Full Paper

Discovery of Novel Aldose Reductase Inhibitors Characterized by an Alkoxy-Substituted Phenylacetic Acid Core

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In continuation of our effort aimed towards the development of novel aldose reductase inhibitors, several phenylacetic acids bearing an alkoxy substituent in position 3 or 4, respectively, were prepared and screened. The latter represent formal ring opening products of the cyclohexylmethyloxyphenylacetic acids **IIa** and **IIb**, recently elaborated in our group. Out of these series, compounds **4aa** and **4ba** characterized by an *n*-heptyloxy subunit turned out to be the most potent inhibitors. Based on these unexpected results, we suggest that such an alkyl side chain acts as a useful surrogate for the 4-bromo-2-fluorobenzyl residue often found in potent aldose reductase inhibitors.

Keywords: Aldose reductase / Enzyme inhibitors / Inhibitor / Substituted phenylacetic acids

Received: March 21, 2006; accepted: May 27, 2006

DOI 10.1002/ardp.200600054

Introduction

Changes in human behaviour and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide [1]. The total number of people with diabetes is projected to rise from 171 million in the year 2000 to 366 million in 2030 [2]. All forms of diabetes mellitus are characterized by chronic hyperglycaemia and the development of diabetes-specific microvascular pathology in the retina, renal glomerulus, and peripheral nerve. As a consequence, diabetes mellitus is a leading cause of blindness, end stage renal disease, and a variety of debilitating neuropathies [3]. These long-term complications can be explained by several biochemical mechanisms, mainly enhanced formation of advanced glycation end-products, activation of protein kinase C isoforms, increase in oxidative stress, and activation of the polyol pathway [4]. This causal link between elevated glucose levels and the above-mentioned pathways can provide the basis for the development of new pharmacological agents. Inhibition of the key enzyme of the polyol pathway (*i.e.* aldose reductase, EC 1.1.1.21, ALR 2) has been recognized as one possible target for preventing the onset or progression of long-term complications. However, so far only a very small number of aldose reductase inhibitors (ARIs) have met the criteria of sufficient potency, oral activity, and an acceptable side-effect profile. Therefore, the area still requires further efforts, in particular with respect to the discovery of new lead structures.

Recently, we have designed several novel types of ARIs which, in fact, turned out to exhibit enzyme inhibition in the micromolar range [5 – 8]. Out of these series, compounds of type **I** were identified as the most potent inhibitors [7]. They appear to possess an acidic function and a lipophilic substructure R (*e.g.* 4-bromo-2-fluorobenzyl) which represent essential structural requisites for an ALR 2 inhibitory effect, in accordance with known pharmacophoric requirements [9 – 13]. On these considerations in a search for new ARIs, we have synthesized derivatives bearing different lipophilic moieties [8]. Surprisingly, within these series, we have found that a cyclohexylmethyl side chain seems to be a useful surrogate for the 4-bromo-2-fluorobenzyl residue. The latter, however, can be often found in potent aldose reductase inhibitors (*e.g.* zenarestat, ponalrestat, minalrestat, and AS-3201 [14]). Based on these results, additional modifications charac-

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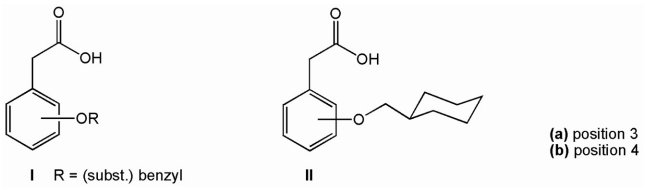


Figure 1. Structures of compounds of type **I** and **II**.

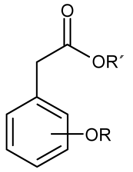
terized by formal replacement of the cyclic substructure by a chain were envisaged. In this context, ring-opened derivatives of compounds of type **IIa** and **IIb** (Fig. 1) bearing an *n*-heptyl or isobutyl residue instead of a cyclohexylmethyl moiety became an object of our interest. Since structure-activity relationships have shown that derivatives of 3-hydroxy- and 4-hydroxyphenylacetic acids are in general favourable to inhibition of the enzyme compared to the corresponding *ortho*-substituted isomers [8], 2-alkoxyphenylacetic acid derivatives were not investigated.

Results and discussion

The target compounds **4aa**, **4ab**, **4ba**, and **4bb** were prepared starting from the hydroxyphenylacetates of type **I** by *O*-alkylation in the presence of potassium carbonate in dry *N,N*-dimethylformamide followed by alkaline hydrolysis of the ester function. Inhibitory activities were evaluated in a spectrometric assay with D,L-glyceraldehyde as the substrate and NADPH as the cofactor. The biological results (IC_{50} values) are given in Table 1.

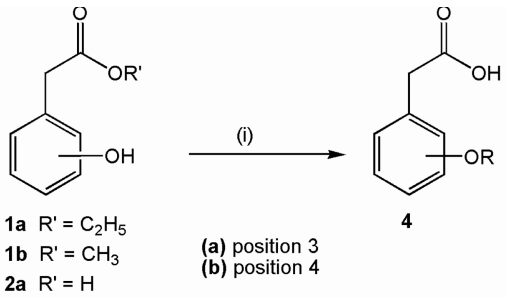
Whereas formal replacement of cyclohexylmethyl by isobutyl leads to a marked decrease in enzyme inhibition, interestingly, both *O*-heptyl substituted phenylacetic acids (**4aa**: IC_{50}: 21.4 μM, **4ba**: IC_{50}: 34.7 μM) exhibit higher activities than the parent compounds **IIa** (IC_{50}: 32.1 μM) and **IIb** (IC_{50}: 45.0 μM). Therefore, these results indicate that, within these series the aromatic subunit R in compounds of type **I** was found to be replaceable even by an *n*-heptyl side chain. Since to our knowledge, no comparable findings for aldose reductase inhibitors have been published before, further studies concerning the influence of the length of the side chain on the activity were performed. Thus, the corresponding derivatives bearing an alkyl substituent formally resulting from introduction as well as removal of one or two methylene units were of interest. These compounds became accessible by treatment of the hydroxy compound **2a** and **1b**, respectively, with the appropriate alkylating agent and subsequent hydrolysis of the ester function (Scheme 1). The biological data revealed that elongation of the side chain (*i.e.* **4ae**, **4af**, **4be**, and **4bf**) does not result in a significant change in inhibitory activity. On the contrary, shortening of the alkyl substituent (*i.e.* **4ac**, **4ad**, **4bc**, and **4bd**) exhibits an effect on enzyme inhibition. Here, reduction of each methylene group decreases the aldose reductase inhibitory effect (factor ranging from 1.3 to 2.6).

Table 1. Aldose reductase inhibition data of compounds of type **4a, 4b**, **I**, and **II.**



N°	R	Position of OR	IC_{50} (μM)* or Inhibition at 100 μM (%)
4aa	*n*-heptyl	3	21.4 (19.0–24.1)
4ab	isobutyl	3	42%
4ac	*n*-pentyl	3	69.4 (59.5–81.0)
4ad	*n*-hexyl	3	56.1 (47.7–66.1)
4ae	*n*-octyl	3	37.1 (28.5–48.4)
4af	*n*-nonyl	3	29.1 (17.9–47.7)
Ia [7]	4-Br-2-F-benzyl	3	20.9 (18.0–24.3)
IIa [8]	$-CH_2$-cyclohexyl	3	32.1 (23.1–44.5)
4ba	*n*-heptyl	4	34.7 (27.2–44.2)
4bb	isobutyl	4	86.5 (75.2–99.4)
4bc	*n*-pentyl	4	81.6 (72.8–91.6)
4bd	*n*-hexyl	4	45.7 (31.1–67.3)
4be	*n*-octyl	4	38.6 (29.8–49.8)
4bf	*n*-nonyl	4	42.9 (40.5–45.5)
Ib [7]	4-Br-2-F-benzyl	4	40.2 (38.3–42.1)
IIb [8]	$-CH_2$-cyclohexyl	4	45.0 (44.1–45.9)

* IC_{50} values (95% CL).

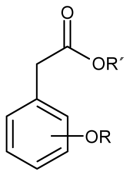


Scheme 1. Synthesis routes of compounds of type **4**. (i) 1) + R-I + K_2CO_3 in DMF, 2) alkaline hydrolysis.

Conclusions

In continuation of our program aimed at the development of *O*-substituted hydroxyphenylacetic acids as ARIs

Table 2. Data of compounds of type **3a** and **3b**.



Nº	R′ OR Formula (MW)	Yield Purification properties	IR (cm^{-1}) MS (EI)	^{1}H-NMR
3aa	C_2H_5 3-OC_7H_{15} $C_{17}H_{26}O_3$ (278.39)	58% cc with CH_2Cl_2/lp (1/1) colourless oil	1737 278	7.19 (d, *J* = 8.4 Hz, 1H, phenyl-H), 6.84-6.76 (m, 3H, phenyl-H), 4.15 (q, *J* = 7.2 Hz, 2H, O**CH$_2$**CH_3), 3.94 (t, *J* = 6.4 Hz, 2H, O**CH$_2$**CH_2), 3.57 (s, 2H, CH_2CO), 1.84 – 1.70 (m, 2H, CH_2), 1.52 – 1.21 (m, 11H, 4 × CH_2, OCH_2CH_3), 0.89 (t, *J* = 6.4 Hz, 3H, CH_3)
3ad	C_2H_5 3-OC_6H_{13} $C_{16}H_{24}O_3$ (264.37)	59% cc with CH_2Cl_2/lp (1/1) colourless oil	1736 264	7.19 (d, *J* =8.4 Hz, 1H, phenyl-H), 6.88 – 6.81 (m, 3H, phenyl-H), 4.15 (q, *J* = 7.1 Hz, 2H, O**CH$_2$**CH_3), 3.94 (t, *J* = 6.6 Hz, 2H, O**CH$_2$**CH_2), 3.57 (s, 2H, CH_2CO), 1.83 – 1.70 (m, 2H, CH_2), 1.52 – 1.21 (m, 9H, 3 × CH_2, O**CH$_2$**CH_3), 0.90 (t, *J* = 6.6 Hz, 3H, CH_3)
3ba	CH_3 4-OC_7H_{15} $C_{16}H_{24}O_3$ (264.37)	61% cc with CH_2Cl_2/lp (9/1) colourless oil	1740 264	7.21 – 7.14 (m, 2H, phenyl-H), 6.88 – 6.81 (m, 2H, phenyl-H), 3.93 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.68 (s, 3H, OCH_3), 3.55 (s, 2H, CH_2CO), 1.83 – 1.70 (m, 2H, CH_2), 1.52 – 1.26 (m, 8H, 4 x CH_2), 0.89 (t, *J* =6.4 Hz, 3H, CH_3)
3bd	CH_3 4-OC_6H_{13} $C_{15}H_{22}O_3$ (250.34)	63% cc with CH_2Cl_2/lp (9/1) colourless oil	1741 250	7.21 – 7.14 (m, 2H, phenyl-H), 6.88 – 6.81 (m, 2H, phenyl-H), 3.93 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.68 (s, 3H, OCH_3), 3.55 (s, 2H, CH_2CO), 1.83 – 1.69 (m, 2H, CH_2), 1.52-1.26 (m, 6H, 3 × CH_2), 0.90 (t, *J* = 6.6 Hz, 3H, CH_3)

we have recently reported that formal replacement of the (substituted) benzyl moiety by cyclohexylmethyl resulted in active compounds **IIa** and **IIb** [8]. In view of this, we have now investigated whether a cyclic moiety is an essential substructure for enzyme inhibition. Interestingly, derivatives bearing the *n*-heptyl side chain which represents a form of the ring opened analogue of cyclohexylmethyl exhibit even higher activities (**4aa**: IC_{50} = 21.4 μM, **4ba**: IC_{50} = 34.7 μM). Moreover, it should be noted that the inhibitory effect of these compounds is comparable with those of the 4-bromo-2-fluorobenzylated congeners **Ia** (IC_{50} = 20.9 μM) and **Ib** (IC_{50} = 40.2 μM). To our knowledge, this structural modification is unique in the class of ARIs. Therefore, we intend to study the utility of an *n*-alkyl as a bioisoster for the 4-bromo-2-fluorobenzyl moiety in highly potent ARIs like zenarestat.

The authors wish to acknowledge Schlachthof Salzburg-Bergheim (Austria) (KR Ing. Sebastian Grießner and Mag. Verena Klob) for providing calf lenses.

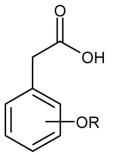
Experimental

Chemistry

Melting points were determined with a Kofler hot-stage microscope (C. Reichert, Vienna, Austria) and are uncorrected. Infrared spectra (KBr pellets) were recorded on a Mattson Galaxy Series FTIR 3000 spectrophotometer (Mattson Instruments, Inc., Madison, WI, USA). Mass spectra were obtained on a Finnigan MAT SSQ 7000 spectrometer (EI, 70 eV or CI, 200 eV, reactant gas: methane; Thermo Electron Corporation, Bremen, Germany). The ^{1}H-NMR spectra were recorded in $CDCl_3$ solution in 5 mm tubes at 30°C on a Varian Gemini 200 spectrometer (199.98 MHz; Varian Inc., Palo Alto, CA, USA) with the deuterium signal of the solvent as the lock and TMS as internal standard. Chemical shifts are expressed in parts per million. Reactions were monitored by TLC using Polygram® SIL G/UV_{254} (Macherey-Nagel) plastic-backed plates (0.25 mm layer thickness). The yields given are not optimized. Light petroleum refers to the fraction of b.p. 40 – 60°C. Elemental analyses were performed by Mag. J. Theiner, Mikroanalytisches Laboratorium, Faculty of Chemistry, University of Vienna, Austria and the data for C and H are within ± 0.4% of the calculated values.

The following compounds are already known but not tested as aldose reductase inhibitors: (4-heptyloxyphenyl)acetic acid **4ba**

Table 3. Data of compounds of type **4a** and **4b**.



N°	OR Formula (MW)	Yield Purification properties	IR (cm^{-1}) MS (CI)	^{1}H-NMR
4aa	3-OC_7H_{15} $C_{15}H_{22}O_3$ (250.34)	88% recryst. from dipe/lp colourless crystals mp 82 – 85°C	1694 251	7.25 – 7.18 (m, 1H, phenyl-H), 6.86 – 6.78 (m, 3H, phenyl-H), 3.93 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.61 (s, 2H, CH_2CO), 1.84 – 1.70 (m, 2H, CH_2), 1.47-1.30 (m, 8H, 4 × CH_2), 0.89 (t, *J* = 6.6 Hz, 3H, CH_3)
4ab	3-$OCH_2CH(CH_3)_2$ $C_{12}H_{16}O_3$ (208.26)	96% recryst. from dipe/lp colourless crystals mp 41 – 44°C	1709 209	7.27 – 7.19 (m, 1H, phenyl-H), 6.87 – 6.79 (m, 3H, phenyl-H), 3.71 (d, *J* = 6.6 Hz, 2H, OCH_2), 3.62 (s, 2H, CH_2CO), 2.17 – 1.97 (m, 1H, CH), 1.02 (d, *J* = 7.0 Hz, 6H, 2 × CH_3)
4ac	3-OC_5H_{11} $C_{13}H_{18}O_3$ (222.29)	86% recryst. from lp colourless crystals mp 82 – 85°C	1699 223	7.27 – 7.18 (m, 1H, phenyl-H), 6.86 – 6.78 (m, 3H, phenyl-H), 3.94 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.61 (s, 2H, CH_2CO), 1.85 – 1.71 (m, 2H, CH_2), 1.52 – 1.28 (m, 4H, 2 × CH_2), 0.93 (t, *J* = 7.2 Hz, 3H, CH_3)
4ad	3-OC_6H_{13} $C_{14}H_{20}O_3$ (236.31)	71% recryst. from dipe/lp colourless crystals mp 80 – 83°C	1698 237	9.74 (br s, 1H, COOH), 7.23 – 7.18 (m, 1H, phenyl-H), 6.86 – 6.79 (m, 3H, phenyl-H), 3.94 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.61 (s, 2H, CH_2CO), 1.84 – 1.70 (m, 2H, CH_2), 1.49–1.29 (m, 6H, 3 × CH_2), 0.90 (t, *J* = 6.6 Hz, 3H, CH_3)
4ae	3-OC_8H_{17} $C_{16}H_{24}O_3$ (264.37)	80% recryst. from lp colourless crystals mp 83 – 84°C	1698 265	7.27 – 7.19 (m, 1H, phenyl-H), 6.86 – 6.78 (m, 3H, phenyl-H), 3.94 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.61 (s, 2H, CH_2CO), 1.84 – 1.70 (m, 2H, CH_2), 1.48 – 1.24 (m, 10H, 5 × CH_2), 0.89 (t, *J* = 6.6 Hz, 3H, CH_3)
4af	3-OC_9H_{19} $C_{17}H_{26}O_3$ (278.39)	82% recryst. from lp colourless crystals mp 83 – 85°C	1698 279	7.26 – 7.18 (m, 1H, phenyl-H), 6.86 – 6.78 (m, 3H, phenyl-H), 3.94 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.61 (s, 2H, CH_2CO), 1.83 – 1.70 (m, 2H, CH_2), 1.48 – 1.28 (m, 12H, 6 × CH_2), 0.88 (t, *J* = 6.4 Hz, 3H, CH_3)
4ba	4-OC_7H_{15} $C_{15}H_{22}O_3$ (250.34)	76% recryst. from dip/lp colourless crystals mp 82 – 84°C	1701 251	9.74 (brs, 1H, COOH), 7.18 (d, *J* = 8.6 Hz, 2H, phenyl-H), 6.85 (d, *J* = 8.6 Hz, 2H, phenyl-H), 3.93 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.57 (s, 2H, CH_2CO), 1.83 – 1.70 (m, 2H, CH_2), 1.46 – 1.30 (m, 8H, 4 × CH_2), 0.89 (t, *J* = 6.6 Hz, 3H, CH_3)
4bb	4-$OCH_2CH(CH_3)_2$ $C_{12}H_{16}O_3$ (208.26)	82% recryst. from dipe/lp colourless crystals mp 79 – 84°C	1709 209	7.18 (d, *J* = 8.6 Hz, 2H, phenyl-H), 6.85 (d, *J* = 8.6 Hz, 2H, phenyl-H), 3.70 (d, *J* = 6.2 Hz, 2H, OCH_2), 3.58 (s, 2H, CH_2CO), 2.17 – 1.97 (m, 1H, CH), 1.01 (d, *J* = 6.6 Hz, 6H, 2 × CH_3)
4bc	4-OC_5H_{11} $C_{13}H_{18}O_3$ (222.29)	73% recryst. from lp colourless crystals mp 79 – 81°C	1696 223	7.21 – 7.14 (m, 2H, phenyl-H), 6.89 – 6.81 (m, 2H, phenyl-H), 3.93 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.58 (s, 2H, CH_2CO), 1.84 – 1.70 (m, 2H, CH_2), 1.52-1.27 (m, 4H, 2 × CH_2), 0.93 (t, *J* = 7.2 Hz, 3H, CH_3)
4be	4-OC_8H_{17} $C_{16}H_{24}O_3$ (264.37)	69% recryst. from lp colourless crystals mp 80 – 82°C	1698 265	7.21 – 7.14 (m, 2H, phenyl-H), 6.89 – 6.81 (m, 2H, phenyl-H), 3.93 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.58 (s, 2H, CH_2CO), 1.83 – 1.69 (m, 2H, CH_2), 1.48 – 1.29 (m, 10H, 5 × CH_2), 0.89 (t, *J* = 6.4 Hz, 3H, CH_3)
4bf	4-OC_9H_{19} $C_{17}H_{26}O_3$ (278.39)	60% recryst. from lp colourless crystals mp 84 – 86°C	1701 279	7.21 – 7.14 (m, 2H, phenyl-H), 6.89 – 6.81 (m, 2H, phenyl-H), 3.93 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.58 (s, 2H, CH_2CO), 1.83 – 1.69 (m, 2H, CH_2), 1.47-1.28 (m, 12H, 6 × CH_2), 0.89 (t, *J* = 6.4 Hz, 3H, CH_3)

lp = light petroleum; dipe = diisopropyl ether.

[15, 16], (4-pentyloxyphenyl)acetic acid **4bc** [15, 17], (4-hexyloxyphenyl)acetic acid **4bd** [15], (4-octyloxyphenyl)acetic acid **4be** [15, 16], (4-*iso*-butoxyphenyl)acetic acid **4bb** [17], (4-nonyloxyphenyl)acetic acid **4bf** [16]. However, with the exception of **4bd** [18] no spectroscopic data are given.

*General procedure for O-alkylation of **1a** and **1b***

Powdered potassium carbonate (4 equiv.) was added to a solution of the appropriate hydroxy compound **1a** or **1b** (2 equiv., 5.6 mmol) in 10 mL of dry *N*,*N*-dimethylformamide under atmosphere of nitrogen. After stirring for 30 min at room temperature, one equivalent of the appropriate alkyliodide was added and the mixture was stirred until TLC indicated no further conversion (room temperature to 50°C). Then, the mixture was poured into cold water, acidified with 2N HCl and the product was extracted exhaustively with diethyl ether or dichloromethane, respectively. The organic layer was washed successively with 2N NaOH, water and brine, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue thus obtained was purified to give **3aa**, **3ad**, **3ba**, and **3bd** (Table 2) or directly used for further conversion (**3ab** and **3bb**).

*General procedure for O-alkylation of **2***

In analogy to the procedure described above, *O*-alkylation was performed by reaction of **2a** (1 equiv., 2.0 mmol) with the appropriate alkyliodide (2.2 equiv.) to give the corresponding alkyl 3-alkyloxyphenylacetates which were directly used for further conversion.

*General procedure for the synthesis of the alkoxyphenylacetic acids of type **4a** and **4b***

A solution of the appropriate ester in ethanol (5 mL/mmol) was treated with 2N NaOH (1.2 equivalents) and stirred overnight at room temperature. The solvent was then evaporated, the residue treated with a small amount of water and the pH adjusted to 5 with 2N HCl. The mixture thus obtained was extracted with diethyl ether or ethyl acetate, respectively; the organic layer was washed successively with water and brine, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue thus obtained was purified as described in Table 3.

Aldose reductase inhibitory assay

NADPH, D,L-glyceraldehyde and dithiothreitol (DTT) were purchased from Sigma Chemical Co. DEAE-cellulose (DE-52) was obtained from Whatman. Sorbinil was a gift from Prof. Dr. Luca Costantino, University of Modena (Italy) and was used as standard [IC_{50} = 0.9 (±0.3) μM]. All other chemicals were commercial samples of good grade. Calf lenses for the isolation of ALR 2 were obtained locally from freshly slaughtered animals. The enzyme was purified by a chromatographic procedure as previously described [19]. Briefly, ALR 2 was released by carving the capsule and the frozen lenses were suspended in potassium phosphate buffer pH 7 containing 5 mM DTT and stirred in an ice-cold bath for 2 h. The suspension was centrifuged at 4000 rpm at 4°C for 30 min and the supernatant was subjected to ion exchange chromatography on DE-52. Enzyme activity was assayed spectrophotometrically on a Cecil Super Aurius CE 3041 spectrophotometer (Cecil Instruments, Cambridge, England) by measuring the decrease in absorption of NADPH at 340 nm which accompanies the oxidation of NADPH catalyzed by ALR 2. The assay was performed at 37°C in a reaction mixture containing 0.25 M potassium phosphate buffer pH 6.8, 0.38 M ammonium sulfate, 0.11 mM NADPH, and 4.7 mM D,L-glyceraldehyde as substrate in a final volume of 1.5 mL. All inhibitors were dissolved in DMSO. The final concentration of DMSO in the reaction mixture was 1%. To correct for the nonenzymatic oxidation of NADPH, the rate of NADPH oxidation in the presence of all the components except the substrate was subtracted from each experimental rate. Each dose-effect curve was generated using at least three concentrations of inhibitor causing an inhibition between 20 and 80%. Each concentration was tested in duplicate and IC_{50} values as well as the 95% confidence limits (95% CL) were obtained by using CalcuSyn software [20] for dose effect analysis.

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