetone/petroleum ether (see Table

1 for chemical data). In the case of **20**, catalytic dehydrogenation was performed instead of hydrolysis.

*4.1.2. 2-Tetrazolylmethyl-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**28**)*

Sodium azide (0.326 g, 5.0 mmol) was added to a solution of trimethyltin chloride (1g, 5.0 mmol) in water (3 ml) and stirred for 3 h, then added to the cyano derivative **27** (0.1 g, 0.4 mmol) dissolved in toluene (3 ml) and the whole refluxed in a nitrogen athmosphere. After 24 h, the solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CH_2Cl_2$:$CH_3OH$ 8:2)

*4.1.3. 5-, 6-, 7-Methoxy- or -phenylmethoxy-1,2,3,4-tetrahydro-1-oxo-2-naphtylideneacetic acids (**52**–**57**)*

A solution of sodium hydroxide (23.2 mmol) in water (12 ml) and ethanol (6 ml) was added to a mixture of substituted tetralone (4.7 mmol) and glyoxylic acid (19.0 mmol) in water (8 ml) at r.t. The mixture was stirred at r.t. for 1 h, then heated at reflux for 2 h. After cooling, the solvent was removed under reduced pressure, the residue acidified with HCl 10 N, and the solid collected and purified by column chromatography ($CH_2Cl_2$:$CH_3OH$:Acetic acid 87.2:12.4:0.4).

*4.1.4. 5-Methoxy-1,2,3,4-tetrahydro-1-oxo-2-naphtylideneacetic acid (**52**)*

Yield 59%, m.p. 140°C; $^1$H NMR: 2.9 (2H, t), 3.3 (2H, dt,), 3.9 (3H, s), 6.6 (1H, t), 7.3 (1H, dd,), 7.4 (1H, t), 7.6 (1H, dd,), 13.0 (1H, s).

*4.1.5. 6-Methoxy-1,2,3,4-tetrahydro-1-oxo-2-naphtylideneacetic acid (**53**)*

Yield 50%, m.p. 170°C; $^1$H NMR: 2.9 (2H, t), 3.3 (2H, dt), 3.9 (3H, s), 6.6 (1H, t), 7.0 (2H, m), 7.9 (1H, d), 13.0 (1H, s).

*4.1.6. 7-Methoxy-1,2,3,4-tetrahydro-1-oxo-2-naphtylideneacetic acid (**54**)*

Yield 68%, m.p. 160°C; $^1$H NMR: 2.9 (2H, t), 3.3 (2H, dt), 3.8 (3H, s), 6.7 (1H, t), 7.2 (1H, dd), 7.3 (1H, d), 7.4 (1H, d), 13.0 (1H, s).

*4.1.7. 5-Phenylmethoxy-1,2,3,4-tetrahydro-1-oxo-2-naphtylideneacetic acid (**55**)*

Yield 29%, m.p. 163–5°C, $^1$H NMR: 2.9 (2H, t), 3.3 (2H, dt), 5.2 (2H, s), 6.6 (1H, t), 7.5 (8H, m), 13.0 (1H, broad s).

*4.1.8. 6-Phenylmethoxy-1,2,3,4-tetrahydro-1-oxo-2-naphtylideneacetic acid (**56**)*

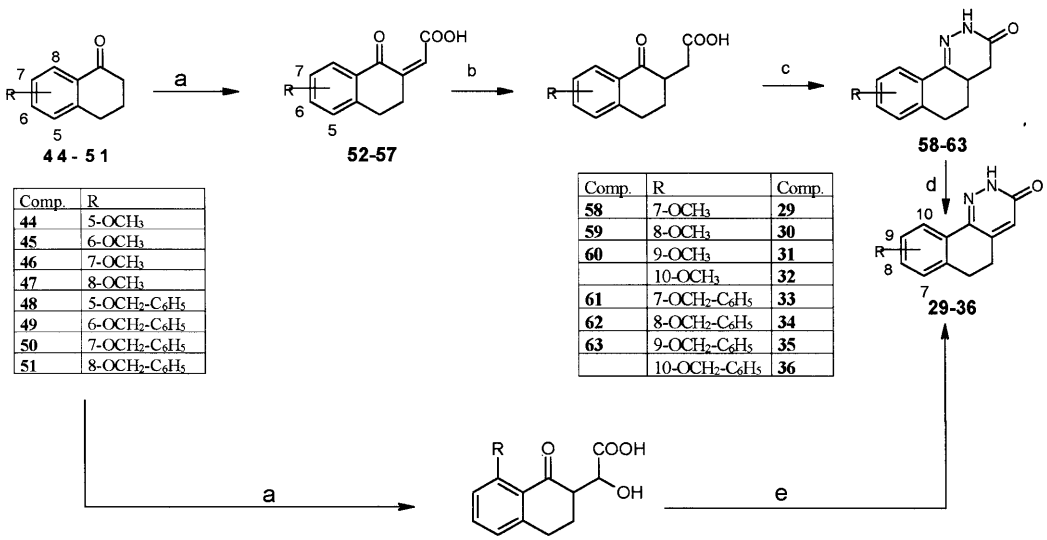
Yield 16%, m.p. 145–8°C, $^1$H NMR: 2.9 (2H, t), 3.3 (2H, broad t), 5.2 (2H, s), 6.7 (1H, t), 7.0 (2H, m), 7.5 (5H, m), 7.9 (1H, m), 13.0 (1H, broad s).

*4.1.9. 7-Phenylmethoxy-1,2,3,4-tetrahydro-1-oxo-2-naphtylideneacetic acid (**57**)*

Yield 30%, m.p. 138–40°C. $^1$H NMR: 2.9 (2H, t), 3.3 (2H, broad t), 5.2 (2H, s), 6.7 (1H, t), 7.4 (8H, m), 12.9 (1H broad s).

*4.1.10. 5-, 6-, 7-Methoxy- or -phenylmethoxy-4,4a,5,6-tetrahydrobenzo[h]cinnolin-3(2H)-ones (**58**–**63**)*

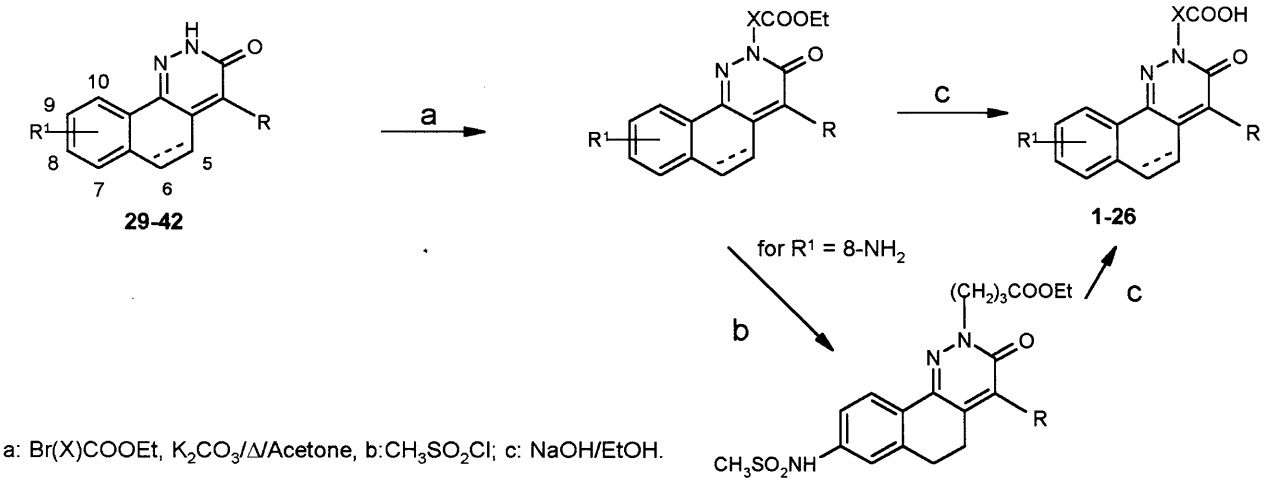
A mixture of the appropriate naphtylideneacetic acid (1.36 mmol) and zinc dust (2.75 mmol) in acetic acid (6



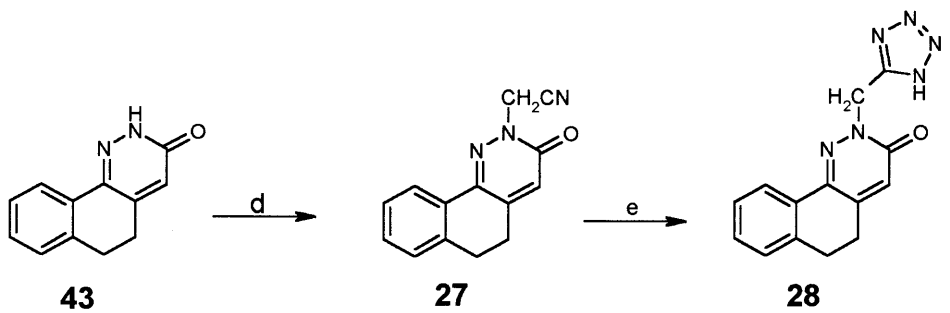

| Comp. | R |
|---|---|
| **44** | 5-$OCH_3$ |
| **45** | 6-$OCH_3$ |
| **46** | 7-$OCH_3$ |
| **47** | 8-$OCH_3$ |
| **48** | 5-$OCH_2$-$C_6H_5$ |
| **49** | 6-$OCH_2$-$C_6H_5$ |
| **50** | 7-$OCH_2$-$C_6H_5$ |
| **51** | 8-$OCH_2$-$C_6H_5$ |

| Comp. | R | Comp. |
|---|---|---|
| **58** | 7-$OCH_3$ | **29** |
| **59** | 8-$OCH_3$ | **30** |
| **60** | 9-$OCH_3$ | **31** |
| | 10-$OCH_3$ | **32** |
| **61** | 7-$OCH_2$-$C_6H_5$ | **33** |
| **62** | 8-$OCH_2$-$C_6H_5$ | **34** |
| **63** | 9-$OCH_2$-$C_6H_5$ | **35** |
| | 10-$OCH_2$-$C_6H_5$ | **36** |

Scheme 1.



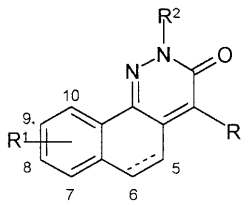

| Comp. | R | $R^1$ | Comp. | $X=(CH_2)_n$ | Comp. | X |
|---|---|---|---|---|---|---|
| **29** | H | 7-$OCH_3$ | **1**<br>**2** | n = 3<br>n = 4 | **22**<br>**24**<br>**25** | -$CH_2$-CH=CH-<br>-CH=CH-$CH_2$-<br>-$C(CH_3)_2$- |
| **30** | H | 8-$OCH_3$ | **3**<br>**4** | n = 3<br>n = 4 | | |
| **31** | H | 9-$OCH_3$ | **5**<br>**6** | n = 3<br>n = 4 | **23**<br>**26** | -$CH_2$-CH=CH-<br>-$CH(CH_2CH_3)$- |
| **32** | H | 10-$OCH_3$ | **7** | n = 3 | | |
| **33** | H | 7-$OCH_2$-$C_6H_5$ | **8** | n = 1 | | |
| **34** | H | 8-$OCH_2$-$C_6H_5$ | **9** | n = 1 | | |
| **35** | H | 9-$OCH_2$-$C_6H_5$ | **10** | n = 1 | | |
| **36** | H | 10-$OCH_2$-$C_6H_5$ | **11** | n = 1 | | |
| **37** | H | 8-$NH_2$ | **12**<br>**16**<br>(8$NHSO_2CH_3$) | n = 3<br>n = 3 | | |
| **38** | H | 8-$NHCOCH_3$ | **13**<br>**14**<br>**15** | n = 1<br>n = 2<br>n = 3 | | |
| **39** | H | 9-$NHCOCH_3$ | **17**<br>**18** | n = 1<br>n = 3 | | |
| **40** | H | H; 5,6-double bond | **19** | n = 1 | | |
| **41** | $COO(CH_2)_3CH_3$ | H | **20** | n = 1 | | |
| **42** | $NH_2$ | H; 5,6-double bond | **21** | n = 1 | | |

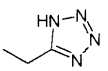



Scheme 2. (a) Glyoxylic acid/$OH^-$/Δ; (b) Zn/$CH_3COOH$, Δ; (c) $NH_2$–$NH_2$·$H_2O$/EtOH/Δ; (d) *m*-nitrobenzensulfonate/$OH^-$ or DDQ/Xylene; (e) $NH_2$–$NH_2$·$H_2O$/$CH_3COOH$/Δ.

ml) and water (3 ml) was heated at 70°C for 1 h. The insoluble was filtered off, water (10 ml) was added to the solution and the mixture was then extracted with dichloromethane (4 × 15 ml). The organic layer was dried ($Na_2SO_4$) and the solvent evaporated under reduced pressure. The residue was dissolved in ethanol (8 ml), added of hydrazine hydrate (1.94 mmol) and refluxed for 3 h. After

Table 2
ALR2 inhibition data of compounds **1**–**28**




| Compd | R | $R^1$ | $R^2$ | ALR2 inh. activity [a] |
|---|---|---|---|---|
| **1** | H | 7-$OCH_3$ | –$(CH_2)_3$–COOH | 19.62 (15.58–24.70) |
| **2** | H | 7-$OCH_3$ | –$(CH_2)_4$–COOH | 23.62 (19.51–28.59) |
| **3** | H | 8-$OCH_3$ | –$(CH_2)_3$–COOH | 25.81 (20.50–32.49) |
| **4** | H | 8-$OCH_3$ | –$(CH_2)_4$–COOH | 17.39 (12.89–23.46) |
| **5** | H | 9-$OCH_3$ | –$(CH_2)_3$–COOH | 4.79 (3.63–6.31) |
| **6** | H | 9-$OCH_3$ | –$(CH_2)_4$–COOH | 7.94 (6.32–9.97) |
| **7** | H | 10-$OCH_3$ | –$(CH_2)_3$–COOH | 73.84 (56.01–97.34) |
| **8** | H | 7-$OCH_2$-$C_6H_5$ | –$CH_2$–COOH | 6.37 (5.04–8.06) |
| **9** | H | 8-$OCH_2$-$C_6H_5$ | –$CH_2$–COOH | 7.85 (6.09–10.119 |
| **10** | H | 9-$OCH_2$-$C_6H_5$ | –$CH_2$–COOH | 38.75 (32.23–46.59) |
| **11** | H | 10-$OCH_2$-$C_6H_5$ | –$CH_2$–COOH | 30.47 (22.59–41.11) |
| **12** | H | 8-$NH_2$ | –$(CH_2)_3$–COOH | 31% inh. (140μM) |
| **13** | H | 8-$NHCOCH_3$ | –$CH_2$–COOH | 14.07 (11.68–16.95) |
| **14** | H | 8-$NHCOCH_3$ | –$(CH_2)_2$–COOH | 32% inh. (100 μM) |
| **15** | H | 8-$NHCOCH_3$ | –$(CH_2)_3$–COOH | 56.02 (44.50–70.52) |
| **16** | H | 8-$NHSO_2CH_3$ | –$(CH_2)_3$–COOH | 39.56 (33.13–47.23) |
| **17** | H | 9-$NHCOCH_3$ | –$CH_2$–COOH | 46.50 (35.27–61.30) |
| **18** | H | 9-$NHCOCH_3$ | –$(CH_2)_3$–COOH | 58.86 (49.07–70.60) |
| **19** | H | H | –$CH_2$–COOH 5,6–double bond | 6.53 (5.30–8.05) |
| **20** | –COO–$(CH_2)_3$–$CH_3$ | H | –$CH_2$–COOH | 34.35 (25.64–46.02) |
| **21** | –$NH_2$ | H | –$CH_2$–COOH 5,6-double bond | 35.36 (28.74–43.50) |
| **22** | H | 7-$OCH_3$ | –$CH_2$–CH=CH–COOH | 4.25 (3.65–4.95) |
| **23** | H | 9-$OCH_3$ | –$CH_2$–CH=CH–COOH | 8.99 (7.98–10.13) |
| **24** | H | 7-$OCH_3$ | –CH=CH–$CH_2$–COOH | 3.90 (3.13–4.86) |
| **25** | H | 7-$OCH_3$ | –$C(CH_3)_2$–COOH | 112. 0 (89.6–140) |
| **26** | H | 9-$OCH_3$ | –$CH(CH_2CH_3)$–COOH | 33% inh. (71 μM) |
| **27** | H | H | –$CH_2$–CN | 24% inh. (79 μM) |
| **28** | H | H |  | 45.57 (32.19–64.52) |
| **I** | H | H | –$CH_2$-COOH | 12.6 (7.14-22.1) [b] |
| **Sorbinil** | | | | 1.19 (1.04–1.36) |
| **Tolrestat** | | | | 0.096 (0.079–0.117) |

[a] $IC_{50}$ (95% C.L.) (μM) or percent inhibition (at a given μM concentration).
[b] From Ref. [2].

cooling, the precipitate was collected and washed with water.

*4.1.11. 7-Methoxy-4,4a,5,6-tetrahydrobenzo[h]cinnolin-3(2H)-one* ***(58)***

Yield 87%, m.p. 178°C (dec.); $^1$H NMR: 1.5 (1H, m), 2.3 (4H, m), 2.5 (1H, m), 3.1 (1H, dt), 3.8 (3H, s), 6.9 (1H, dd), 7.2 (1H, t), 7.6 (1H, dd), 10.9 (1H, s).

*4.1.12. 8-Methoxy-4,4a,5,6-tetrahydrobenzo[h]cinnolin-3(2H)-one* ***(59)***

Yield 77%, m.p. 180°C (dec.); $^1$H NMR: 1.5 (1H, m), 2.3 (3H, m), 2.8 (3H, m), 3.8 (3H, s), 6.8 (2H, m), 7.9 (1H, d), 10.8 (1H, s).

*4.1.13. 9-Methoxy-4,4a,5,6-tetrahydrobenzo[h]cinnolin-3(2H)-one* ***(60)***

Yield 73%, m.p. 155°C; $^1$H NMR: 1.5 (1H, m), 2.2 (3H, m), 2.8 (3H, m), 3.8 (3H, s), 6.9 (1H, dd), 7.2 (1H, d), 7.5 (1H, d), 10.9 (1H, s).

*4.1.14. 7-Phenylmethoxy-4,4a,5,6-tetrahydrobenzo[h]-cinnolin-3(2H)-one* ***(61)***

Yield 72%, m.p. 217°C; $^1$H NMR: 1.5 (1H, m), 2.3 (5H, m), 2.8 (1H, m), 5.1 (2H, s), 7.1 (1H, d), 7.2 (1H, t), 7.4 (5H, m), 7.7 (1H, d), 10.9 (1H, broad s).

*4.1.15. 8-Phenylmethoxy-4,4a,5,6-tetrahydrobenzo[h]-cinnolin-3(2H)-one (**62**)*

Yield 82%, m.p. 220°C; $^1$H NMR: 1.5 (1H, m), 2.6 (6H, m), 5.1 (2H, s), 6.9 (2H, m), 7.4 (5H, m), 7.9 (1H, d), 10.8 (1H, broad s).

*4.1.16. 9-Phenylmethoxy-4,4a,5,6-tetrahydrobenzo[h]-cinnolin-3(2H)-one (**63**)*

Yield 70%, m.p. 142–5°C; $^1$H NMR: 1.5 (1H, m), 2.5 (6H, m), 5.1 (2H, s), 7.0 (1H, dd), 7.2 (1H, d), 7.1 (5H, m), 7.6 (1H, d), 10.9 (1H, broad s).

*4.1.17. 7-, 8-, and 9-Methoxy-and 7-phenylmethoxy-5,6-dihydrobenzo[h]cinnolin-3(2H)-ones (**29**–**31**, **33**)*

The corresponding 4,4a-dihydroderivatives were transformed into **29**–**31** and **33** by a standard procedure, using sodium *m*-nitrobenzenesulfonate in alkaline medium [3].

*4.1.18. 7-Methoxy-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**29**)*

Yield 97%, m.p. 270°C, $^1$H NMR: 2.8 (4H, s), 3.8 (3H, s), 6.8 (1H, s), 7.0 (1H, dd), 7.3 (1H, t), 7.6 (1H, dd), 12.9 (1H, s).

*4.1.19. 8-Methoxy-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**30**)*

Yield 95%, m.p. 235°C, $^1$H NMR: 2.8 (4H, s), 3.8 (3H, s), 6.7 (1H, s), 6.9 (2H, m), 7.8 (1H, d), 12.9 (1H, s).

*4.1.20. 9-Methoxy-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**31**)*

Yield 90%, m.p. 245°C; $^1$H NMR: 2.8 (4H, s), 3.8 (3H, s), 6.8 (1H, s), 6.9 (1H, dd), 7.2 (1H, d), 7.4 (1H, d), 13.0 (1H, s).

*4.1.21. 7-Phenylmethoxy-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**33**)*

Yield 6.7%, m.p. 192°C, $^1$H NMR: 2.8 (4H, m), 5.2 (2H, s), 6.8 (1H, s), 7.4 (8H, m), 12.9 (1H, broad s).

*4.1.22. 8- and 9-Phenylmethoxy-5,6-dihydrobenzo[h]-cinnolin-3(2H)-one (**34**,**35**)*

A mixture of **62** or **63** (0.10 g, 0.32 mmol) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (0.070 g, 0.31 mmol) in xylene (5 ml) was refluxed for 12 h. After cooling, the precipitate was collected and purified by column chromatography ($CH_2Cl_2$:$CH_3OH$ 9.5:0.5)

*4.1.23. 8-Phenylmethoxy-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**34**)*

Yield 51%, m.p. 200–3°C, $^1$H NMR: 2.8 (4H, s), 5.2 (2H, s), 6.8 (1H, s), 7.0 (2H, m), 7.4 (5H, m), 7.8 (1H, m), 13.8 (1H, broad s).

*4.1.24. 9-Phenylmethoxy-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**35**)*

Yield 41%, m.p. 215–8°C, $^1$H NMR: 2.8 (4H, s), 5.2 (2H, s), 6.8 (1H, s), 7.0 (1H, dd), 7.4 (7H, m), 13.8 (1H, broad s).

*4.1.25. 10-Substituted-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**32**, **36**)*

A solution of sodium hydroxide (23.2 mmol) in water (12 ml) and ethanol (6 ml) was added to a mixture of substituted tetralone **47** or **51** (4.7 mmol) and glyoxylic acid (19.0 mmol) in water (8 ml) at r.t. The mixture was stirred at r.t. for 1 h, then heated at reflux for 2 h. After cooling, the solvent was removed under reduced pressure, the residue acidified with 10 N HCl, and the solid collected to give the corresponding α-hydroxy-2-tetralone acetic acid, which was dissolved in EtOH (10 ml). Hydrazine hydrate (5.47 mmol) was added and the solution refluxed for 3 h. After cooling the solvent was concentrated under reduced pressure. The precipitate thus formed was constituted by **32** or **36**.

*4.1.26. 10-Methoxy-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**32**)*

Yield 78%, m.p. 186–7°C; $^1$H NMR ($CDCl_3$): 2.8 (4H, m), 3.8 (3H, s), 6.8 (1H, s), 7.1 (3H, m), 13.0 (1H, s).

*4.1.27. 10-Phenylmethoxy-5,6-dihydrobenzo[h]cinnolin-3(2H)-one (**36**)*

Yield 27.5%, m.p. 180–2°C, $^1$H NMR ($CDCl_3$): 2.8 (4H, m), 5.2 (2H, s), 6.8 (1H, s), 7.2 (8H, m), 11.6 (1H, s).

*4.2. Enzyme section*

Calf lenses for the purification of ALR2 were obtained locally from freshly-slaughtered animals. The capsule was incised and the frozen lenses were suspended in sodium potassium phosphate buffer, pH 7 (standard buffer) containing 5 mM DTT (1 g tissue/3.5 ml) and stirred in an ice-cold bath for 1 h. The suspension was then centrifuged at 22 000 × *g* at 4°C for 40 min and the supernatant was subjected to ion exchange chromatography on DE52 [2]. Enzyme activity for all tested enzymes was measured by monitoring the change in absorbance at 340 nm, which accompanies the oxidation of NADPH catalyzed by ALR2. The assay was performed at 37°C as previously described, using 4.7 mM D,L-glyceraldehyde as substrate in 0.25 M sodium phosphate buffer pH 6.8, containing 0.38 M ammonium sulphate and 0.11 mM NADPH. The sensitivity of ALR2 to inhibition by different ARIs was tested in the above assay conditions by including the inhibitors dissolved in DMSO at the desired concentration in the

reaction mixture. DMSO in the assay mixture was kept at constant concentration of 1%. A reference blank containing all the above reagents except the substrate was used to correct for the non enzymatic oxidation of NADPH. $IC_{50}$ values (the concentration of the inhibitor required to produce 50% inhibition of the enzyme catalyzed reaction) were determined from least squares analyses of the linear portion of the log dose-inhibition curves. Each curve was generated using at least three concentrations of inhibitor causing an inhibition between 20 and 80% with two replicates at each concentration [2]. The 95% confidence limits (95% CL) were calculated from $T$-values for $n-2$, where $n$ is the total number of determinations [11].

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