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Original article

Synthesis and structure–activity relationships for 1-(4-(piperidin-1-ylsulfonyl)phenyl)pyrrolidin-2-ones as novel non-carboxylate inhibitors of the aldo-keto reductase enzyme AKR1C3

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ABSTRACT

High expression of the aldo-keto reductase enzyme AKR1C3 in the human prostate and breast has implicated it in the development and progression of leukemias and of prostate and breast cancers. Inhibitors are thus of interest as potential drugs. Most inhibitors of AKR1C3 are carboxylic acids, whose transport into cells is likely dominated by carrier-mediated processes. We describe here a series of (piperidinosulfonamidophenyl)pyrrolidin-2-ones as potent (<100 nM) and isoform-selective non-carboxylate inhibitors of AKR1C3. Structure–activity relationships identified the sulfonamide was critical, and a crystal structure showed the 2-pyrrolidinone does not interact directly with residues in the oxyanion hole. Variations in the position, co-planarity or electronic nature of the pyrrolidinone ring severely diminished activity, as did altering the size or polarity of the piperidino ring. There was a broad correlation between the enzyme potencies of the compounds and their effectiveness at inhibiting AKR1C3 activity in cells.

1. Introduction

Enzymes belonging to the aldo-keto reductase (AKR) 1C subfamily are important modulators of both the steroid hormone and the leukotriene cascades. Of the AKR1C enzymes, the C3 isoform (also known as type 5 17-β-hydroxysteroid dehydrogenase) is of particular interest because of its high expression in the human prostate and breast, where it is responsible for producing (and over-producing) testosterone, 17-β-estradiol and 20-α-hydroxyprogesterone [1]. As such, AKR1C3 is implicated in the development and progression of leukemias and of prostate and breast cancers [2], and to a lesser extent leukemias through its prostaglandin F synthase activity [3]. This has made them interesting targets for the development of inhibitory small-molecule drugs that could be of potential use in controlling these diseases, and a number of such compounds have been reported [4,5].

Important issues in such drug development are obtaining selectivity between the four isoforms AKR1C1-4, which play different biological roles [6] and between these and the cyclo-oxygenase (COX) enzymes. The latter is highlighted by the fact that many inhibitors of the AKR1C subfamily are structurally related to the NSAID class of COX inhibitors, such as the anthranilates flufenamic [7] (**1**), meclofenamic [8] (**2**) and mefenamic [9] (**3**) acids (Table 1). We have determined the crystal structures for a range of NSAIDs bound in the AKR1C3 active site [10,11] that show, along with flufenamic acid [7], that the drug carboxylate group occupies the oxyanion hole, making H-bonds to the Y55 and H117 residues. We have also recently shown [4] that a series of (dihydroisoquinolyl)sulfonamidobenzoic acids (e.g., **4**) are potent and

Abbreviations: AKR, aldo-keto reductase; COX, cyclo-oxygenase; DCM, dichloromethane; DMSO, dimethyl sulfoxide; NADPH, nicotinamide adenine dinucleotide phosphate; NSAID, non-steroidal anti-inflammatory drugs; PDB, Protein Data Bank; ROCS, rapid overlay of chemical structures; TEA, triethylamine; TFA, trifluoroacetic acid.

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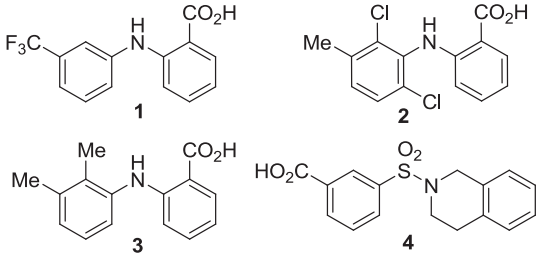
Table 1
Potency and selectivity of carboxylate-based AKR1C inhibitors.



No	IC_{50} (μM)[a]					
	AKR1C1	AKR1C2	AKR1C3	AKR1C4	COX1[b]	COX2[b]
1	2.64	3.14	0.41	>100	3.0	9.3
2	3.16	8.74	0.54	>100	0.22	0.7
3	3.91	6.97	0.56	>100	25	2.9
4	20.3	>30	0.013	>30	>10	>10

[a] IC_{50} values are the average of 2 or more determinations.
[b] COX data for compounds **1**–**3** from Ref. [18]; for compound **4** from Ref. [4].

very selective inhibitors of AKR1C3 (Table 1), with the carboxylate binding similarly in the oxyanion hole of the enzyme.

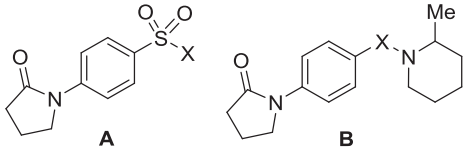
The majority of known AKR1C inhibitors are carboxylic acids, whose transport into cells is likely dominated by carrier-mediated processes rather than passive diffusion [12], and thus difficult to predict. Therefore, we were interested that one of the hits from a high-throughput screen seeking novel and selective inhibitors of AKR1C3 was the pyrrolidine **12**, which was a very potent (IC_{50} 0.094 μM) and selective inhibitor [4]. Furthermore, in a cell-based assay evaluating analogues for their ability to block AKR1C3 from metabolising a known dinitrobenzamide substrate [4], **12** was more potent relative to its enzyme inhibitory activity than a carboxylic acid analogue (ratio IC_{50}(enz)/IC_{50}(cell) is 0.48 for **4** versus 8.5 for **12**), suggesting a pharmacological disadvantage for the acids in cells. We therefore undertook a study of the structure–activity relationships around the pyrrolidine lead **12**, and an investigation of its binding to the enzyme.

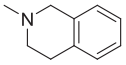
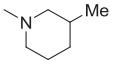
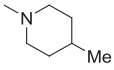
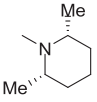
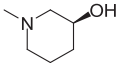
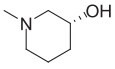
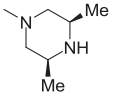
2. Chemistry

Compounds of Table 2, where the amide-containing ring was varied, were prepared from the halogenated sulfonamide intermediates **38a**–**k**, which were in turn prepared from known sulfonyl chlorides **36a**,**b** and substituted piperidines **37a**–**k** (Scheme 1). Reaction of **38a**–**k** with pyrrolidinone in the presence of CuI, *N*,*N*'-dimethylethylenediamine and K_2CO_3 gave compounds **10**, **12**–**21** in moderate yields. Compound **11** was prepared similarly from sulfonamide **39**. Compounds **22** and **23**, where the sulfonamide was replaced by amide and amine respectively, were prepared by coupling the benzoyl chloride **40** and benzyl bromide **41** with 2-methylpyrrolidine, and reacting the resultant intermediates **42**, **43** with 2-pyrrolidinone as above (Scheme 1).

The compounds of Table 3 fix the 2-methylpiperidine ring of **12** and vary the key pyrrolidone unit. Coupling of 2-methylpiperidine **37a** with substituted benzenesulfonic acids **36a**,**b**,**i**–**p** gave the corresponding sulfonamide intermediates **38a**,**b**,**i**–**p**, which were then reacted with 2-pyrrolidinine as before to give compounds **24**–**30** (Scheme 2). Reduction of the 4-nitro intermediate **38q** gave the amine **38r**, which was reacted with succinic or phthalic anhydride, pivaloyl or isobutyryl chloride or 3-chlorosulfonyl chloride to give respectively compounds **31**–**35** of Table 3.

Table 2
Variation of the lipophilic binding moiety.



No	Fm	X	AKR (IC_{50}, μM)[a]			
			1C1	1C2	1C3	1C4
9	A		>30	>30	0.052	>30
10	A		>30	>30	0.14	>30
11	A				>30	
12	A		>30	>30	0.094	>30
13	A				11.88	
14	A		>30	>30	0.088	>30
15	A		>30	>30	0.084	>30
16	A		>30	>30	0.20	>30
17	A		>30	>30	0.056	>30
18	A		>30	>30	8.98	>30
19	A		>30	>30	1.55	>30
20	A				>30	
21	A				21.2	
22	B	C=O			>30	
23	B	CH_2			>30	

[a] IC_{50} values <10 μM are the mean for 2 or more determinations.

3. Enzyme biochemistry and cell biology

The structures of the new compounds and their activities in the enzyme and cellular assays are shown in Tables 2 and 3. The inhibitory activity of the compounds against the AKR1 isoforms C1–C4 was performed with a competitive fluorescence assay,