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New cyclopentane derivatives as inhibitors of steroid metabolizing enzymes AKR1C1 and AKR1C3

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ABSTRACT

A series of cyclopentane derivatives was synthesized and evaluated for inhibition of the steroid metabolizing enzymes AKR1C1 and AKR1C3. Selective inhibitors that are active in the low micromolar range were identified. These compounds represent promising starting points in the development of new anticancer agents for the treatment of hormone-dependent forms of cancer and other diseases where AKR1C1 and AKR1C3 are involved.

1. Introduction

Hydroxysteroid dehydrogenases (HSDs) are crucial enzymes that are involved in both the synthesis and inactivation of all classes of steroid hormones. HSDs belong to two distinct protein superfamilies: the short-chain dehydrogenase/reductase (SDR) [1,2] superfamily and the aldo–keto reductase (AKR) superfamily [3]. Human AKR HSDs belong to the AKR1C subfamily and regulate the concentrations of active and inactive androgens, estrogens and progestins in target tissues, and thus regulate ligand occupancy and *trans*-activation of the nuclear steroid hormone receptors [4–6].

AKR1C1 acts preferentially as a 20α-HSD and inactivates progesterone by converting it to 20α-hydroxyprogesterone (20α-OHP; Fig. 1), which has a low affinity for progesterone receptors [6]. AKR1C1 converts 3α,5α-tetrahydroprogesterone (5α-THP), an allosteric modulator of the γ-aminobutyric acid $(GABA)_A$ receptor into the inactive 5α-pregnane-3α,20α-diol. It thereby exhibits anaesthetic, analgesic, anxiolytic and anticonvulsant effects [6–8]. Inhibitors of AKR1C1 are thus very interesting as potential agents for the treatment of endometrial cancers, premenstrual syndrome, catamenial epilepsy, and depressive disorders, and for the maintenance of pregnancy [9,10].

AKR1C3 is a peripheral 17β-HSD (type 5) that reduces the weak androgen androstenedione into the potent androgen testosterone, and the weak estrogen estrone into the potent estrogen 17β-estradiol (Fig. 1) [11]. AKR1C3 is thus an interesting target for the development of agents for treating hormone-dependent forms of cancer, such as prostate, breast and endometrial cancers. AKR1C3 also catalyzes the reduction of 3-ketosteroids, 20-ketosteroids and prostaglandin D_2 (PGD_2), so it has been known variously as 3α-HSD type 2 [12] and PGD_2 11-ketoreductase [13]. By converting PGD_2 into $PGF_{2\alpha}$, AKR1C3 prevents the conversion of PGD_2 into 15-$\Delta^{12,14}$-PGJ_2, which is a natural ligand for the peroxisome-proliferator-activated receptor-γ (PPARγ). Inhibitors of AKR1C3 are thus potential antineoplastic agents also, because they can indirectly activate the PPARγ receptor by diverting PGD_2 catabolism to the generation of J-series prostanoids. Activation of the PPARγ receptor induces differentiation, is anti-proliferative, and causes apoptosis in many cell types and cancers [14,15].

Although both AKR1C1 and AKR1C3 are promising therapeutic targets, only a few inhibitors have been reported to date. Dietary phytoestrogens were shown to inhibit both of these enzymes in low micromolar concentrations [16,17], as were some other small molecule compounds, including benzodiazepines [18], benzofuranes and

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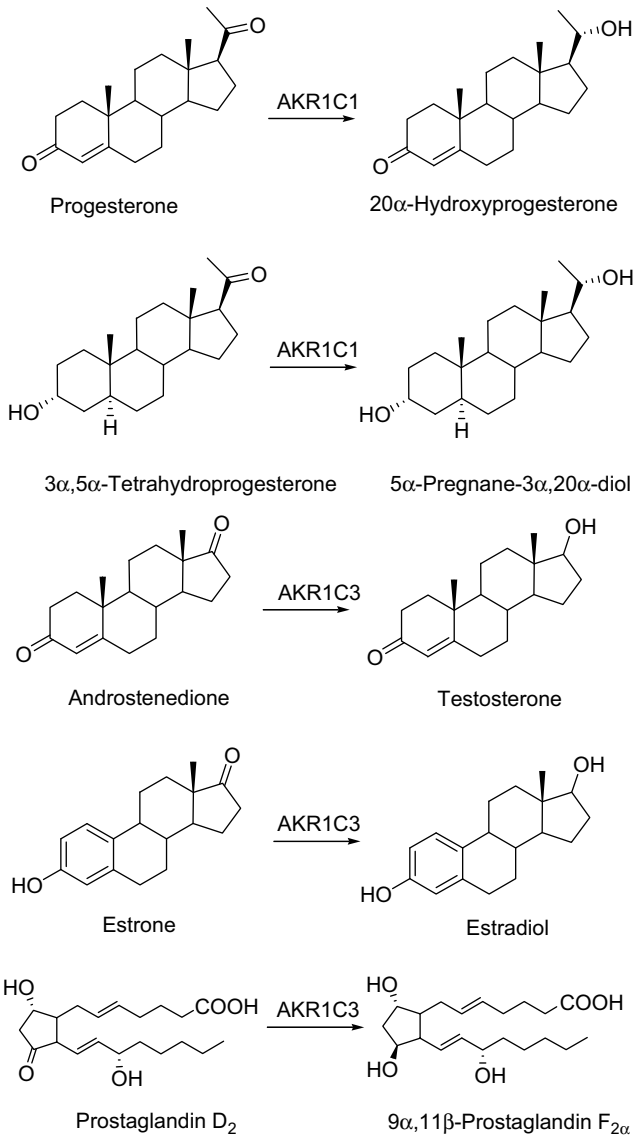


Fig. 1. Reactions catalyzed by AKR1C1 and AKR1C3 *in vivo*.

phenolphthalein derivatives [12]. Indomethacin, flufenamic acid, *N*-phenylanthranilic acid derivatives and some related non-steroidal anti-inflammatory agents are very potent inhibitors [13,19–21]. Steroid carboxylates [19], benzoyl benzoic acids [21], 3-phenoxybenzoic acid [21] and cinnamic acids [22] have also been reported to inhibit AKR1C3. Recently, structurally diverse inhibitors of AKR1C1 were also discovered by two virtual high-throughput screenings [23,24] and synthetic efforts [25]. The X-ray crystal structures of AKR1C1 in complex with 20α-OHP [26] and AKR1C3 complexed with indomethacin [20a], flufenamic acid [20], PGD_2 [27], rutin [27] and androstenedione [28] were reported, providing an excellent basis for the structure-based design of new and improved inhibitors. Furthermore, an indomethacin analogue as a selective inhibitor of aldo–keto reductase 1C3 (type 2 3α-HSD, type 5 17β-HSD, and prostaglandin F synthase) have been discussed by Penning et al. [20b].

2. Chemistry

The synthesis of all of these compounds is presented in Schemes 1–3. The benzyl-substituted cyclopentane derivatives were synthesized according to Scheme 1. 2-(4-Chlorobenzylidene)cyclopentanone (**2**) was obtained by base-promoted aldol condensation of cyclopentanone **1** and 4-chlorobenzaldehyde (Scheme 1). Further, reductions under Luche conditions [29] were carried out at room temperature in methanol or ethanol, giving the allylic ethers **3** and **4**, respectively. Treatment of **1** with LDA at −5 °C with the subsequent addition of benzyl bromide resulted in the formation of a mixture of benzyl-substituted cyclopentanones (**5**–**8**) that were partially separated by column chromatography (SiO_2). An inseparable mixture of isomers **6** and **7** (formed in the ratio 5:1) was taken directly into the next step. Benzylation of **1** was followed by reduction under Luche conditions, producing the desired cyclopentanol derivatives **9**–**11** in reasonable yields (Scheme 1).

The common precursors of several substrates were aziridine **12**, which was easily accessible by Sharpless aziridination [30], and the epoxy derivative **13** (Scheme 2). Tosylamino functionalised cyclopentane derivatives **14** and **15** were prepared from **12** *via* nucleophilic aziridine ring opening, providing these products at good yields [31]. The selective hydrolysis of **14** resulted in the formation of the corresponding amide **16** and carboxylic acid **17** [32] (Scheme 2). Treatment of the mixture of the epoxide **13** and aniline with equimolar amounts of $ZrCl_4$ proved to be an efficient method for producing the desired amino alcohol **18** at good yield [33]. However, use of $BiCl_3$ [34] as a Lewis acid was much less efficient for this epoxide ring opening, giving **18** only at a 53% yield.

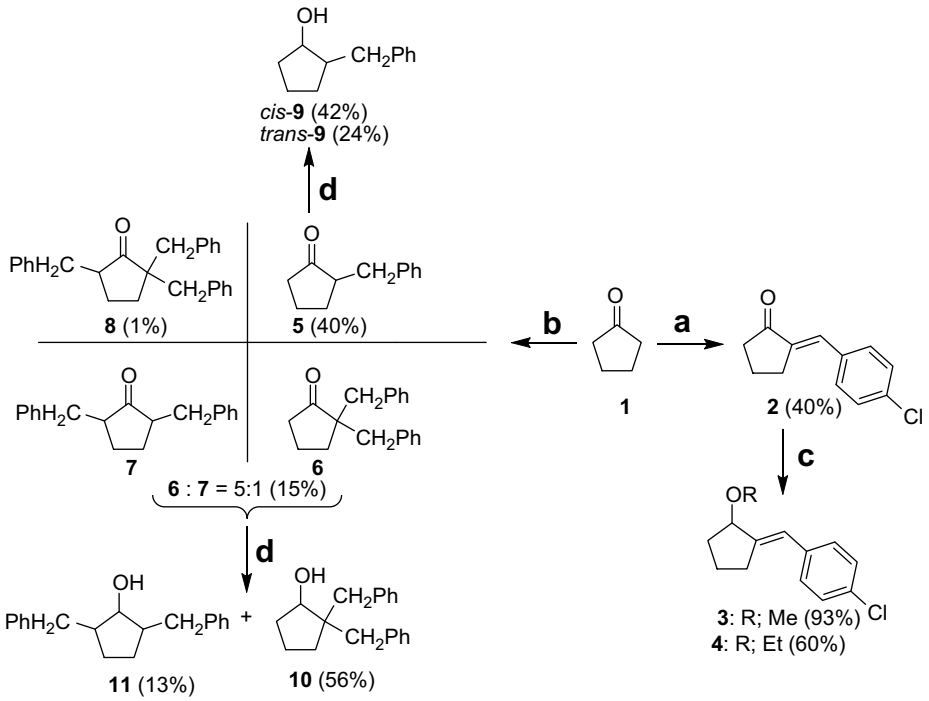
The preparation of 3-phenylcyclopentanecarboxylic acid (**20**) is presented in Scheme 3 and it is based on peroxyselenium-promoted 4-phenylcyclohexanone (**19**) ring contraction [35], deriving the desired **20** at moderate yields.

3. Results and discussion

In our search for new inhibitors of the AKR1C1 and AKR1C3 isoforms as potential anticancer agents, we decided to investigate only compounds that consist of a non-steroid core, and are thus devoid of residual steroidogenic activity. Since one of the substrates of AKR1C3 is a cyclopentane-containing PGD_2 (Fig. 1), we focused our attention on derivatives of cyclopentane. Preliminary docking experiments revealed that correctly substituted cyclopentanes should bind to the active sites of AKR1C1 and AKR1C3 and inhibit their enzymatic activities. To confirm this hypothesis, a series of cyclopentane derivatives was synthesized and evaluated for inhibition of AKR1C1 and AKR1C3.

Most of the compounds synthesized in the present study were evaluated for their inhibitory activities against recombinant human AKR1C1 and AKR1C3 (Table 1). Of the 4-chlorobenzylidene cyclopentane derivatives evaluated, ketone **2** was the most active inhibitor of AKR1C1, with a K_i value of 17.2 μM, K_i value for AKR1C3 was 33.4 μM. In a kinetic experiment, competitive inhibition pattern was obtained for AKR1C1 with compound **2** (Section 5.2.2). For the remainder of the compounds K_i values were calculated as described under Experimental protocols. Methyl ether **3** was the best inhibitor of AKR1C3 arising from this study with K_i value of 16.2 μM. As this compound has a lower affinity towards AKR1C1 it represents an excellent starting point for development of selective inhibitors of AKR1C3. Ethyl ether **4** inhibited both AKR1C1 and AKR1C3, with K_i values of 30.5 μM and 20.5 μM, respectively.

Cyclopentanone derivatives **5** and **8** were insoluble. Cyclopentanol derivatives **9**–**11**, tosylate **12** and aminocyclopentanes **14**–**18** were poor inhibitors of both AKR1C1 and AKR1C3. The only exceptions here are the compounds **10** and **11**, which inhibited AKR1C3 for 67% and 46% at 50 μM, respectively. However, due to poor solubility of the compounds at higher concentrations we were unable to determine the IC_{50} and K_i values. Interesting inhibitory



Scheme 1. a) NaOH, 4-chlorobenzaldehyde, H_2O, 25 °C, 14 h; b) LDA, BnBr, THF, −5 °C, 1 h, 25 °C, 24 h; c) $NaBH_4$, $CeCl_3 \times 6H_2O$, ROH, 25 °C, 2 h then HCl (1 M), 15 h 25 °C; d) $NaBH_4$, $CeCl_3 \times 6H_2O$, MeOH, 25 °C, 1.5 h.

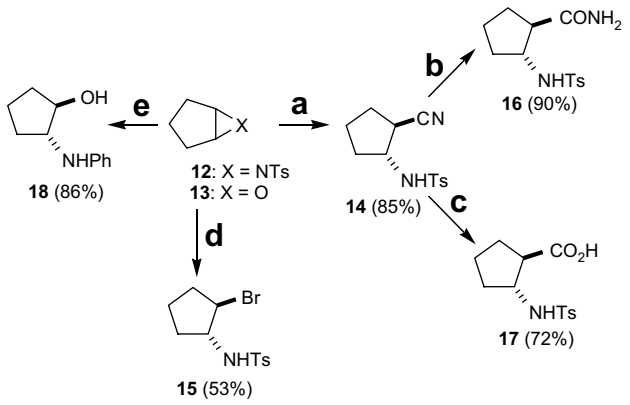
activities were also seen for 3-phenylcyclopentanecarboxylic acid (**20**) and its isomer, the commercially available 1-phenylcyclopentanecarboxylic acid (**21**). The former inhibited both AKR1C1 and AKR1C3 (K_i values of 48.5 μM and 69.6 μM, respectively), while the latter was inhibitor of AKR1C1 ($K_i = 35.8$ μM) with no affinity towards AKR1C3.

The X-ray crystal structures of AKR1C1 and AKR1C3 reveal substrate-binding sites that consist mainly of hydrophobic aromatic amino acid side chains (Leu54, His222, Trp227, Thr307 and Leu308 for AKR1C1; Tyr24, Tyr55, Leu54, Trp227 and Phe306 for AKR1C3). The four conserved amino acids Asp50, Tyr55, Lys84 and His117 have been proposed to form a catalytic tetrad that is involved in the oxidation of alcohol or reduction of ketone functional groups *via* a "push–pull" mechanism [36]. To investigate the possible binding mode of the best inhibitor of AKR1C1, compound **21** was docked into the AKR1C1 active site (pdb code 1MRQ, co-crystal structure with 20α-OHP) using AutoDock 3.0 with the Lamarckian genetic algorithm [37]. According to the docking calculations, inhibitor **21** should occupy the bottom of the hydrophobic pocket of the active site, making hydrophobic interactions with Leu54, Trp227 and Leu308 (Fig. 2). The carboxylate moiety of inhibitor is pointing towards the catalytic tetrade and the nicotinamide moiety of the coenzyme (which form the oxyanion hole). This highest ranked docking pose had a mean docked energy of −6.53 kcal/mol and was found six times out of 100 docking runs.

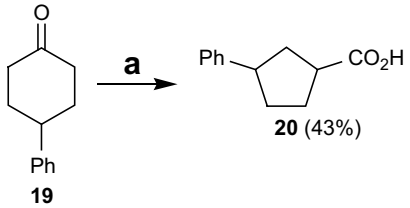
Compound **3** was docked into the active site of AKR1C3 (pdb code 1RY0, co-crystal structure with prostaglandin D2). The docking position is presented in Fig. 3. Also inhibitor **3** is located at the bottom of the enzyme active site where it makes hydrophobic interactions with Tyr24, Tyr55 and Trp227. This docking pose was found as the lowest energy pose with the predicted docked energy of −7.91 kcal/mol and was found eight times out of 100 runs.

4. Conclusion

To conclude, we have presented the synthesis and biochemical evaluation of new selective inhibitors of the steroid metabolizing enzymes AKR1C1 and AKR1C3 that were based on the cyclopentane scaffold. These compounds represent promising starting points for development of agents for treating hormone-dependent forms of cancers and other diseases where AKR1C1 and AKR1C3 are involved.



Scheme 2. a) TMSCN, TBAF, THF, 25 °C, 4 days; b) HCl (conc.), 25 °C, 21 h; c) HCl (conc.), 80 °C, 9 h; d) PPh_3, CBr_4, MeOH, MW, 130 °C, 10 min.; e) $PhNH_2$, $ZrCl_4$, 25 °C, 0.5 h.



Scheme 3. a) $(PhSe)_2$, H_2O_2 (30%), *t*-BuOH, reflux, 4 days.

Table 1
Inhibitors of AKR1C1 and AKR1C3.

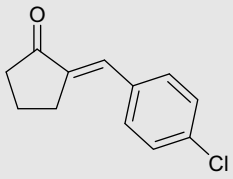
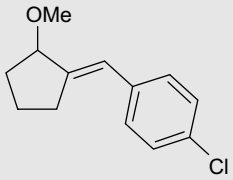
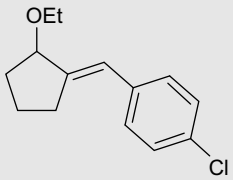
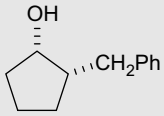
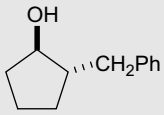
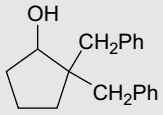
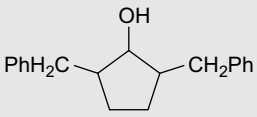
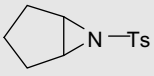
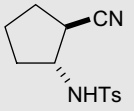
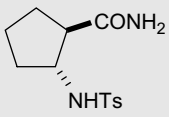
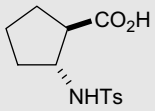
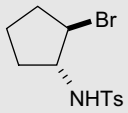
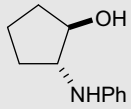
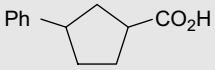
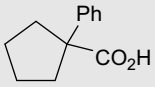
Compound	Structure	AKR1C1 inhibition (%) ± SD[a]	AKR1C3 inhibition (%) ± SD[b]
2		77.8 ± 0.4 IC_{50} = 35.0 μM[c] K_i = 17.2 μM	88.8 ± 2.6 IC_{50} = 36.7 μM[c] K_i = 33.4 μM
3		40.1 ± 0.8	87.4 ± 1.1 IC_{50} = 17.8 μM[c] K_i = 16.2 μM
4		52.9 ± 3.9 IC_{50} = 62.1 μM[c] K_i = 30.5 μM	83.9 ± 5.1 IC_{50} = 22.5 μM[c] K_i = 20.5 μM
cis-**9**		9.7 ± 5.3	NI
trans-**9**		13.8 ± 4.0	NI
10		15.9 ± 1.2[d]	67.3 ± 1.8[e]
11		11.5 ± 1.3[d]	46.6 ± 0.8[e]
12		2.1 ± 1.5	2.7 ± 1.1
14		15.9 ± 5.4	NI
15		12.9 ± 3.4	NI

Table 1 (*continued*)

Compound	Structure	AKR1C1 inhibition (%) ± SD[a]	AKR1C3 inhibition (%) ± SD[b]
16		26.1 ± 4.5	NI
17		20.3 ± 0.8	9.2 ± 1.7
18		14.7 ± 1.3	NI
20		55.4 ± 0.9 IC_{50} = 98.6 μM[c] K_i = 48.5 μM	52.7 ± 2.3 IC_{50} = 76,6 μM[c] K_i = 69.6 μM
21		53.5 ± 2.3 IC_{50} = 72.9 μM[c] K_i = 35.8 μM	5.8 ± 0.8

NI – no inhibition detected.
[a] Percentage of enzyme inhibition at 30 μM acenaphthenol and 100 μM of each inhibitor. The results of at least three independent experiments are shown as means ± SD.
[b] Percentage of enzyme inhibition at 100 μM acenaphthenol and 100 μM of each inhibitor. The results of at least three independent experiments are shown as means ± SD.
[c] IC_{50} values were determined as described.
[d] Percentage of enzyme inhibition at 30 μM acenaphthenol and 50 μM of each inhibitor. The results of at least three independent experiments are shown as means ± SD.
[e] Percentage of enzyme inhibition at 100 μM acenaphthenol and 50 μM of each inhibitor. The results of at least three independent experiments are shown as means ± SD.

5. Experimental protocols

5.1. Chemistry

Solvents and starting compounds (including compounds **13** and **21**) were obtained from commercial sources (Fluka, Sigma and Aldrich). All reactions requiring dry conditions were carried out in dry solvents. Light petroleum refers to the fraction with the distillation range 40–60 °C. TLC was carried out on Fluka silica-gel TLC-cards. All mps were determined on a hot stage and are uncorrected. IR spectra were recorded on a BioRad FTS 3000MX instrument. NMR spectra were recorded on a Bruker Avance 300 DPX spectrometer at 302 K. Chemical shifts are reported in δ ppm, referenced to an internal TMS standard for ^{1}H NMR, chloroform-*d*

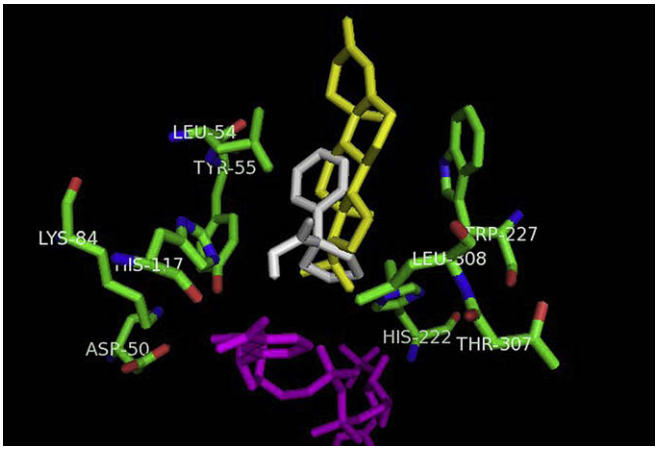


Fig. 2. Superimposition of the computer model of compound **21** (white) on the X-ray structure of 20α-hydroxyprogesterone (yellow) and NAD^+ (magenta) bound to AKR1C1. The highest ranked position of the inhibitor is presented (as calculated by AutoDock 3.0). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

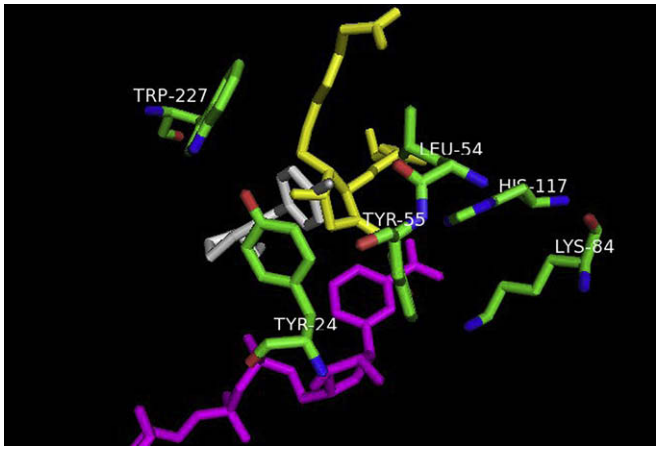


Fig. 3. Superimposition of the computer model of compound **3** (white) on the X-ray structure of prostaglandine D2 (yellow) and $NADP^+$ (magenta) bound to AKR1C3. The highest ranked position of the inhibitor is presented (as calculated by AutoDock 3.0). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(δ 77.0), DMSO-d_6 (δ 39.5) for ^{13}C NMR. The ^{1}H–^{13}C HMBC spectra were obtained with 512 time increments and 32 scans per t_1 increment. Microanalyses were performed on a Perkin–Elmer 2400 series II CHNS/O analyser. Mass spectra and high-resolution mass measurements were performed on a VG-Analytical Autospec EQ instrumet.

5.1.1. Synthesis

*5.1.1.1. 2-(4-Chlorobenzylidene)cyclopentanone (**2**) [38].* A mixture of cyclopentanone (4.00 g, 47.6 mmol) and 4-chlorobenzaldehyde (3.37 g, 24 mmol) was treated with NaOH (0.2 M, 350 mL) at 25 °C. The reaction mixture was stirred at 25 °C for 14 h, diluted with water (350 mL), acidified with HCl (5%, 50 mL) pH = 5, and extracted with CH_2Cl_2 (3 × 150 mL). The combined extracts were dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by SiO_2 column chromatography (light petroleum:EtOAc = 20:1) yielding title compound **2** as white crystals, 1.98 g (40%). Mp = 74–76 °C; IR (KBr, cm^{-1}): 2983, 2947, 1713, 1623, 1584, 1487, 1460, 1405, 1301, 1275, 1230, 1172, 1089, 1005, 905, 871, 817. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (tt, J = 7.5 Hz, 15.0 Hz, 2H); 2.41 (t, J = 7.5 Hz, 2H); 2.95 (dt, J = 2.5 Hz, 2H); 7.33 (t, J = 2.5 Hz, 1H); 7.36–7.40 (m, 2H); 7.45–7.48 (m, 2H).

*5.1.1.2. General procedure for 2-(4-chlorobenzylidene)cyclopentyl methyl ether (**3**) and 2-(4-chlorobenzylidene)cyclopentyl ethyl ether (**4**).* A mixture of 2-(4-chlorobenzylidene)cyclopentanone (**2**) (200 mg, 0.97 mmol) and $CeCl_3 \times 6H_2O$ (687 mg, 1.94 mmol) in MeOH (10 mL) or EtOH (10 mL) was treated with $NaBH_4$ (74.0 mg, 1.94 mmol). After stirring of the reaction mixture at 25 °C for 45 min, HCl (1 M, 25 mL) was added and the reaction mixture stirred for the additional 15 h. The reaction mixture was than extracted with EtOAc (3 × 25 mL). Combined organic layers were washed with $NaHCO_3$ (5%, 2 × 50 mL), H_2O (50 mL), brine (50 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The residue, colourless liquid, was purified by column chromatography on SiO_2 (light petroleum/EtOAc = 10:1) yielding 200 mg (93%) of product **3**. 2-(4-Chlorobenzylidene)cyclopentyl methyl ether (**3**); colourless liquid; R_f 0.57 (light petroleum:EtOAc = 5:1); IR (NaCl, cm^{-1}): 3029, 2963, 2884, 2817, 1491, 1462, 1406, 1339, 1276, 1196, 1150, 1088, 1055, 1012, 926, 885, 821; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.69–1.79 (m, 2H), 1.81–1.91 (m, 1H), 1.94–2.04 (m, 1H), 2.43–2.55 (m,1H), 2.58–2.69 (m, 1H), 3.38 (s, 3H), 4.07–4.10 (m, 1H), 6.48–6.50 (m, 1H), 7.28 (bs, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.1, 136.3, 132.1, 129.7, 128.4, 123.8, 86.0, 56.1, 31.3, 28.9, 22.9. EI-MS, m/z (relative intensity) 222 (46%); HRMS calcd for $C_{13}H_{15}ClO$: 222.0811; found 222.0817. 2-(4-Chlorobenzylidene)cyclopentyl ethyl ether (**4**); colourless liquid; R_f 0.54 (light petroleum:EtOAc = 7:1); IR (film, cm^{-1}): 3407, 2967, 2873, 1491, 1442, 1403, 1327, 1300, 1078, 882, 820; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, J = 7.0 Hz, 3H), 1.64–1.75 (m, 2H), 1.80–2.03 (m, 2H), 2.43–2.54 (m, 1H), 2.57–2.65 (m,1H), 3.52–3.59 (m, 2H), 4.19 (bs, 1H), 6.48 (bs, 1H), 7.24–7.28 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 145.5, 136.3, 131.9, 129.6, 128.2, 123.2, 84.1, 63.9, 31.7, 28.9, 22.7, 15.5; EI-MS, m/z (relative intensity) 236 (36%); HRMS calcd for $C_{14}H_{17}ClO$: 236.0968; found 236.0961.

*5.1.1.3. Synthesis of compounds **5**–**8**.* To a solution of cyclopentanone (3.00 g, 35.7 mmol) in THF (50 mL) cooled to 0 °C LDA (18 mL, 35.7 mmol) was added drop-wise. After being stirred at −5 °C for 20 min, the solution of benzyl bromide (30.50 g, 178.5 mmol) in THF (10 mL) was added and the reaction mixture was stirred at room temperature for 24 h. After the reaction was complete, the mixture was treated with HCl (0.5 M, 300 mL) and extracted with EtOAc (3 × 200 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was dissolved in pyridine (50 mL) and stirred at room temperature overnight. The precipitated material was filtered off and mother-liquid treated with water (300 mL) and extracted with Et_2O (2 × 200 mL). The combined organic extracts were washed with HCl (1 M, 250 mL), saturated $NaHCO_3$ (250 mL), and brine (250 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The residue, colourless oil, was purified by SiO_2 column chromatography (light petroleum:EtOAc = 20:1). 2-Benzyl cyclopentanon (**5**); colourless oil, 2.39 g (40%); R_f 0.18 (light petroleum:EtOAc = 20:1); IR (film, cm^{-1}): 3454, 3061, 3028, 2963, 2876, 1950, 1881, 1805, 1738, 1639, 1603, 1495, 1450, 1405, 1341, 1310, 1267, 1219, 1154, 1111, 1067, 1028, 1002, 924, 845, 802, 760, 725, 700; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48–1.62 (m, 1H), 1.67–1.79 (m, 1H), 1.89–2.00 (m, 1H), 2.04–2.16 (m, 2H), 2.28–2.40 (m, 2H), 2.54 (dd, J = 9.5, 14.0 Hz, 1H), 3.15 (dd, J = 4.0, 14.0 Hz, 1H), 7.15–7.30 (m, 5H). EI-MS, m/z (relative intensity) 174 (13%); HRMS calcd for $C_{12}H_{14}O$: 174.1045; found 174.1049. Mixture of isomers 2,2-dibenzylcyclopentanone (**6**) and 2,5-dibenzylcyclopentanone (**7**); yellowish oil, 1.43 g (15% overall); ratio of isomers **6**:**7** = 5:1; R_f 0.42 (light petroleum:EtOAc = 7:1); EI-MS: m/z = 264 (16%); HRMS calcd for $C_{19}H_{20}O$: 264.1514; found 264.1521. 2,2,5-Tribenzylcyclopentanone (**8**); colourless solid, 173 mg (1%); R_f 0.47 (light petroleum:-EtOAc = 7:1); mp = 94 − 96 °C; IR (KBr, cm^{-1}): 3443, 3028, 2935, 2908, 1961, 1896, 1827, 1731, 1638, 1599, 1494, 1454, 1342, 1320, 1306, 1236, 1167, 1125, 1075, 1028, 976, 913, 861, 822, 755, 728, 706. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72–0.88 (m, 1H), 1.44–1.52 (m, 1H), 1.68–1.76 (m, 1H), 1.78–1.90 (m, 2H), 2.05 (dd, J = 10.5, 14.0 Hz, 1H), 2.58 (dd, J = 10.5, 13.0 Hz, 2H), 2.92 (d, J = 13.0 Hz, 1H), 3.02 (dd, J = 4.0, 14.0 Hz, 1H), 3.10 (d, J = 13.0 Hz, 1H), 6.94–6.96 (m, 2H), 7.05–7.31 (m, 13H). EI-MS, m/z (relative intensity) 354 (3.3%); HRMS calcd for $C_{26}H_{26}O$: 354.1984; found 354.1990.

5.1.1.4. cis- *and* trans-*2-Benzylcyclopentanol (*cis- *and* trans-***9***) [39].* To a mixture of 2-benzylcyclopentanone (**5**) (150 mg, 0.86 mmol) and $CeCl_3 \times 6H_2O$ (581 mg, 1.56 mmol) in MeOH (10 mL), $NaBH_4$ (62.8 mg, 1.65 mmol) was added. The reaction mixture was stirred at 25 °C for 1.5 h and then treated with HCl (1 M, 50 mL), extracted with EtOAc (3 × 50 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by SiO_2 column chromatography (light petroleum:EtOAc = 5:1) yielding *cis* and *trans* isomer in ratio 1:1. *cis*-2-Benzylcyclopentanol (*cis*-**9**); colourless oil; 64 mg (42%); R_f 0.26 (light petroleum:-EtOAC = 5:1); IR (film, cm^{-1}): 3564, 3387, 3062, 3026, 2957, 2872, 1944, 1873, 1805, 1603, 1494, 1454, 1404, 1305, 1199, 1145, 1109, 1030, 986, 908, 751, 700. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46–1.61 (m, 3H), 1.63–1.74 (m, 2H), 1.67–1.91 (m, 2H), 1.95–2.08 (m, 1H), 2.68 (dd, J = 8.0, 13.5 Hz, 1H), 2.85 (dd, J = 8.0, 13.5 Hz, 1H), 4.09 (br, 1H), 7.16–7.31 (m, 5H). EI-MS, m/z (relative intensity) 176 (7.9%); HRMS calcd for $C_{12}H_{16}O$: 176.1201; found 176.1206. *trans*-2-Benzylcyclopentanol (*trans*-**9**); colourless oil; 44 mg (29%); R_f 0.17 (light petroleum:EtOAc = 5:1); IR (film, cm^{-1}): 3345, 3062, 3026, 2955, 2872, 2402, 1944, 1873, 1804, 1603, 1495, 1453, 1343, 1257, 1170, 1071, 1031, 967, 913, 746, 700. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21–1.33 (m, 2H), 1.50–2.09 (m, 6H), 2.55 (dd, J = 8.0, 13.5 Hz, 1H), 2.76 (dd, J = 8.0, 13.5 Hz, 1H), 3.88–3.94 (m, 1H), 7.16–7.31 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.1, 128.8, 128.4, 126.0, 78.6, 49.9, 39.8, 34.2, 29.9, 21.5. EI-MS, m/z (relative intensity) 176 (8.0%).

*5.1.1.5. 2,2-Dibenzylcyclopentanol (**10**) and 2,5-dibenzylcyclopentanol (**11**).* To a mixture of isomers **7** and **8** (223 mg, 0.85 mmol) and $CeCl_3 \times 6H_2O$ (599 mg, 1.69 mmol) in MeOH (10 mL), $NaBH_4$ (63.9 mg, 1.69 mmol) was added. The reaction mixture was stirred at 25 °C for 1.5 h and then treated with HCl (1 M, 50 mL), extracted with EtOAc (3 × 50 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by SiO_2 column chromatography (light petroleum:EtOAc = 10:1). 2,2-Dibenzylcyclopentanol (**10**);

colourless oil; 105 mg (56%); R_f 0.32 (light petroleum:EtOAc = 7:1); IR (film, cm^{-1}): 3582, 3399, 3060, 3026, 2953, 2875, 1949, 1881, 1810, 1601, 1494, 1454, 1397, 1338, 1072, 1031, 975, 913, 865, 753, 703. ^1H NMR (300 MHz, $CDCl_3$): δ 1.19–1.29 (m, 1H), 1.39–1.52 (m, 2H), 1.54–1.67 (m, 2H), 1.69–1.83 (m, 1H), 1.90–2.02 (m, 1H), 2.52 (d, J = 13.0 Hz, 1H), 2.73 (dd, J = 5.0, 14.0 Hz, 3H), 3.89 (t, J = 7.5 Hz, 1H), 7.09–7.12 (m, 2H), 7.16–7.33 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.3, 138.4, 130.8, 130.8, 128.0, 127.9, 126.0, 126.0, 76.5, 49.1, 40.6, 37.4, 31.2, 30.0, 18.5. EI-MS, m/z (relative intensity) 266 (2%); HRMS calcd for $C_{19}H_{22}O$: 266.1671; found 266.1677. 2,5-Dibenzylcyclopentanol (**11**); white solid; 24 mg (13%); R_f 0.38 (light petroleum:EtOAc = 7:1); mp = 49–51 °C; IR (KBr, cm^{-1}): 3592, 3325, 3024, 2949, 2919, 2850, 1604, 1495, 1450, 1345, 1121, 1060, 1030, 936, 880, 739, 698. ^1H NMR (300 MHz, $CDCl_3$): δ 1.15–1.27 (m, 2H), 1.43–1.56 (m, 1H), 1.65–1.75 (m, 1H), 1.86–1.97 (m, 1H), 2.14–2.27 (m, 2H), 2.48–2.64 (m, 2H), 2.68–2.76 (m, 1H), 2.88 (dd, J = 7.0, 13.5 Hz, 1H), 3.84–3.85 (m, 1H), 7.15–7.30 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.7, 141.0, 128.8, 128.7 (2C), 128.3, 125.9, 125.8, 78.8, 49.5, 45.4, 40.7, 35.3, 29.1, 28.9. EI-MS, m/z (relative intensity) 266 (0.5%); HRMS calcd for $C_{19}H_{22}O$: 266.1671; found 266.1680.

*5.1.1.6. 6-Tosyl-6-azacicyclo[3.1.0]hexane (**12**) [30].* White solid; 2.78 g (80%); R_f 0.22 (light petroleum:EtOAc = 5:1); mp = 69–71 °C; IR (KBr, cm^{-1}): 3353, 3260, 3048, 2958, 2856, 2412, 1950, 1682, 1595, 1491, 1437, 1400, 1367, 1341, 1317, 1200, 1154, 1092, 1012, 972, 929, 870, 828, 722, 676; ^1H NMR (300 MHz, $CDCl_3$): δ 1.52–1.68 (m, 4H); 1.91–1.98 (m, 2H); 2.44 (s, 3H); 3.33 (s, 2H); 7.31–7.33 (m, 2H); 7.80–7.82 (m, 2H).

*5.1.1.7. N-(2-Cyanocyclopentyl)-4-methylbenzenesulfonamide (**14**) [31].* White solid; 380 mg (85%); R_f 0.32 (light petroleum:-EtOAc = 5:3); mp = 106–108 °C; IR (KBr, cm^{-1}): 3906, 3251, 3036, 2954, 2879, 2244, 1927, 1813, 1600, 1452, 1401, 1333, 1269, 1159, 1093, 1019, 937, 896, 820, 674; ^1H NMR (300 MHz, $CDCl_3$): δ 1.42–1.54 (m, 1H), 1.70–1.80 (m, 2H), 1.84–1.95 (m, 1H), 1.98–2.17 (m, 2H), 2.45 (s, 3H), 2.81–2.87 (m, 1H), 3.72–3.81 (m, 1H), 5.18 (bd, J = 6.5 Hz, 1H), 7.31–7.36 (m, 2H), 7.75–7.82 (m, 2H).

*5.1.1.8. N-(2-Bromocyclopentyl)-4-methylbenzenesulfonamide (**15**) [40].* Aziridine **12** (237 mg, 1 mmol) was dissolved in methanol (5 mL), Ph_3P (525 mg, 2 mmol), and CBr_4 (667 mg, 2 mmol) were added. The mixture was then heated under microwave conditions at 130 °C for 10 min. After this time the solvent was evaporated and the crude material was purified by SiO_2 column chromatography (light petroleum:EtOAc = 10:1) to provide the product **15** (167 mg, 53%) as a white solid; R_f 0.10 (light petroleum:EtOAc = 7:1); mp = 82–85 °C; IR (KBr, cm^{-1}): IR (KBr, cm^{-1}): 3438, 3263, 2973, 2877, 1597, 1439, 1412, 1324, 1162, 1095, 1077, 1036, 901, 847, 806, 665; ^1H NMR (300 MHz, $CDCl_3$): δ 1.36–1.47 (m,1H), 1.66–1.89 (m, 2H), 1.91–2.02 (m, 1H), 2.15–2.32 (m, 2H), 2.44 (s, 3H), 3.62–3.70 (m, 1H), 4.06–4.11 (m, 1H), 4.62 (bd, J = 6.0 Hz, 1H), 7.32–7.34 (m, 2H), 7.77–7.79 (m, 2H). EI-MS, m/z (relative intensity) 319 (30%); HRMS calcd for $C_{12}H_{26}BrNSO_2$: 317.0085; found 317.0095.

*5.1.1.9. 2-(4-Methylphenylsulfonamido)cyclopentanecarboxamide (**16**) [32].* *N*-(2-Cyanocyclopentyl)-4-methylbenzenesulfonamide (50 mg, 0.19 mmol) was dissolved in concentrated HCl (2 mL). After the reaction mixture was stirred at 25 °C for 21 h it was diluted with H_2O (10 mL) and the product was extracted with EtOAc (5 × 15 mL), which was dried (Na_2SO_4), filtered, and evaporated under reduced pressure yielding pure product **16**, 48 mg (90%). White solid; R_f 0.18 (light petroleum:EtOAc = 5:3); mp = 174–175 °C; IR (KBr, cm^{-1}): IR (KBr, cm^{-1}): 3398, 3291, 3190, 3117, 2977, 2883, 2790, 2739, 1929, 1668, 1623, 1445, 1351, 1308, 1149, 1096, 955, 918, 822, 708; ^1H NMR (300 MHz, DMSO): δ 1.16–1.27 (m, 1H), 1.38–1.54 (m, 4H), 1.74–1.88 (m, 1H), 2.38 (s, 3H), 2.42–2.47 (m, 1H), 3.63–3.72 (m, 1H), 6.70 (s, 1H), 7.11 (s, 1H), 7.35–7.37 (m, 2H), 7.59 (bd, J = 8.0 Hz, 1H), 7.65–7.67 (m, 2H).

*5.1.1.10. 2-(4-Methylphenylsulfonamido)cyclopentanecarboxylic acid (**17**) [32].* *N*-(2-Cyanocyclopentyl)-4-methylbenzenesulfonamide (50 mg, 0.19 mmol) was dissolved in concentrated HCl (2 mL). After the reaction mixture was heated at 80 °C for 9 h it was diluted with H_2O (5 mL). The precipitated material was filtered off, washed with H_2O (3 mL) and dried under the vacuum providing the pure product **17**, 36 mg (72%). White solid; R_f 0.10 (light petroleum:EtOAc = 5:3); mp = 120–122 °C; IR (KBr, cm^{-1}): IR (KBr, cm^{-1}): 3302, 3035, 2959, 2873, 2654, 1690, 1599, 1454, 1331, 1290, 1220, 1160, 1093, 1050, 1017, 930, 814; ^1H NMR (300 MHz, $CDCl_3$): δ 1.41–1.53 (m, 1H), 1.55–1.74 (m, 2H). 1.77–1.89 (m, 1H), 1.95–2.10 (m, 2H), 2.42 (s, 3H), 2.69–2.77 (m, 1H), 3.73–3.83 (m, 1H), 4.83 (bd, J = 6.0 Hz, 1H), 7.29–7.32 (m, 2H), 7.75–7.77 (m, 2H); EI-MS, m/z (relative intensity) 284 (0.3%); HRMS calcd for $C_{13}H_{18}NO_4S$: 284.0957; found 284.0968.

*5.1.1.11. 2-(Phenylamino)cyclopentanol (**18**) [33,34].* White solid; 175 mg (86%); R_f 0.10 (light petroleum:EtOAc = 5:1); mp = 57–58 °C; IR (KBr, cm^{-1}): IR (NaCl, cm^{-1}) 3394, 3087, 3051, 3021, 2961, 2874, 1921, 1830, 1603, 1505, 1470, 1432, 1317, 1284, 1180, 1154, 1107, 1076, 1041, 978, 871, 837, 750, 694; ^1H NMR (300 MHz, $CDCl_3$): δ 1.35–1.47 (m, 1H), 1.59–1.91 (m, 4H), 1.94–2.05 (m, 1H), 2.23–2.34 (m, 1H), 3.59–3.65 (m, 1H), 4.05–4.10 (m, 1H), 6.64–6.73 (m, 3H), 7.14–7.21 (m, 2H).

*5.1.1.12. 3-Phenylcyclopentanecarboxylic acid (**20**) [35].* To the stirred mixture of the corresponding cyclohexanone **19** (1.394 g, 8 mmol), diphenyldiselenide (25 mg, 0.08 mmol), and *t*-BuOH (4 mL), H_2O_2 (30%, 6 mL, 48 mmol) was added. The reaction was heated in sealed tube for 4 days. After the reaction was complete, Pd/C (10%, 50 mg) was added and the solvent was distilled off. The residue was treated with aqueous Na_2CO_3 (10%, 100 mL) and washed with CH_2Cl_2 (3 × 10 mL). The aqueous phase was adjusted to pH 1 with HCl (1%, ca. 170 mL) and extracted with CH_2Cl_2 (3 × 25 mL), dried with Na_2SO_4, and evaporated under reduced pressure. The residue, colourless oil, was distilled under the vacuum (7×10^{-3} mbar, 110 °C) yielding colourless oil, mixture of isomers *cis* and *trans*, 1.10 g (70%); R_f 0.15 (light petroleum:-EtOAc = 5:3); IR (film, cm^{-1}): 3028, 2963, 2932, 2670, 1708, 1602, 1493, 1452, 1413, 1287, 1246, 1169, 1072, 1030, 935, 760, 701; ^1H NMR (300 MHz, $CDCl_3$): δ 1.62–1.88 (m, 1H), 1.90–2.20 (m, 4H), 2.36–2.46 (m, 1H), 2.90–2.31 (m, 2H), 7.10–7.32 (m, 5H), 11.30 (bs, 1H); EI-MS, m/z (relative intensity) 190 (69%); HRMS calcd for $C_{12}H_{14}NO_2$: 190.0994; found 190.0986.

5.2. Pharmacology

5.2.1. Expression and purification of recombinant AKR1C1 and AKR1C3

pGex-AKR1C1 (constructed from a pcDNA3-AKR1C1 vector provided by Dr. Trevor M. Penning) [17] and pGex-AKR1C3 (provided by Dr. Jerzy Adamski) were transferred into the BL21 *Escherichia coli* strain. The cells were then grown in Luria-Bertani medium containing 100 μg/ml ampicilin, at 37 °C in a rotary shaker, until an OD_{600} of 1.0 had been reached. Expression of AKR1C1 and AKR1C3 was induced by IPTG at a final concentration of 1 mM and 0.5 mM, respectively, and the incubations were continued for 16 h at 24 °C and 3 h at 37 °C, respectively [16,41]. The preparation of cell extracts, the purification of glutathione-*S*-transferase (GST)-fusion proteins by affinity binding to glutathione-Sepharose, and the cleavage of these with thrombin were all performed as described in the GST Gene Fusion System Handbook (Amersham Biosciences). Protein concentrations were determined using the

Bradford method, with bovine serum albumin as standard, and the homogeneity of the proteins was checked by SDS PAGE followed by Coomassie Blue staining.

5.2.2. Inhibition assays

Human recombinant AKR1C1 and AKR1C3 catalyze the oxidation of the 1-acenaphthenol in the presence of the coenzyme NAD^+. The reaction was followed spectrophotometrically by measuring the increase in NADH absorbance ($\varepsilon_{\lambda 340} = 6220\ M^{-1}\ cm^{-1}$) in the absence and presence of each of the compound. The assays for AKR1C1 were carried out in a 0.6-mL volume that included 100 mM phosphate buffer (pH 6.5), 0.005% triton X-114 and 1.5% *N*,*N*-dimethylformamide (DMF) as a co-solvent. A substrate concentration of 30 μM was used, with 2.3 mM coenzyme and 0.8 μM enzyme. AKR1C1 converted 1-acenaphthenol with a specific activity of 1.3 μmol 1-acenaphthenol oxidised/min/mg. The concentrations of compounds ranged from 1.2 μM to 300 μM. The assays for AKR1C3 were carried out in a 0.6-mL volume that included 100 mM sodium phosphate buffer (pH 9.0), 0.005% triton X-114 and 1.7% DMF as a co-solvent. A substrate concentration of 100 μM was used, with 2.3 mM coenzyme and 1.0 μM enzyme. AKR1C3 converted 1-acenaphthenol with a specific activity of 208 nmol 1-acenaphthenol oxidised/min/mg at 100 μM 1-acenaphtehenol. The concentrations of compounds ranged from 4.5 μM to 300 μM. The measurements were performed on a Beckman DU7500 spectrophotometer, initial reaction velocities were calculated, and the IC_{50} values were determined graphically from plots of $\log_{10}$ [inhibitor concentration] versus % inhibition, using GraphPad Prism Version 4.00 (GraphPad Software, Inc.). Representative inhibition pattern was determined for AKR1C1 with compound **2**. Here, the concentrations of the inhibitor were in the range 0.5–2.0 × IC_{50} and that of the substrate in the range 0.5–5.0 × K_m. Initial velocity data were fitted to competitive, non-competitive and uncompetitive inhibition models using SigmaPlot 8.0 software (Fig. 4). Using the Cheng–Prusoff relationship, K_i values were calculated for the remainder of the compounds.

5.3. Molecular docking

Automated docking was used to determine the orientation of inhibitors bound in the active site of AKR1C1 and AKR1C3. A genetic algorithm method, implemented in the program AutoDock 3.0, was employed [37]. The structures of inhibitors were prepared using HyperChem 7.5 (HyperChem, version 7.5 for Windows. Hypercube, Inc.: Gainesville, FL, 2002). The crystal structures of AKR1C1 and AKR1C3 were retrieved from the RCSB protein database (PDB codes 1MRQ and 1RY0, respectively) and all water molecules and substrates removed. Polar hydrogen atoms were added and Kollman charges [42], atomic solvation parameters and fragmental volumes were assigned to the protein using AutoDock Tools (ADT). For docking calculations, Gasteiger–Marsili partial charges [43] were assigned to the ligands and cofactors and nonpolar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The grid map, which was large enough to cover the inhibitors and the enzyme's active site, was generated with AutoGrid. Lennard–Jones parameters 12-10 and 12-6, supplied with the program, were used for modeling H-bonds and van der Waals interactions, respectively. The distance-dependent dielectric permittivity of Mehler and Solmajer [44] was used to calculate the electrostatic grid maps. Random starting points, random orientation, and torsions were used for all ligands. The translation, quaternion, and torsion steps were taken from default values in AutoDock 3.0. The Lamarckian genetic algorithm and the pseudo-Soils and Wets methods were applied for minimization, using default parameters. The number of docking runs was 100, the population in the genetic algorithm was 250, the number of energy evaluations was 500,000, and the maximum number of iterations 27,000.

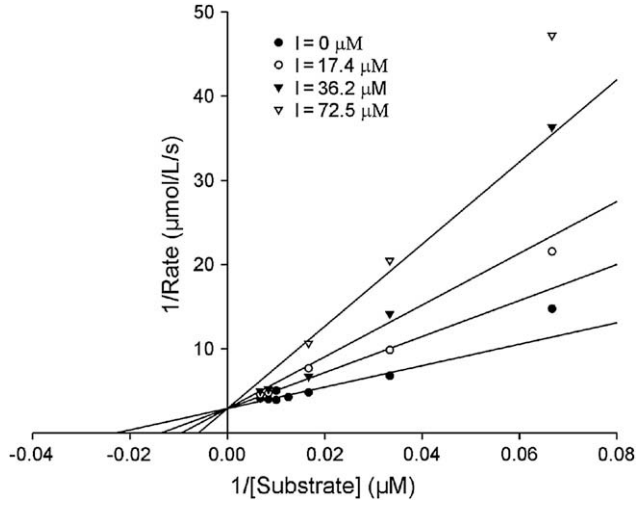


Fig. 4. Kinetic analysis of inhibition by compound **2**. Lineweaver–Burk plot of the reciprocal of the initial velocity versus the reciprocal concentrations of 1-acenphthenol at different fixed concentrations of compound **2**. Compound **2** concentrations were: 0 μM (●), 17.4 μM (○), 36.2 μM (▼) and 72.5 μM (▽).

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References

[1] B. Persson, M. Krook, H. Jornvall, Eur. J. Biochem. 200 (1991) 537–543.
[2] H. Jornvall, B. Persson, M. Krook, S. Atrian, R. Gonzales-Duarte, J. Jefferey, D. Ghosh, Biochemistry 34 (1995) 6003–6013.
[3] T.M. Penning, Hum. Reprod. Update 9 (2003) 193–205.
[4] I. Dufort, P. Rheault, X.F. Huang, P. Soucy, V. Luu, Endocrinology 140 (1999) 568–574.
[5] I. Dufort, F. Labrie, V. Luu-The, J. Clin. Endocrinol. Metab. 86 (2001) 841–846.
[6] T.M. Penning, M.E. Burczynski, J.M. Jez, H.-K. Lin, H. Ma, M. Moore, N. Palackal, K. Ratnam, Biochem J. 351 (2000) 67–77.
[7] L.D. Griffin, S.H. Mellon, Proc. Natl. Acad. Sci. 96 (1999) 13512–13517.
[8] S. Nobel, L. Abrahamsen, U. Oppermann, Eur. J. Biochem. 268 (2001) 4113–4125.
[9] T. Lanišnik Rižner, T. Šmuc, R. Rupreht, J. Šinkovec, T.M. Penning, Mol. Cell. Endocrinol. 248 (2006) 126–135.
[10] Y. Higaki, N. Usami, S. Shintani, S. Ishikura, O. El-Kabbani, O. Hara, Chem. Biol. Interact. 503 (2003) 143–144.
[11] T.M. Penning, M.E. Burczynski, J.M. Jez, H.-K. Lin, H. Ma, M. Moore, K. Ratnam, N. Palackal, Mol. Cell. Endocrinol. 171 (2001) 137–149.
[12] M. Khanna, K.N. Qin, R.W. Wang, K.C. Cheng, J. Biol. Chem. 270 (1995) 20162–20168.
[13] K. Matsuura, H. Shiraishi, A. Hara, K. Sato, Y. Deyashiki, M. Ninomiya, S. Sakai, J. Biochem. (Tokyo) 124 (1998) 940–946.
[14] J.C. Desmond, J.C. Mountford, M.T. Drayson, E.A. Walker, M. Hewison, J.P. Ride, Q.T. Luong, R.E. Hayden, E.F. Vanin, C.M. Bunce, Cancer Res. 63 (2003) 505–512.
[15] M. Wick, G. Hurteau, C. Dessev, D. Chan, M.W. Geraci, R.A. Winn, L.E. Heasley, R.A. Nemenoff, Mol. Pharmacol. 62 (2002) 1207–1214.
[16] A. Krazeisen, R. Breitling, G. Möller, J. Adamski, Mol. Cell. Endocrinol. 171 (2001) 151–162.
[17] P. Brožič, T. Šmuc, S. Gobec, T. Lanišnik Rižner, Mol. Cell. Endocrinol. 259 (2006) 30–42.

[18] N. Usami, T. Yamamoto, S. Shintani, Y. Higaki, S. Ishikura, Y. Katagiri, A. Hara, Biol. Pharm. Bull. 25 (2002) 441–445.
[19] D.R. Bauman, S. Rudnick, L.M. Szewczuk, Y. Jin, S. Gopishetty, T.M. Penning, Mol. Pharmacol. 67 (2005) 60–68.
[20] (a) A.L. Lovering, J.P. Ride, C.M. Bunce, J.C. Desmond, S.M. Cummings, S.A. White, Cancer Res. 64 (2004) 1802–1810;
(b) M.C. Byrns, S. Steckelbroeck, T.M. Penning, Biochem. Pharmacol. 75 (2008) 484–493.
[21] S. Gobec, P. Brožič, T. Lanišnik Rižner, Bioorg. Med. Chem. Lett. 15 (2005) 5170–5175.
[22] P. Brožič, B. Golob, N. Gomboc, T. Lanišnik Rižner, S. Gobec, Molec. Cell. Endocrinol. 248 (2006) 233–235.
[23] U. Dhagat, V. Carbone, R.P.T. Chung, T. Matsunaga, S. Endo, A. Hara, O. El-Kabbani, Med. Chem. 3 (2007) 546–550.
[24] P. Brožič, S. Turk, T. Lanišnik Rižner, S. Gobec, Mol. Cell. Endocrinol., in press.
[25] P. Brožič, J. Cesar, A. Kovač, M. Davies, P.A. Johnson, C.W.G. Fishwick, T. Lanišnik Rižner, S. Gobec, Chem. Biol. Interact. 178 (2009) 158–164.
[26] J.-F. Couture, P. Legrand, L. Cantin, V. Luu-The, F. Labrie, R. Breton, J. Mol. Biol. 331 (2003) 593–604.
[27] J. Komoto, T. Yamada, K. Watanabe, F. Takusagawa, Biochemistry 43 (2004) 2188–2198.
[28] W. Qiu, M. Zhou, F. Labrie, S.-X. Lin, Mol. Endocrinol. 18 (2004) 1798–1809.
[29] (a) J.-L. Luche, J. Am. Chem. Soc. 100 (1978) 2226–2227;
(b) A.L. Gemal, J.L. Luche, J. Am. Chem. Soc. 103 (1981) 5454–5459.
[30] J.U. Jeong, B. Tao, I. Sagasser, H. Henniges, K.B. Sharpless, J. Am. Chem. Soc. 120 (1998) 6844–6845.
[31] J. Wu, X.L. Hou, L.X. Dai, J. Org. Chem. 65 (2000) 1344–1348.
[32] M. Preiml, H. Honig, N. Kleimper, J. Mol. Catal. B: Enzym. 29 (2004) 115–121.
[33] A.K. Chakraborti, A. Kondaskar, Tetrahedron Lett. 44 (2004) 8315–8319.
[34] T. Ollevier, G. Lavie-Compin, Tetrahedron Lett. 43 (2002) 7891–7893.
[35] M. Giurg, J. Młochowski, Synth. Commun. 29 (1999) 2281–2291.
[36] B.P. Schlegel, J.M. Jez, T.M. Penning, Biochemistry 37 (1998) 3538–3548.
[37] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, J. Comput. Chem. 19 (1998) 1639–1662.
[38] U.P. Kreher, A.E. Rosamilija, C.L. Raston, J.L. Scott, C.R. Strauss, Org. Lett. 5 (2003) 3107–3110.
[39] N.F. Janes, B.P.S. Khambay, Magn. Reson. Chem. 27 (1989) 197–203.
[40] L.S. Hegedus, J.M. Mckearin, J. Am. Chem. Soc. 104 (1982) 2444–2451.
[41] J.F. Couture, L. Cantin, P. Legrand, V. Luu-The, F. Labrie, R. Breton, Acta. Crystallogr. D 58 (2002) 135–139.
[42] S.J. Weiner, P.A. Kollman, D.A. Case, U.C. Singh, C. Ghio, G. Alagona, S. Profeta, P. Weiner, J. Am. Chem. Soc. 106 (1984) 765–784.
[43] J. Gasteiger, M. Marsili, Tetrahedron 36 (1980) 3219–3228.
[44] E.L. Mehler, T. Solmajer, Protein Eng. 4 (1991) 903–910.