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Morpholylureas are a new class of potent and selective inhibitors of the type 5 17-β-hydroxysteroid dehydrogenase (AKR1C3)

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ABSTRACT

Inhibitors of the aldo–keto reductase enzyme AKR1C3 are of interest as potential drugs for leukemia and hormone-related cancers. A series of non-carboxylate morpholino(phenylpiperazin-1-yl)methanones were prepared by palladium-catalysed coupling of substituted phenyl or pyridyl bromides with the known morpholino(piperazin-1-yl)methanone, and shown to be potent ($IC_{50} \sim 100$ nM) and very isoform-selective inhibitors of AKR1C3. Lipophilic electron-withdrawing substituents on the phenyl ring were positive for activity, as was an H-bond acceptor on the other terminal ring, and the ketone moiety (as a urea) was essential. These structure–activity relationships are consistent with an X-ray structure of a representative compound bound in the AKR1C3 active site, which showed H-bonding between the carbonyl oxygen of the drug and Tyr55 and His117 in the 'oxyanion hole' of the enzyme, with the piperazine bridging unit providing the correct twist to allow the terminal benzene ring to occupy the lipophilic pocket and align with Phe311.

1. Introduction

The type 5 17-β-hydroxysteroid dehydrogenase (AKR1C3) is a member of the aldo–keto reductase (AKR) superfamily of enzymes, responsible for reducing androst-4-ene-3,17-dione, estrone and progesterone to, respectively, the growth-stimulatory steroid hormones testosterone, 17β-estradiol and 20α-hydroxprogesterone,[1] making it a potential therapeutic target in both breast and prostate cancer. Inhibitors of AKR1C3 have become of particular interest since it was suggested[2] that the broad observation that non-steroidal anti-inflammatory drugs (NSAIDs) appear to protect against a variety of cancers[3] may be due in part to their demonstrated inhibition of AKR1C3. Several NSAIDs including flufenamic acid (**1**), indomethacin (**2**), naproxen (**3**), meclofenamic acid (**4**), *S*(+)-ibuprofen (**5**) and flurbiprofen (**6**),[4] and their analogues,[5,6] are known to have AKR1 isoform inhibitory activity (Fig. 1). Crystal structures of complexes of several NSAIDs,[2,7] show that the carboxylate group of the drugs form direct H-bonds to Y55 and H117 residues in the 'oxyanion hole' of the enzyme. More recently we have shown[7] that 3-(3,4-dihydroisoquinolin-2(1*H*)-ylsulfonyl)benzoic acids (e.g., **7**) are a novel class of highly potent and selective inhibitors of AKR1C3, with the carboxylate adopting a similar orientation.

Since carboxylic acids are likely to be transported into cells by carrier-mediated processes rather than passive diffusion,[8] there are potential advantages in finding non-carboxylate inhibitors, and we have recently reported[9] a new class of phenylpyrrolidin-2-ones (e.g., **8**) that achieve this without forming a direct interaction with the enzymes oxyanion hole. A potential advantage of these non-carboxylate inhibitors is that they show relatively higher activity than the carboxylate-based inhibitors in cell-based assays (relative to their enzyme inhibitory potencies), arguably because of superior uptake/transport properties.

In a previously-described[10] high-throughput screen we identified the morpholino(phenylpiperazin-1-yl)methanone **24** (Fig. 1) as a novel, reasonably potent ($IC_{50} \sim 100$ nM) and very isoform-selective non-carboxylate inhibitor of AKR1C3. In this paper we report the synthesis and structure–activity relationship (SAR) studies of a new class of morpholino(phenylpiperazin-1-yl)methanone AKR1C3 inhibitors derived from this lead.

Abbreviations: AKR, aldo–keto reductase; COX, cyclo-oxygenase; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMSO, dimethyl sulfoxide; NADPH, nicotinamide adenine dinucleotide phosphate; NSAID, non-steroidal anti-inflammatory drugs; PBD, Protein Data Bank; TFA, trifluoroacetic acid.

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2. Chemistry

The bulk of the compounds of Table 1 were prepared by palladium-catalysed coupling of substituted phenyl or pyridyl bromides with the known morpholino(piperazin-1-yl)methanone (**64**), which was prepared as reported[11] from *tert*-butyl piperazine-1-carboxylate (**62**) and morpholine-4-carbonyl chloride (**63**) (Scheme 1A). The 4-iodide **26** was prepared from Cu-catalysed halogen exchange of 4-bromide **25**, and was in turn used to prepare the 4-ethyne **21** via a Sonogashira reaction[12] with TMS-acetylene to give the TMS adduct **65** which was then deblocked with K_2CO_3 to give **21**. The 4-ester **27** was hydrolysed to acid **28**, which was used to make amides **29**–**31**. Reduction of the 4-nitro analogue **33** gave amine **66**, which was in turn used to prepare acetamide **34** and sulfonamide **36**. The methylene-linked analogue **41** was prepared by reaction of mono-BOC piperazine (**62**) and 4-chlorobenzaldehyde (**67**) to give **68**, followed by coupling of this with morpholine-4-carbonyl chloride (**63**) (Scheme 1B). The carbonyl-linked analogue **42** was prepared by direct coupling of **64** with 4-chlorobenzoyl chloride (**69**) (Scheme 1C).

The bulk of the compounds of Table 2 were prepared from either 1-(4-chlorophenyl)piperazine (**70**) and appropriate carbonyl chlorides, or from the corresponding 4-(4-chlorophenyl)piperazine-1-carbonyl chloride (**71**) and appropriate amines. The sulfoxide **51** was prepared from the sulfide **50** by oxidation with H_2O_2. The acid **55** was prepared by basic hydrolysis of ester **72**. Hydroxyamide **56** was prepared from the intermediate *t*-butyl ester **73** by hydrolysis with TFA to **74**, coupling of this with 3-chloro-3-oxopropyl acetate to give **75**, followed by C_2CO_3-mediated hydrolysis (Scheme 2).

Sulfonylurea **58** and thiourea **59** of Table 3 were prepared by base-catalysed condensation of 1-(4-chlorophenyl)piperazine (**70**) with morpholine-4-sulfonyl chloride (**77**) or morpholine-4-carbothioyl chloride (**78**), respectively. Compounds **60** and **61** were similarly prepared from base-catalysed condensation of morpholine-4-carbonyl chloride (**63**) with 1-(4-chlorophenyl)-1,4-diazepane[13] (**79**) or 2-(4-chlorophenyl)- 2,5-diazabicyclo [2.2.1]heptane[14] (**80**) respectively (Scheme 3).

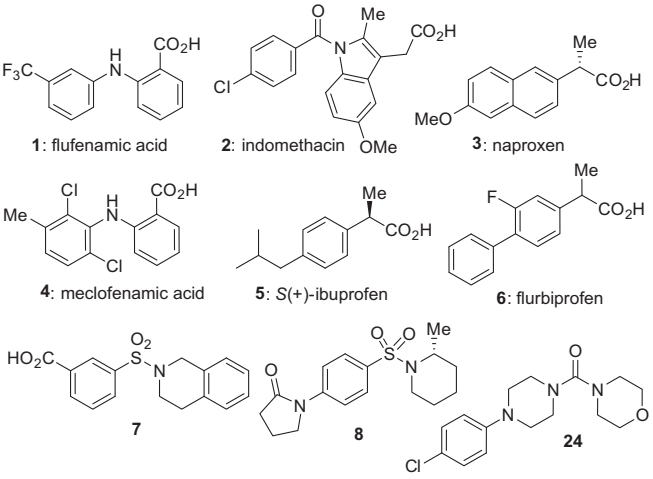
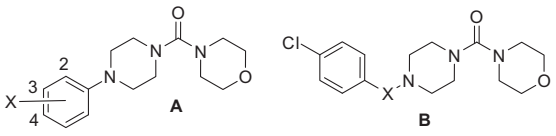


Figure 1. Representative inhibitors of AKR1C3.

Table 1
Structural and biological data for phenyl-substituted analogues



No.	Fm	X	AKR IC_{50}[a] (μM)				
			1C1	1C2	1C3	1C4	1C3 (cells)[b]
9	A	H	>30	>30	6.1	>30	
10	A	2-Me			12.1		
11	A	2-OMe			20.3		
12	A	2-Cl	>30	>30	2.3	>30	
13	A	2-CF_3	>30	>30	0.70	>30	
14	A	3-Cl	>30	>30	5.3	>30	
15	A	3-$CONH_2$			>30		
16	A	4-Me	>30	>30	0.36	>30	
17	A	4-OMe	>30	>30	0.35	>30	
18	A	4-Ph			>30		
19	A	4,5-Benz	>30	>30	4.9	>30	
20	A	3-Aza-4,5-benz	>30	>30	2.9	>30	
21	A	4-C≡CH	>30	>30	0.66	>30	
22	A	4-CF_3	>30	>30	0.068	>30	0.022
23	A	4-F	>30	>30	0.50	>30	
24	A	4-Cl	>30	>30	0.11	>30	0.018
25	A	4-Br	>30	>30	0.14	>30	0.012
26	A	4-I	>30	>30	0.067	>30	0.01
27	A	4-CO_2Me	>30	>30	>30	>30	
28	A	4-CO_2H			>30		
29	A	4-$CONH_2$			>30		
30	A	4-CONHMe			>30		
31	A	4-$CONMe_2$			>30		
32	A	4-SO_2Me	>30	>30	5.52	>30	
33	A	4-NO_2	>30	>30	0.57	>30	
34	A	4-NHCOMe			>30		
35	A	4-$NHSO_2Me$			>30		
36	A	2,4-diCl	>30	>30	0.025	>30	0.003
37	A	2-Me, 4-Cl	>30	>30	0.11	>30	0.013
38	A	2-CH_2OH, 4-Cl	>30	>30	0.16	>30	0.013
39	A	3-Aza, 4-Cl	>30	>30	0.53	>30	
40	A	2-Aza, 4-Br	>30	>30	0.10	>30	0.031
41	B	CH_2	>30	>30	0.35	>30	
42	B	CO	>30	>30	0.48	>30	

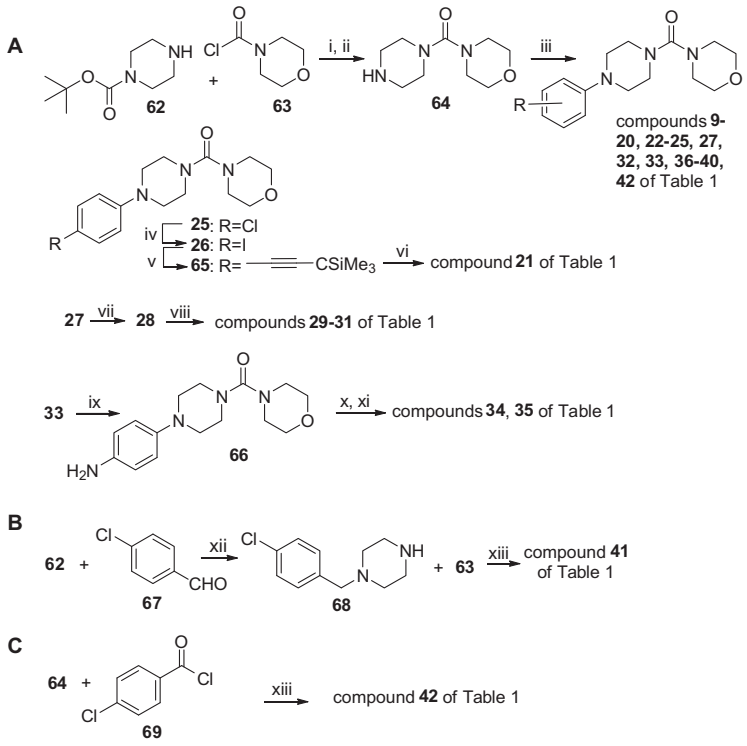
[a] IC_{50} values were determined using a competitive fluorescence assay; see Section 7. Values <1 μM represent the mean for 2 or more determinations.
[b] Only compounds with AKR1C3 IC_{50} values <0.3 μM were evaluated in the cellular assay.

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 1–3. The inhibition assay of the compounds against the AKR1 isoforms C1–C4 was performed with a competitive fluorescence assay, where a non-fluorescent ketone probe (probe 5)[15] selective for the AKR1C enzyme isoforms is reduced to a fluorescent alcohol in the presence of AKR1C enzyme and NADPH. Compounds with biochemical IC_{50} values <0.3 μM, plus the two most potent morpholine-ring replacements (**56** and **57**) were tested for activity at inhibiting the AKR1C3-dependent aerobic reduction of the dinitrobenzamide PR-104A to its hydroxylamine metabolite in an AKR1C3-overexpressing HCT116 cell line. Compound IC_{50} values were calculated by fitting the inhibition data to a four-parameter logistic sigmoidal dose–response curve using Prism 5.02 (GraphPad, La Jolla, CA, USA). The methanone compounds are competitive inhibitors of AKR1C3; their effectiveness can be reduced by increasing substrate concentration (data not shown).

4. Crystallography and modelling

The structure of **24** bound in the AKR1C3 active site was determined by X-ray crystallography, as detailed previously[10] (see Table 4 for crystallographic parameters). Figure 2 shows that the carbonyl interacts with the oxyanion hole residues Ty55 and His117, while the terminal benzene ring aligns with Phe311 of the SP1 pocket.[16] The morpholine oxygen may contact Leu-54 via



Scheme 1. Reagents and conditions. (i) DIPEA, CH_2Cl_2; (ii) TFA; (iii) ArBr, Cs_2CO_3, BINAP, $Pd(OAc)_2$, toluene, reflux; (iv) (1*R*,2*R*)-N^1,N^2-dimethylcyclohexane-1,2-diamine, CuI, NaI, dioxane; (v) TMS-acetylene, CuI, $(Ph_3P)PdCl_2$, Et_3N, DMF, then Cs_2CO_3, MeOH; (vi) K_2CO_3, MeOH; (vii) aqueous NaOH; (viii) $(COCl)_2$, DMF, DCM, then RNH_2/RRNH; (ix) Pd-C, H_2, THF; (x) Ac_2O; (xi) MsCl; (xii) BBN, then $Pd(dppf)Cl_2$, 4-bromo-1-chlorobenzene, K_2CO_3, DMF, then TFA; (xiii) DIPEA, DCM.

a structured water molecule. Analogues were docked with the core of the molecule, represented by **9**) restrained to the binding mode of **24** and then used to find hotspots within the SP1 pocket that can improve affinity.

5. Results and discussion

The inhibitory properties of the compounds for the four AKR1C enzyme isoforms used a competitive fluorescence binding assay where a non-fluorescent ketone probe is reduced to a fluorescent alcohol in the presence of the AKR1C enzymes isoforms.[10] Compounds **9**–**39** explore SAR around the aromatic ring, which is shown in the crystal structure of the 4-chloro compound **24** bound to AKR1C3 (Fig. 2) to occupy the SP1 pocket bounded by the side chains of Ser118, Met120, Asn167, Tyr216, Phe306, Phe311, Tyr317, Pro318 and Tyr319 and the ordered water HOH28. The unsubstituted analogue **9** and the 2-substituted compounds **10**–**13** showed good AKR1C3 selectivity and moderate to weak potencies (IC_{50}s 0.7–20 μM). The 3-Cl analogue **14** also had modest activity, while the polar 3-$CONH_2$ substituent in **15** abolished activity. The 4-position was much more favoured (as illustrated by the 2-, 3- and 4-Cl analogues **12**, **14**, **24** retrieving IC_{50}s of 2.3, 5.3 and 0.011 μM, respectively), so 4-substituents were studied in more detail (compounds **16**–**34**). There was a clear distinction between the utility of lipophilic (compounds **16**–**26**; IC_{50}s 0.07–4.9 μM) and polar H-bond donor and acceptor containing compounds (compounds **27**–**35**; IC_{50}s >30 μM) substituents. The relative effectiveness of these substituents suggests that interactions with the lipophilic binding pocket are largely non-specific van der Waals interactions, with the potential for little specific H-bond contribution, although steric effects may also contribute to the negative effect on activity.

Exceptions included compound **18** (4-phenyl), which was inactive, but this was likely due steric hindrance, a point noted in a previous study.[7] Of more interest were compounds **32** and **33**, bearing the polar but very electron-withdrawing substituents SO_2-Me and NO_2, respectively, and which showed weak to moderate activity (IC_{50}s 5.5 and 0.57 μM, respectively).

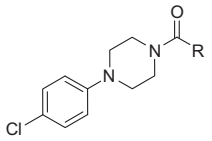
The disubstituted compounds **36**–**40** showed patterns of activity consistent with the above. The 2,4-dichloro analogue **36** was the most potent compound in the series (IC_{50} 0.025 μM), while the 2-CH_2OH, 4-Cl compound **38** and the pyridine analogues **39** and **40** also had modest potency. Collectively these results suggested that both the lipophilicity and electron-withdrawing properties of substituents might make positive contributions, and this was supported by a QSAR study (Eq. 1) of all the active compounds with no H-bond donors in Table 1 for which descriptive parameter values were available (compounds **9**–**14**, **16**–**18**, **21**–**26**, **31**, **33**, **36**, **37**)

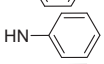
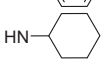
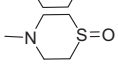
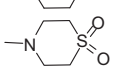
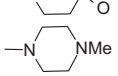
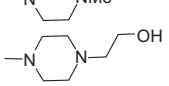
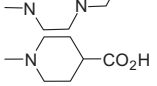
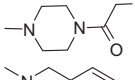
$$\text{Log } IC_{50}(\text{AKR1C3}) = -0.70(\pm 0.24)\sigma - 0.70(\pm 0.54)\pi + 0.33 \quad (1)$$

$N = 19$ $R = 0.62$ $F = 4.64$

Compounds **43**–**58** in Table 3 explore the binding site for the terminal substituent of the ketone moiety that binds the oxyanion hole. In the crystal structure of **24** bound to AKR1C3 (Fig. 2) the morpholine oxygen is within hydrogen bonding distance to a structured water (HOH556) that is part of a network located in the SP3 pocket, and the importance of this was examined. The complete inactivity of compounds **43**–**47** suggest that there is a requirement for a secondary aliphatic nitrogen (i.e., a urea moiety) and an H-bond acceptor, although **48** and **49** show that maintenance of only one of these can result in some level of AKR1C3 activity and (in the case of **49**) isoform selectivity. The thiourea **50** shows a low level of inhibitory activity (9.1 μM), but the sulfoxide **51**, the sulfone **52** and the *N*-methyl- and *N*-(2-hydroxyethyl)-piperazines **53** and **54** were all inactive. The 4-carboxypiperidine **55** showed some minimal activity (IC_{50} 22 μM), while the 2-hydroxyacetamide **56** was respectably active (IC_{50} 0.46 μM), along with the tetrahydroisoquinoline **57** (IC_{50} 0.35 μM).

Table 2
Structural and biological data for morpholine ring replacements

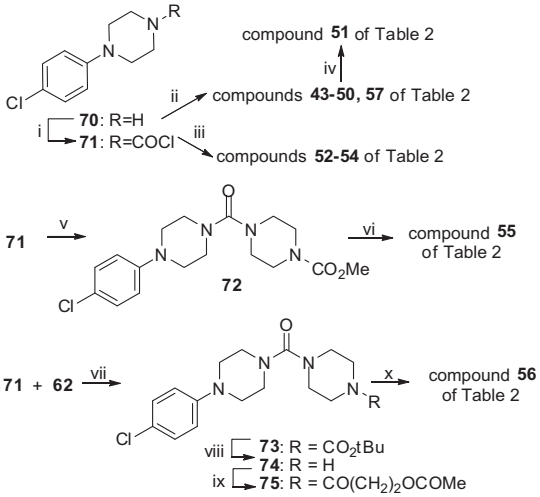


No.	R	AKR IC_{50}[a] (μM)				
		1C1	1C2	1C3	1C4	1C3[b] (cells)
43				>30		
44				>30		
45				>30		
46				>30		
47				>30		
48				12.6		
49		>30	>30	3.8	>30	
50		>30	>30	9.1	>30	
51				>30		
52				>30		
53				>30		
54				>30		
55				21.9		
56		>30	>30	0.46	>30	0.059
57		>30	>30	0.35	>30	0.39

[a] As for Table 1.
[b] Only compounds with AKR1C3 IC_{50} values <0.5 μM were evaluated in the cellular assay.

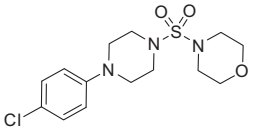
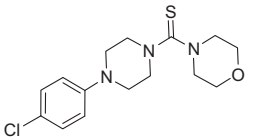
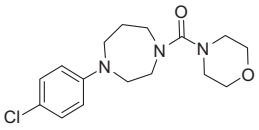
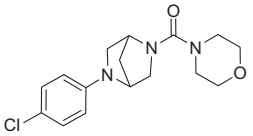
Analogues **58**–**61** in Table 3 demonstrate the importance of the oxyanion binding moiety and the piperazine bridge that links this to the aromatic ring. Displacing or replacing the ketone that makes the two H-bond contacts with the Tyr55 and His117 residues in the oxyanion hole with either the sulfone **58** or thioketone **59** abolishes binding. The crystal structure (Fig. 2 and Table 4) also shows the importance of the piperazine bridging unit in providing the correct twist to allow the benzene ring to occupy the SP1 pocket.[5,16] Altering this alignment with either a homo (**60**) or bridged (**61**) piperazine respectively abolishes or greatly reduces AKR1C3 inhibitory activity.

To find SP1 pocket hot spots that improved the IC_{50}, compounds from Table 1 substituted around the phenyl ring that retrieved IC_{50} values lower than that of compound **9** (6.1 μM) were docked into the AKR1C3 active site using the X-ray determined binding mode of compound **24** to position the core of the molecule. Compounds with lipophilic substitutions at the 4-position retrieved IC_{50} values less than 0.2 μM (**22**, **26**, **36**, **24**, **25**, **37**, **38** and **40**) and were predicted to probe a subsite within the SP1 pocket defined by the side chains of amino acids Tyr319, Tyr317, Phe311, Met120 and Pro318, with the larger halide based substitutions of (**22** (Fig. 3) and **26**)

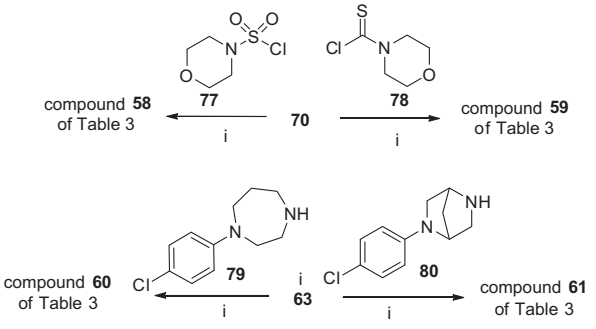


Scheme 2. Reagents and conditions: (i) Triphosgene, pyridine, DCM; (ii) HN-RR, DIPEA, DCM; (iii) RRN-COCl, DIPEA, DCM. (iv) H_2O_2; (v) methyl piperidine-4-carboxylate, DIPEA; (vi) MeOH, NaOH; (vii) DIPEA; (viii) TFA, DCM; (ix) 3-chloro-3-oxopropyl acetate, DIPEA; (x) Cs_2CO_3.

Table 3
Structural and biological data for morpholides with varying linker groups

No.	R	AKR IC_{50}[a] (μM)			
		1C1	1C2	1C3	1C4
58				>30	
59				>30	
60				30	
61		>30	>30	3.7	>30

[a] As for Table 1.



Scheme 3. Reagents: (i) DIPEA, DCM.

Table 4
X-ray data collection and refinement statistics for compound **24**

Data collection:		Refinement:	
Wavelength (Å)	1.54182	Resolution	18.53–2.40 (2.46–2.40)
Space group	$P2_12_12_1$	R_{work}[a]	0.219 (0.394)
Unit cell parameters (Å; °)	58.0, 64.1, 96.4	R_{free}[b]	0.297 (0.532)
Resolution (Å)	18.53–2.40 (2.49–2.40)	No. of working reflections	13,488 (806)
Number of molecules/AU	1	No. of free reflections	702 (44)
Total number of reflections	47,000 (3213)	Average B factor protein main-chain/side-chain (Å^2)	30.0/31.1
Unique reflections	14,191 (1277)	Average B factor ligands (Å^2)	26.0
Multiplicity	3.3 (2.5)	Average B factor water (Å^2)	30.3
Completeness (%)	97.6 (90.7)	RMSD bond (Å)	0.0150
Mean <I/σI>	12.3 (2.8)	RMSD angles (°)	1.7795
R_{merge}	0.068 (0.246)	Ramachandran most favoured/outliers (%)	97.0/0.3
Wilson B (Å^2)	49.9	Molprobity all-atom clash score	6.3

Data were collected using a Rigaku Saturn 944+ CCD detector mounted on a Rigaku Micromax-007 HF rotating anode X-ray generator.
Numbers in parentheses represent data in the highest resolution shell.
[a] $R_{work} = \Sigma||F(obs)|-|F(calc)||/\Sigma|F(obs)|$ for the 95% of the reflection data used in refinement.
[b] $R_{free} = \Sigma||F(obs)|-|F(calc)||/\Sigma|F(obs)|$ for the remaining 5%.

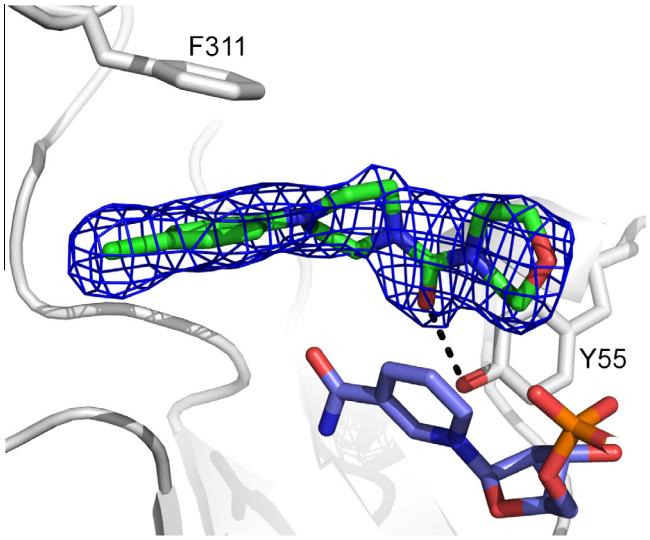


Figure 2. Crystal structure of **24** complexed with AKR1C. PDB: Protein Data Bank (PDB ID: 4HMN). Soaking experiment: AKR1C3+NADP$^+$ crystal soaked with 5 mM compound **24** for 3 days. Space group $P2_12_12_1$; cell dimensions: $a = 58.0$, $b = 64.1$, $c = 96.4$ Å. Resolution = 2.4 Å).

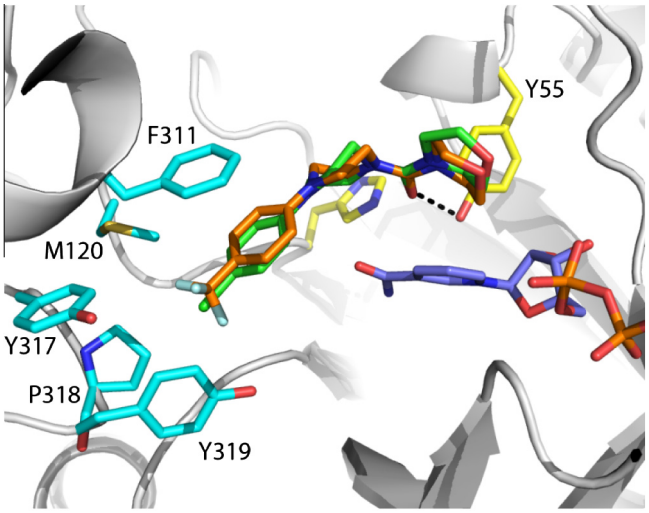


Figure 3. Predicted binding mode for **22** in the AKR1C3 active site. The oxy-anion hole residues Tyr-55 and His117 are shown in white sticks, and NADP$^+$ in purple sticks. Compound **24** is shown in green sticks, and the interaction between its central carbonyl group and the side chain hydroxyl group of Tyr55 is shown as a black dashed line. The SP1 pocket region that the 4-phenyl-CF_3 of **22** is predicted to interact is shown in cyan sticks and **22** is shown in orange sticks.

better suited to this site. This subsite is also used by the non-steroidal anti-inflammatory drugs naproxen (PDB codes 3UFY, 3R58) ibuprofen (PDB code 3R8G), zomepirac (PDB code 3R8H) and flurbiprofen (PDB code 3R94).[7] The 4-substituted **33** was predicted to place an acceptor group close to the side chain hydroxyl of Tyr319, part of the water binding site created by Glu192, Tyr216 and Tyr319. The top ranked predicted mode binding for the 3-substituted, compound **13**, consistent with the orientation of compound **24**, placed the chloride toward the empty water binding site created by Tyr216, Tyr319 and Glu192.

Comparison of the X-ray crystal structures of **24** and **7** (PDB code 4FAM) bound in the AKR1C3 active site showed that the N-linked phenyl group of **24** occupied a different region of the SP1 binding pocket to the dihydroisoquinoline moiety of **7**. The side chains of Trp227 and Phe311 as well as the loop containing Phe311 adopted an alternate conformation to accommodate the dihydroisoquinoline moiety. While the potency of **7** was reported to be tolerant to many dihydroisoquinoline substitutions,[10] modelling the likely binding modes of these analogues based on that of **7** indicated that 5-position halide and amine substitutions extended toward the same space as the 4-chloro substituted phenyl ring of **24**.

AKR1C isoform selectivity was determined for all compounds with AKR1C3 IC_{50}s <10 μM, with all compounds tested found to have minimal inhibitory activity for AKR1C1, 2 and 4 (IC_{50}s >30 μM). A representative compound, **24**, was also screened for inhibition of COX-1 and COX-2, but was inactive in both assays at 10 μM.

Compounds of interest or with IC_{50}s <0.3 μM against isolated AKR1C3 were also evaluated in an AKR1C3 overexpressing HCT-116 human colon cancer cell line for their effectiveness in inhibiting the 2-electron reduction of PR-104A, an exogenous dinitrobenzamide substrate that is exclusively metabolised by AKR1C3 under aerobic conditions to its cytotoxic 4-hydroxylamine (PR-104H) and 4-amine (PR-104 M) metabolites[17] as described previously.[10] For these compounds (**22**, **24**–**27**, **36**–**38**, **46**, **56**, **57**), there was a broad rank order correlation between their enzymatic and cellular activity, but an average 3-fold higher potency in cells. This is in contrast with analogues of the carboxylic acid **7** which, while showing a similar broad rank order correlation between enzymatic and cellular activity, were on average 4-fold less potent in cells than in isolated enzyme assays.[10]

6. Conclusions

While a considerable number of inhibitors of AKR1C3 have been reported, the vast majority are carboxylates, where this moiety binds to the 'oxyanion hole' of the enzyme. The present series provides a new class of non-carboxylate inhibitors that bind selectively to AKR1C3 via the carbonyl oxygen of the central urea linker. This activity is favored by lipophilic electron-withdrawing substituents on the phenyl ring that probe specific regions of the SP1 pocket and H-bond acceptors on the other terminal ring. This is consistent with the X-ray structure of **24** bound in the AKR1C3 active site, which shows interactions between the carbonyl oxygen of the drug and Tyr55 and His117 in the 'oxyanion hole' of the enzyme, with the piperazine bridging unit providing the correct twist to allow the terminal benzene ring to occupy the lipophilic pocket and align with Phe311. These neutral compounds had an average 3-fold higher potency for inhibition of AKR1C3 in cells compared with isolated enzymes. Compound **24** has been used, in conjunction with the pan-AKR1 fluorogenic substrate coumberone, to evaluate AKR1C3 activity in human leukaemia cells.[18]

7. Experimental section

7.1. COX assays

These were were carried out by GVK Biosciences Ltd., Biology, 28A, IDA Nacharam, Hyderabad 500 076 India (www.gvkbio.com). For details see Ref. 10.

7.2. Measurement of AKR1C enzyme activity

Recombinant AKR1C protein was produced and purified as described previously.[10] A competitive fluorescence assay was used to measure AKR1C enzyme activity, where a non-fluorescent ketone probe (probe 5)[15] selective for the AKR1C enzyme isoforms is reduced to a fluorescent alcohol in the presence of AKR1C enzyme and NADPH. Briefly, purified protein (2 μg/mL AKR1C1, 1 μg/mL AKR1C2, 2 μg/mL AKR1C3 and 5 μg/mL AKR1C4) was incubated with 40 μM probe 5, test compounds and 50 μM NADPH in an assay buffer of 10 mM MOPS (pH = 7.2), 130 mM NaCl, 1 mM DTT and 0.01% Triton-X-100 for 1 h at 37 °C. The reaction was stopped by addition of 35 mM NaOH and fluorescence was read in a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at excitation/emission wavelengths of 420/510 nM. The compounds and known AKR1C3 inhibitors (flufenamic acid, indomethacin, naproxen, meclofenamic acid, S(+)-ibuprofen and flurbiprofen; Sigma–Aldrich, Auckland, New Zealand) were tested at multiple concentrations between 0.1 nM and 100 μM in 2% DMSO to generate AKR1C enzyme inhibition data. Compound IC_{50} values were calculated by fitting the inhibition data to a four-parameter logistic sigmoidal dose–response curve using Prism 5.02 (GraphPad, La Jolla, CA, USA).

AKR1C3 activity was determined in HCT-116 cells engineered to over-express AKR1C3 by measuring the major metabolite (PR-104H) of PR-104A produced by 2-electron reduction under aerobic conditions, a reaction catalysed selectively by AKR1C3.[17] The synthesis of PR-104A, PR-104H and tetradeuterated PR-104H,[19,20] the transfection and maintenance of the HCT-116/AKR1C3 cell line[17] and the measurement of PR-104H by LC–MS/MS have been described previously.[21] The compounds were administered 2 h prior to 100 μM PR-104A at multiple concentrations between 1 nM and 3 μM. PR-104H formation was quantitated against a PR-104H standard curve ranging from 1 to 1000 nM. Compound IC_{50} values were calculated from four-parameter logistic sigmoidal dose–response curves that were fitted to the inhibition data using Prism 5.02. Representative compounds were tested over repeat assays to ensure assay reproducibility.

7.3. Chemistry

Combustion analyses were performed by the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal IA9100 melting point apparatus, and are as read. NMR spectra were measured on a Bruker Avance 400 spectrometer at 400 MHz for ^1H and are referenced to Me_4Si. Chemical shifts and coupling constants are recorded in units of ppm and Hertz, respectively. High-resolution electron impact (HREIMS) and fast atom bombardment (HRFABMS) mass spectra were determined on a VG-70SE mass spectrometer at nominal 5000 resolution. High-resolution electrospray ionisation (HRESIMS) and atmospheric pressure chemical ionisation (HRAPCIMS) mass spectra were determined on a Bruker micrOTOF-Q II mass spectrometer. Low-resolution atmospheric pressure chemical ionisation (APCI) mass spectra were measured for organic solutions on a ThermoFinnigan Surveyor MSQ mass spectrometer, connected to a Gilson autosampler. Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F_{254}), with exposure to I_2, or by staining in permanganate and phosphomolybdic acid dips and by UV light (254 nm & 365 nm). Column chromatography was carried out on silica gel (Merck 230–400 mesh).

7.3.1. Compounds of Table 1 (Scheme 1). (4-(4-Chlorophenyl)piperazin-1-yl)(morpholino)methanone (24)

A solution of tert-butyl piperazine-1-carboxylate (**62**) (4.0 g, 21.5 mmol) in DCM (30 mL) was treated with DIPEA (5.61 mL, 1.5 equiv) and this solution was cooled to 0 °C and treated dropwise with a solution of morpholine-4-carbonyl chloride (**63**) (3.85 g, 25.7 mmol) in DCM (10 mL). The mixture was stirred at 20 °C for 30 min, then diluted with DCM, washed with aqueous Na_2CO_3, dried and evaporated. The residue was triturated with EtOH, then dissolved in DCM (30 mL) and treated with TFA (30 mL). The solution was stirred at 20 °C for 2 h and evaporated. The residue was shaken with DCM and 4 N NH_4OH, the aqueous layer was back-extracted with DCM (4×) and the combined organic extracts were dried and evaporated to give morpholino(piperazin-1-yl)methanone[7] (**64**) (70%). ^1H NMR ($CDCl_3$) δ 3.55 (t, J = 4.7 Hz, 4H), 3.14–3.02 (m, 8H), 2.64 (t, J = 4.9 Hz, 4H), 2.25 (br s, 1H). HPLC purity 98.8%

A mixture of **64** (1.075 g, 5.5 mmol), 1-bromo-4-chlorobenzene (1.03 g, 5.4 mmol), Cs_2CO_3 (2.28 g, 7 mmol) and BINAP (0.05 equiv) in toluene (60 mL) was purged with N_2, $Pd(OAc)_2$ (110 mg, 0.22 mmol) was added and the mixture was stirred under reflux for 16 h, then cooled, diluted with EtOAc, filtered and evaporated. The residue was chromatographed on silica gel, with EtOAc/CH_2Cl_2 (2:1) eluting **24** (0.62 g. 37%), mp (EtOAc/pet ether) 121–123 °C. ^1H NMR ($CDCl_3$) δ 7.24 (br d, J = 4.5 Hz, 2H), 6.95 (br d, J = 4.5 Hz, 2H, 3.57 (t, J = 4,7 Hz, 4H), 3.32–3.25 (m, 4H), 3.20–3.08 (m, 8H). HPLC purity 99.3%.

7.3.2. Morpholino(4-phenylpiperazin-1-yl)methanone (9)

Similar reaction of **64** and 1-bromobenzene gave **9** (45%), mp (hexane) 67–68 °C. ^1H NMR ($CDCl_3$) δ 7.22 (br dd, J = 8.8, 7.3 Hz, 2H), 6.94 (br dd, J = 8.8, 1.0 Hz, 2H), 6.80 (t, J = 7.3 Hz, 1H), 3.58 (t, J = 4.7 Hz, 4H), 3.32–3.24 (m, 4H), 3.17 (t, J = 4.8 Hz, 4H), 3.15–3.09 (m, 4H). HPLC purity 100%.

7.3.3. Morpholino(4-(*o*-tolyl)piperazin-1-yl)methanone (10)

Similar reaction of **64** and 1-bromo-2-methylbenzene gave **10** (40%), mp (hexane) 134–135 °C. 7.20–7.11 (m, 2H), 7.04–6.93 (m, 2 H), 3.58 (t, J = 4.7 Hz, 4H), 3.32 (t, J = 4.8 Hz, 4H), 3.17 (t,

J = 4.7 Hz, 4H), 2,81 (t, *J* = 4,8 Hz, 4H), 2.26 (s, 3H). HPLC purity 99.0%.

7.3.4. (4-(2-Methoxyphenyl)piperazin-1-yl)(morpholino)methanone (11)

Similar reaction of **64** and 1-bromo-2-methoxybenzene gave **11** (65%), mp (EtOAc/pet ether) 113–114 °C. ^{1}H NMR ($CDCl_3$) δ 6.98–6.81 (m, 4H), 3.76 (s, 3H), 3.56 (t, *J* = 4.6 Hz, 4H), 3.33–3.20 (m, 4 H), 3.14 (t, *J* = 4.6 Hz, 4H), 2.91 (t, *J* = 4.7 Hz, 4H). HPLC purity 100%.

7.3.5. (4-(2-Chlorophenyl)piperazin-1-yl)(morpholino)methanone (12)

Similar reaction of **64** and 1-bromo-2-chlorobenzene gave **12** (48%), mp (EtOAc/pet ether) 137–139 °C. ^{1}H NMR ($CDCl_3$) δ 7.42 (dd, *J* = 7.9, 1,5 Hz, 1H), 7.33–7.26 (m, 1H), 7.16 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.06 (td, *J* = 7.6, 1.4 Hz, 1H), 3.58 (t, *J* = 4.7 Hz, 4H), 3.34 (t, *J* = 4.8 Hz, 4H), 3.17 (t, *J* = 4.7 Hz, 4H), 2.95 (t, *J* = 4.8 Hz, 4H). HPLC purity 100%.

7.3.6. Morpholino(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)methanone (13)

Similar reaction of **64** and 1-bromo-2-(trifluoromethyl)benzene gave **13** (63%), mp (hexane) 110–112 °C. ^{1}H NMR ($CDCl_3$) δ 7.71–7.62 (m, 2H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 3.58 (t, *J* = 4.7 Hz, 4H), 3.32–3.20 (m, 4H), 3.17 (t, *J* = 4.7 Hz, 4H), 2.84 (t, *J* = 4.8 Hz, 4H). HPLC purity 88.1%.

7.3.7. (4-(3-Chlorophenyl)piperazin-1-yl)(morpholino)methanone (14)

Similar reaction of **64** and 1-bromo-3-chlorobenzene gave **14** (57%), mp (EtOAc/pet ether) 83–84 °C. ^{1}H NMR ($CDCl_3$) δ 7.22 (t, *J* = 8.1 Hz, 1H), 6.95 (t, *J* = 2.1 Hz, 1H), 6.92–6.87 (m, 1H), 6.83–6.78 (m, 1H), 3.60–3.55 (m, 4H), 3.32–3.26 (m, 4H), 3.21–3.14 (m, 8H). HPLC purity 98.3%.

7.3.8. 3-(4-(Morpholine-4-carbonyl)piperazin-1-yl)benzamide (15)

Similar reaction of **64** and 3-bromobenzamide gave **15** (43%), mp (CH_2Cl_2/pet ether) 239–242 °C. ^{1}H NMR ($CDCl_3$) δ 7.88 (s, 2H), 7.43 (s, 2H), 7.33–7.20 (m, 3H), 7.09 (dt, *J* = 4.7 Hz, 1H), 3.58 (m, 4H), 3.22–3.13 (m. 8H). HPLC purity 97.9%.

7.3.9. Morpholino(4-(*p*-tolyl)piperazin-1-yl)methanone (16)

Similar reaction of **64** and 1-bromo-4-methylbenzene gave **16** (83%) mp (hexane) 97–98 °C. ^{1}H NMR ($CDCl_3$) δ 7.03 (br d, *J* = 8.3 Hz, 2H), 6.84 (be d, *J* = 8.6 Hz, 2H), 3.57 (t, *J* = 4.7 Hz, 4H), 3.31–3.23 (m, 4H), 3.17 (t, *J* = 4.7 Hz, 4H), 3.05 (t, *J* = 5.0 Hz, 4H), 2.20 (s, 3H). HPLC purity 96.2%.

7.3.10. (4-(4-Methoxyphenyl)piperazin-1-yl)(morpholino)methanone (17)

Similar reaction of **64** and 1-bromo-4-methoxybenzene gave **17** (52%), mp (hexane) 116–117 °C. ^{1}H NMR ($CDCl_3$) δ 6.90 (br d, *J* = 9.1 Hz, 2H), 6.82 (br d, *J* = 9.1 Hz, 2H), 3.68 (s, 3H), 3.60–3.55 (m, 4H), 3.31–3.24 (m, 4H), 3.20–3.13 (m, 4H), 3.02–2.95 (m, 4H). HPLC purity 99.4%.

7.3.11. (4-(Biphenyl-4-yl)piperazin-1-yl)(morpholino)methanone (18)

Similar reaction of **64** and 1-bromobiphenyl gave **18** (52%), mp (EtOAc)185–186 °C. ^{1}H NMR ($CDCl_3$) δ 7.63–7.57 (m, 2H), 7.55 (br d, *J* = 8.8 Hz, 2H), 7.44–7.37 (m, 2H), 7.30–7.24 (m, 1H), 7.03 (br d, *J* = 8.9 Hz, 2H), 3.58 (t, *J* = 4.7 Hz, 4H), 3.37–3.25 (m, 4H), 3.22–3.12 (m, 4H). HPLC purity 95.9%.

7.3.12. Morpholino(4-(naphthalen-2-yl)piperazin-1-yl)methanone (19)

Similar reaction of **64** and 2-bromonaphthalene gave **19** (68%), mp (EtOAc/pet ether) 149–152 °C. ^{1}H NMR ($CDCl_3$) δ 7.79–7.69 (m, 3H), 7.42–7.34 (m, 2H), 7.30–7.24 (m, 1H), 7.18 (d, *J* = 2.3 Hz, 1H), 3.59 (t, *J* = 4.7 Hz, 4H), 3.39–3.32 (m, 4H), 3.28–3.16 (m, 4H). HPLC purity 96.9%.

7.3.13. Morpholino(4-(quinolin-3-yl)piperazin-1-yl)methanone (20)

Similar reaction of **64** and 3-bromoquinoline gave **20** (62%), mp (EtOAc/pet ether) 156–157 °C. ^{1}H NMR ($CDCl_3$) δ 8.87 (d, *J* = 2.9 Hz, 1H), 7.90–7.84 (m, 1H), 7.82–7.75 (m, 1H), 7.56 (d, *J* = 2.8 Hz, 1H), 7.54–7.46 (m, 2H), 3.59 (t, *J* = 4.7 Hz, 4H), 3.42–3.34 (m, 4H), 3.34–3.26 (m, 4H), 3.20 (t, *J* = 4.7 Hz, 4H).m, 4H). HPLC purity 98.0%.

7.3.14. (4-(4-Ethynylphenyl)piperazin-1-yl)(morpholino)methanone (21)

A mixture of (4-(4-iodophenyl)piperazin-1-yl)(morpholino)methanone (**26**) (80 mg. 0.2 mmol), CuI (3 mg, 0.01 mmol) and $(Ph_3P)_2PdCl_2$ (104 mg, 0.01 mmol)) in dry THF (2 mL) was flushed with N_2. A solution of trimethylsilylacetylene (25 mg, 0.25 mmol) in Et_3N (2 mL) was added and the reaction was stirred at 20 °C for 15 h. The mixure was evaporated and the residue was chromatographed on silica gel to give crude morpholino(4-(4-((trimethylsilyl)ethynyl)phenyl)piperazin-1-yl)methanone (**65**) (58%). A mixture of **65** (41 mg, 0.11 mmol) and powdered K_2CO_3 (41 mg, 0.30 mmol) in MeOH (5 mL) was stirred at 20 °C for 3 h, then evaporated to dryness. The residue was extracted with EtOAc and the solution was filtered through silica gel to give **21** (30 mg, 91%), mp (EtOAc/pet ether) 159–161 °C. ^{1}H NMR ($CDCl_3$) δ 7.31 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 8.9 Hz, 2H), 3.93 (s, 1H), 3.60–3.54 (m, 4H), 3.32–3.24 (m, 4H), 3.24–3.14 (m, 8H). HPLC purity 99.0%.

7.3.15. Morpholino-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)methanone (22)

Similar reaction of **64** and 1-bromo-4-(trifluoromethyl)benzene gave **22** (63%), mp (hexane) 117–118 °C. ^{1}H NMR ($CDCl_3$) δ 7.52 (d, *J* = 8.7 Hz, 2H), 7.06 (d, *J* = 8.7 Hz, 2H, 3.58 (t, *J* = 4.7 Hz, 4H), 3.32–3.22 (m, 8H), 3.18 (t, *J* = 4.7 Hz, 4H. HPLC purity 99.8%.

7.3.16. (4-(4-Fluorophenyl)piperazin-1-yl)(morpholino)methanone (23)

Similar reaction of **64** and 1-bromo-4-fluorobenzene gave **23** (86%), mp (hexane) 102–103 °C. ^{1}H NMR ($CDCl_3$) δ 7.10–6.92 (m, 4H), 3.62–3.53 (m, 4H), 3.31–3.23 (m, 4H), 3.21, 3.14 (m, 4H), 3.10–3.02 (m, 4H). HPLC purity 98.7%.

7.3.17. (4-(4-Bromophenyl)piperazin-1-yl)(morpholino) methanone (25)

Similar reaction of **64** and 1,4-dibromobenzene gave **25** (52%), mp (EtOAc/pet ether) 145–147 °C. ^{1}H NMR ($CDCl_3$) δ 7.35 (d, *J* = 9.0 Hz, 2H), 6.90 (d, *J* = 9.1 Hz, 2H), 3–60–3.55 (m, 4H), 3.31–3.22 (m, 4H) (after D_2O), 3.20–3.10 (m, 8H). HPLC purity 97.3%.

7.3.18. (4-(4-Iodophenyl)piperazin-1-yl)(morpholino)methanone (26)

A mixture of **25** (177 mg, 0.50 mmol), NaI (150 mg, 1.0 mmol), CuI (5 mg, 0.025 mmol) and (1*S*,2*S*)-N^1,N^2-dimethylcyclohexane-1,2-diamine (7 mg, 0.04 mmol) in dioxane (1 mL) was purged with N_2, then heated in a sealed tube at 110 °C for 48 h. The mixture was evaporated, shaken with water and DCM, filtered and the organic layer was dried and evaporated. The residue was dissolved in EtOAc and filtered through silica gel to give **26** (75%), mp (EtOAc/

pet ether) 151–153 °C. ^{1}H NMR ($CDCl_3$) δ 7.50 (d, J = 9.0 Hz, 2H), 6.79 (d, J = 9.0 Hz, 2H), 3.51–3.54 (m, 4H), 3.31–3.24 (m, 4H), 3.20–3.08 (m, 8H). HPLC purity 94.2%.

7.3.19. Methyl 4-(4-(morpholine-4-carbonyl)piperazin-1-yl)benzoate (27)

Similar reaction of **64** and methyl 4-bromobenzoate gave **27** (81%), mp (EtOAc/pet ether) 149–150 °C. ^{1}H NMR ($CDCl_3$) δ 7.80 (br t, J = 9.1 Hsz, 2H), 6.98 (br t, J = 9.1 Hz, 2H), 3.78 (s, 3H), 3.58 (t, J = 4.7 Hz, 4H), 3.36–3.28 (m, 8H), 3.18 (t, J = 4.7 Hz, 4H). HPLC purity 98.8%.

7.3.20. 4-(4-(Morpholine-4-carbonyl)piperazin-1-yl)benzoic acid (28)

A solution of **27** (508 mg, 1.53 mmol) in MeOH/water (1:1, 3 mL) and 1 N aqueous NaOH (1.52 mL, 1 equiv) was heated under reflux for 2 h, then diluted with water and extracted with DCM. The aqueous layer was acidified with AcOH and the resulting solid was collected and washed to give **28** (362 mg, 74%), mp (MeOH/EtOAc) 240–243 °C. ^{1}H NMR ($(CD_3)_2SO$) δ 12.26 (s, 1H), 7.78 (br d, J = 9.0 Hz, 2H), 6.96 (br d, J = 9.1 Hz, 2H), 3.58 (t, J = 4.7 Hz, 4H), 3.29 (s, 8H), 3.18 (t, J = 4.7 Hz, 4H), HPLC purity 98.6%.

7.3.21. 4-(4-(Morpholine-4-carbonyl)piperazin-1-yl)benzamide (29)

A suspension of **28** (200 mg, 0.62 mmol) in DCM (2 mL), $(COCl)_2$ (20 mg, 2 equiv) and a trace of DMF was stirred at 20 °C for 30 min, then evaporated to dryness. The residue of crude acid chloride was dissolved in THF and the solution was cooled to 0 °C and treated with excess NH_3 gas, then evaporated to dryness. The residue was partitioned between EtOAc and 2 N NH_4OH, and the organic layer was evaporated. The residue was chromatographed on silica gel, eluting with EtOAc/MeOH (9:1) to give **29** (86%), mp (EtOAc/pet ether) 255–257 °C. ^{1}H NMR ($CDCl_3$) δ 7.76 (br d, J = 8.9 Hz, 2H), 7.68 (br s, 1H), 6.98 (br S, 1H), 6.98 (br d, J = 9,0 Hz, 2H), 3.56 (t, J = 4.7 Hz, 4H), 3.34–3.22 (m, 8H), 3.18 (t, J = 4.7 Hz, 2H). HPLC purity 98.9%.

7.3.22. *N*-Methyl-4-(4-(morpholine-4-carbonyl)piperazin-1-yl) benzamide (30)

Similar treatment of the crude acid chloride of **28** with excess aqueous methylamine gave **30** (82%), mp 217–220 °C. ^{1}H NMR ($CDCl_3$) δ 8.12 (d, J = 4.5 Hz, 1H), 7.72 (d, J = 9.0 Hz, 2H), 6.96 (d, J = 9.0 Hz, 2H), 3.58 (t, J = 5.0 Hz, 4H), 3.38–3,22 (m, 4H), 3.20–3.14 (m, 4H), 2.75 (d, J = 4.0 Hz, 3H). HPLC purity 98.3%.

7.3.23. *N,N*-Dimethyl-4-(4-(morpholine-4-carbonyl)piperazin-1-yl)benzamide (31)

Similar treatment of the crude acid chloride of **28** with excess dimethylamine in THF gave **31** (78%), mp (EtOC/pet ether) 130–131 °C. ^{1}H NMR ($CDCl_3$) δ 7.31 (d, J = 9.5 Hz, 2H), 6.94 (d, J = 9.5 Hz, 2H), 3.58 (t, J = 5.0 Hz, 2H), 3.73–3.68 (m, 4H), 3.63–3.56 (m, 8H). HPLC purity 99.6%.

7.3.24. (4-(4-(Methylsulfonyl)phenyl)piperazin-1-yl)(morpholino)methanone (32)

Similar reaction of **64** and 1-bromo-4-(methylsulfonyl)benzene gave **32** (45%), mp (EtOAc/pet ether) 180–181 °C. ^{1}H NMR ($CDCl_3$) δ 7.69 (br d, J = 12 Hz, 2H), 7.07 (br d, J = 12 Hz, 2H), 3.58 (t, J = 4.8 Hz, 2H), 3.38–3.27 (m, 8 H), 3.18 (t, J = 4.8 Hz, 2H), 3.09 (s, 3H). HPLC purity 99.6%.

7.3.25. Morpholino(4-(4-nitrophenyl)piperazin-1-yl)methanone (33)

Similar reaction of **64** and 1-bromo-4-nitrobenzene gave **33** (48%), mp (EtOAc/iPr_2O) 190–192 °C. ^{1}H NMR ($CDCl_3$) δ 8.07 (br d, J = 9.4 Hz, 2H), 7.02 (br d, J = 9.5 MHz, 2H), 3.58 (t, J = 4.7 Hz, 4H), 3.52–3.45 (m, 4H), 3.36–3.28 (m, 4H), 3.19 (t, J = 4.7 Hz, 4H). HPLC purity 99.8%.

7.3.26. *N*-(4-(4-(Morpholine-4-carbonyl)piperazin-1-yl)phenyl) acetamide (34)

A solution of morpholino(4-(4-nitrophenyl)piperazin-1-yl)methanone (**33**) (520 mg, 1.63 mmol) in THF (60 mL) was hydrogenated over 5% Pd–C at 40 psi for 1 h. The catalyst was filtered off and the solution of crude (4-(4-aminophenyl)piperazin-1-yl)(morpholino)methanone (**66**) was treated with Ac_2O (333 mg, 3.26 mmol) and a trace of $HClO_4$ at 20 °C for 5 min. The mixture was then evaporated, and the residue was dissolved in EtOAc, washed with aqueous Na_2CO_3 and evaporated. The crude product was chromatographed on silica gel, eluting with EtOAc/MeOH (9:1) to give **34** (92%), mp (EtOAc) 193–195 °C. ^{1}H NMR ($CDCl_3$) δ 9.67 (s, 1H), 7.42 (br d, J = 9.1 Hz, 2H), 6.88 (br d, J = 9.1 Hz, 2H), 3.57 (t, J = 4.7 Hz, 4H), 3.31–3.23 (m, 4H) (after D2O), 3.17 (t, J = 4.7 Hz, 4H), 3.08–3.01 (m, 4H), 1.99 (s, 3H). HPLC purity 99.9%.

7.3.27. *N*-(4-(4-(Morpholine-4-carbonyl)piperazin-1-yl)phenyl) methanesulfonamide (35)

The crude amine (**66**) from evaporation of the hydrogenation of **33** (110 mg, 0.34 mmol) was dissolved in pyridine (2 mL) and treated with MsCl (35 μL, 0.41 mmol) at 20 °C for 15 min. The reaction was then diluted with aqueous $KHCO_3$, extracted with EtOAc, and the organic layer was washed, dried and evaporated. The residue was chromatographed on silica gel, eluting with EtOAc/MeOH (9:1), to give **35** (96 mg, 76%), mp (EtOAc/iPr_2O) 189–192 °C. ^{1}H NMR ($CDCl_3$) δ 9.24 (s, 1H), 7.09 (br d, J = 9.0 Hz, 2H), 6.93 (br d, J = 9.0 Hz, 2H), 3.60–3.55 (m, 4H), 3.32–3.26 (m, 4H), 3.20–3.15 (m. 4H), 3.11–3.06 (m, 4H), 2.57 (s, 3H). HPLC purity 99.6%.

7.3.28. (4-(2,4-Dichlorophenyl)piperazin-1-yl)(morpholino)methanone (36)

Similar reaction of **64** and 1-bromo-2,4-dichlorobenzene gave **36** (65%), mp (EtOAc/pet ether) 135–136 °C. ^{1}H NMR ($CDCl_3$) δ 7.55 (d, J = 2.5 Hz, 2H), 7.38 (dd, J = 8.7, 2.5 Hz, 1H), 7.17 (d, J = 8.7 Hz, 1H), 3.58 (t, J = 4.7 Hz, 4H), 3.33 (t, J = 4.8 Hz, 4H), 3.16 (t, J = 4.7 Hz, 4H), 2.94 (t, J = 4.8 Hz, 4H). HPLC purity 98.9%.

7.3.29. (4-(4-Chloro-2-methylphenyl)piperazin-1-yl)(morpholino)methanone (37)

Similar reaction of **64** and 1-bromo-4-chloro-2-methylbenzene gave **37** (61%), mp (EtOAc/pet ether) 129–131 °C. ^{1}H NMR ($CDCl_3$) δ 7.24 (d, J = 2.3.Hz, 1H), 7.18 (dd, J = 8.5, 2.4 Hz, 1H), 7.02 (d, J = 8.5 Hz, 1H), 3.58 (t, J = 4.6 Hz, 4H), 3.33–3.27 (m, 4H) after D_2O), 3.16 (t, J = 4.6 Hz, 4H), 2.79 (t, J = 4.7 Hz, 4H), 3.25 (s, 3H). HPLC purity 99.6%.

7.3.30. (4-(4-Chloro-2-(hydroxymethyl)phenyl)piperazin-1-yl)(morpholino)methanone (38)

Similar reaction of **64** and (2-bromo-5-chlorophenyl)methanol gave **38** (8%), mp (EtOAc/pet ether) 160–162 °C. ^{1}H NMR ($CDCl_3$) δ 7.44 (d, J = 2.6 Hz, 1H), 7.24 (dd, J = 8.5, 2.7 Hz, 1H), 7.05 (d, J = 8.6 Hz, 1H), 5.25 (t, J = 5.7 Hz, 1H), 4.53 (d, J = 5.7 Hz, 2H), 3.57 (t, J = 4.7 Hz, 4H), 3.33–3.27 (m, 4H), 3.16 (t, J = 4.7 Hz, 4H), 2.79 (t, J = 4.7 Hz, 4H). HPLC purity 98.5%.

7.3.31. (4-(6-Chloropyridin-3-yl)piperazin-1-yl)(morpholino)methanone (39)

Similar reaction of **64** and 5-bromo-2-chloropyridine gave **39** (34%), mp (EtOAc/pet ether) 132–134 °C. ^{1}H NMR ($CDCl_3$) δ 8.07

(d, J = 3.0 Hz, 1H), 7.43 (dd, J = 8.7, 3.2 Hz, 1H), 7.31 (d, J = 8.8 Hz, 1H), 3.60–3.54 m(m, 4 H), 3.32–3.26 (m., 4 H), 3.23–3.14 (m, 4H). HPLC purity 98.0%.

7.3.32. (4-(6-Bromopyridin-2-yl)piperazin-1-yl)(morpholino) methanone (40)

Similar reaction of **64** and 2,5-dibromopyridine gave **40** (52%), mp (EtOAc/pet ether) 141–142 °C. ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 2.3 Hz, 1H), 7.70 (dd, J = 9.0, 2.3 Hz, 1H), 6.83 (d, J = 9.2 Hz, 1H), 3.61–3.54 (m, 4H), 3.52–3.45 (m, 4H), 3.18–3,22 (m, 4H), 3.20–3.14 (m, 4H). HPLC purity 99.3%.

7.3.33. (4-(4-Chlorobenzyl)piperazin-1-yl)(morpholino)meth anone (41)

A solution of tert-butyl piperazine-1-carboxylate (**62**) (2.0 g, 10.8 mmol) and 4-chlorobenzaldehyde (**67**) (1.28 g, 9.08 mmol) in DCE (30 mL) were treated with $NaBH(OAc)_3$ (3.12 g, 14.7 mmol) and the mixure was stirred at 20 °C under N_2 for 6 h, then diluted with DCM and washed with 1 N aqueous NaOH. The organic layer was dried and evaporated, and the residue was filtered through a short column of silica gel. The crude product was dissolved in DCM/TFA (1:1, 40 mL) and kept at 20 °C for 1 h, then evaporated. The residue was dissolved in DCM and washed with 4 N NH_4OH, dried and evaporated. The residue was redissolved in 2 N aqueous AcOH, and the aqueous layer was basified with concd NH_4OH to give crude 1-(4-chlorobenzyl)piperazine (**68**). A cold solution of **68** (100 mg, 0.47 mmol) and DIPEA (20 μL, 2 equiv) in DCM (3 mL) was treated with morpholine-4-carbonyl chloride (**63**) (110 mg, 0.74 mmol). The mixture was stirred at 20 °C for 30 min, then diluted with DCM, washed with Na_2CO_3, dried and evaporated. Chromatography on silica gel, eluting with EtOAc/MeOH (from 95:5) containing 0.5% concd NH_4OH gave **41**, mp (hexane) 80–81 °C. ^{1}H NMR ($CDCl_3$) δ 7.38 (br d, J = 8.5 Hz, 2H), 7.32 (br d, J = 8,5 Hz, 2H), 3.54 (t, J = 4.7 Hz, 4H), 3.47 (s, 2H), 3.15 (t, J = 4.9 Hz, 4H), 3.10 (t, J = 4.7 Hz, 4H), 2.33 (t, J = 4.9 Hz, 4H), HPLC purity 98.2%.

7.3.34. (4-(4-Chlorobenzoyl)piperazin-1-yl)(morpholino)meth anone (42)

A solution of **64** (70 mg, 0.35 mmol) in DCM (3 mL) and DIPEA (92 μL, 1.5 equiv) was treated at 0 °C with a solution of 4-chlorobenzoyl chloride (**69**) (68 mg, 1.1 equiv) in DCM (1 mL). The mixture was stirred at 20 °C for 15 min, then diluted with DCM, washed with Na_2CO_3, evaporated and triturated with iPr_2O to give **42** (89%), mp (EtOAc/pet ether) 147–149 °C. ^{1}H NMR ($CDCl_3$) δ 7.54–7.49 (m, 4H), 7.47–7.43 (m, 4H) 3.55 (t, J = 5.0 Hz, 4H), 3.20–3.10 (m, 8H). HPLC purity 98.3%.

7.3.35. Compounds of Table 2 (Scheme 2). (4-(4-Chlorophenyl) piperazin-1-yl)(tetrahydro-2*H*-pyran-4-yl)methanone (43)

A solution of 1-(4-chlorophenyl)piperazine (**70**) (80 mg, 0.41 mmol) and DIPEA (0.13 mL, 2 equiv) in DCM (4 mL) was treated at 0 °C with tetrahydro-2*H*-pyran-4-carbonyl chloride (74 mg, 1.2 equiv) in DCM (1 mL) for 15 min. The mixture was then diluted with DCM, washed with Na_2CO_3 and evaporated to give **43** (115 mg, 91%), mp (EtOAc/pet ether) 153–154 °C. ^{1}H NMR ($CDCl_3$) δ 7.25 (br d, J = 9.0 Hz, 2H), 6.96 (br d, J = 9.1 Hz, 2H), 3.89–3.80 (m, 2H), 3.11–3.53 (m, 4H), 3.39 (td, J = 11.5, 2.7 Hz, 4H), 3.19–3.04 (m, 4H), 2.97–2.86 (m, 1H), 1.67–1.49 (m, 4H). HPLC purity 98.9%.

7.3.36. (4-(4-Chlorophenyl)piperazin-1-yl)(cyclohexyl)met hanone (44)

Similar reaction of **70** and cyclohexanecarbonyl chloride gave **44** (87%), mp (hexane) 79–81 °C. ^{1}H NMR ($CDCl_3$) δ 7.24 (br d, J = 9.0 Hz, 2H), 6.96 (br d, J = 9.1 Hz, 2H), 3.68–3.51 (m, 4H), 3.19–3.01 (m, 4H), 2.68–2.55 (m, 1H), 1.76–1.58 (m, 5H), 1.40–1.09 (m, 5H). HPLC purity 100%.

7.3.37. (4-(4-Chlorophenyl)piperazin-1-yl)(phenyl)methanone (45)

Similar reaction of **70** and benzoyl chloride gave **45** (77%), mp (EtOAc/pet ether) 81–82 °C. ^{1}H NMR ($CDCl_3$) δ 7.50–7.40 (m, 5H), 7.25 (br d, J = 9.0 Hz, 2H), 6.96 (br d, J = 9.1 Hz, 2H), 3.86–3.36 (m, 4H), 3.18 (br s, 4H). HPLC purity 99.9%.

7.3.38. 4-(4-Chlorophenyl)-*N*-phenylpiperazine-1-carboxamide (46)

Similar reaction of **70** (21 mg) and phenylisocyanate gave **46** (84%), mp (EtOAc/pet ether) 195–198 °C. ^{1}H NMR ($CDCl_3$) δ 8.58 (s, 1H), 7.47 (br d, J = 8.6 Hz, 2H), 7.78–7.19 (m, 4H), 7.00 (br d, J = 9.0 Hz, 2H), 6.94 (br d, J = 7.3 Hz, 1H), 3.59 (t, J = 5.1 Hz, 4H), 3.17 (t, J = 5.1 Hz, 4H). HPLC purity 99.4%.

7.3.39. 4-(4-Chlorophenyl)-*N*-cyclohexylpiperazine-1-carbo xamide (47)

Similar reaction of **70** and isocyanatocyclohexane gave **47** (90%), mp (EtOAc/pet ether) 166–169 °C. ^{1}H NMR ($CDCl_3$) δ 7.23 (be d, J = 9.0 Hz, 2H), 6.96 (br d, J = 9.1 Hz, 2H), 6.22 (d, J = 7.7 Hz, 1H), 3.46–3.36 (m, 5H), 3.07 (t, J = 5.1 Hz, 4H), 1.79–1.51 (m, 5H), 1.30–1.00 (m, 5H), HPLC purity 99.5%.

7.3.40. (4-(4-Chlorophenyl)piperazin-1-yl)(pyridin-4-yl)meth anone (48)

Similar reaction of **70** and isonicotinoyl chloride gave **48** (91%), mp (EtOAc/pet ether) 148–149 °C. ^{1}H NMR ($CDCl_3$) δ 8.69 (dd, J = 4.3, 1.6 Hz, 2H), 7.43 (dd, J = 4.4, 1.6 Hz, 2H), 7.25 (br d, J = 9.0 Hz, 2H), 6.96 (br s, 2H), 3.76 (br s, 2H), 3.40 (br s, 2H), 3.25 (br s, 2H), 3.13 (br s, 2H), HPLC purity 99.7%.

7.3.41. (4-(4-Chlorophenyl)piperazin-1-yl)(piperidin-1-yl)met hanone (49)

Similar reaction of **70** and piperidine-1-carbonyl chloride gave **49** (88%), mp (hexane) 100–101 °C. ^{1}H NMR ($CDCl_3$) δ 7.23 (br d, J = 9.0 Hz, 2H), 6.95 (br d, J = 9.1 Hz, 2H), 3–27–3–20 (m, 4H), 3.20–3.07 (m, 8H), 1.59–1.43 (m, 6H). HPLC purity 99.8%.

7.3.42. (4-(4-Chlorophenyl)piperazin-1-yl)(thiomorpholino) methanone (50)

Similar reaction of **70** and thiomorpholine-4-carbonyl chloride gave **50** (80%), mp (EtOAc/pet ether) 119–121 °C. ^{1}H NMR ($CDCl_3$) δ br d, J = 9.0 Hz, 2H), br d, J = 9.1 Hz, 2H), 3.47–3.40 (m, 4H), 3.29–3.22 (m, 4H), 3.16–3.08 (m, 4H), 2.63–2.57 (m, 4H). HPLC purity 99.8%.

7.3.43. (4-(4-Chlorophenyl)piperazin-1-yl)(1-oxido-4-thiomor pholino)methanone (51)

A solution of **50** (50 mg, 0.15 mmol) in EtOH (4 mL) was treated with excess 27% aqueous H_2O_2 under reflux for 1 h, then evaporated. The residue was chromatographed over silica gel, with EtOAc/MeOH (85:15) eluting **51** (74%), mp (EtOAc/pet ether) 162–165 °C. ^{1}H NMR ($CDCl_3$) δ 7.24 (br d, J = 9.0 Hz), 6.96 (brd d, J = 9.1 Hz, 2H), 3.69–3.45 (m, 4H), 3.69–3.45 (m, 4H), 3.35–3.22 (m, 4H), 3.19–3.08 (m, 4H), 2.96–2.84 (m, 4H), 2.76–2.65 (m, 4H). HPLC purity 98.2%.

7.3.44. Thiomorpholine, 4-((4-chlorophenyl)-4-piperazinylcarb onyl)-, 1,1-dioxide (52)

A solution of triphosgene (165 mg, 0.56 mmol) in DCM (4 mL) and pyridine (0.26 mL, 3.3 mmol) at 20 °C was treated dropwise over 20 min with a solution of 1-(4-chlorophenyl)piperazine (**70**)

(295 mg, 1.5 mmol) and stirred at 20 °C for a further 3 h to give a solution of crude 4-(4-chlorophenyl)piperazine-1-carbonyl chloride[22] (**71**) (258 mg, 1.0 mmol). This was added dropwise to an ice-cold solution of thiomorpholine 1,1-dioxide[23] (0.11 g, 0.81 mmol) and DIPEA (1.5 equiv) in DCM (4 mL), and the mixture was allowed to warm to 20 °C over 1 h. The mixture was diluted with water and the organic layer was separated, washed with water, dried and evaporated. The crude product was chromatographed on silica gel, eluting with EtOAc/DCM (1:1) to give **52** (42%), mp (EtOAc/pet ether) 180–182 °C. ^{1}H NMR ($CDCl_3$) δ 7.24 (br d, J = 8.9 Hz, 2H), 6.96 (br d, J = 9.0 Hz, 2H), 3.67–3.53 (m, 4H), 3.40–3.28 (m, 4H), 3.23–3.07 (m, 8H). HPLC purity 97.7%.

7.3.45. (4-(4-Chlorophenyl)piperazin-1-yl)(4-methylpiperazin-1-yl)methanone (53)

A solution of crude **71** (50 mg, 0.19 mol) in DCM (3 mL) was cooled to 0 °C and added to a cold solution of 1-methylpiperazine (85 µL, 3 equiv) in DCM (2 mL). After stirring at 20 °C for 2 h the mixture was diluted with DCM, washed with aqueous Na_2CO_3, dried and evaporated. The residue was extracted with dilute aqueous AcOH, filtered and basified with aqueous Na_2CO_3 to precipitate **53** (32 mg, 42%), mp (EtOAc/pet ether) 130–132 °C. ^{1}H NMR ($CDCl_3$) δ 7.24 (br d, J = 9.0 Hz, 2H), 6.95 (br d, J = 9.1 Hz, 2H), 3.30–3.27 (m, 12H), 2.28 (t, J = 4.6 Hz, 4H), 2.17 (s, 3H). HPLC purity 94.6%.

7.3.46. (4-(4-Chlorophenyl)piperazin-1-yl)(4-(2-hydroxyethyl) piperazin-1-yl)methanone (54)

A solution of crude **71** (70 mg, 0.27 mmol) in DCM (4 mL) was cooled to 0 °C and treated dropwise with an ice-cold solution of 2-(piperazin-1-yl)ethanol (181 µL, 3 equiv) in DCM (2 mL). The mixture was stirred at 20 °C for 2 h, diluted with DCM, washed with aqueous Na_2CO_3, dried and evaporated. The residue was chromatographed on silica gel, eluting with EtOAc/MeOH (85:15) plus 1% NH_3. The resulting product was triturated with iPr_2O to give **54** (52%), mp (EtOAc/pet ether) 120–121 °C. ^{1}H NMR ($CDCl_3$) δ 7.24 (br d, J = 9.0 Hz, 2H), 6.95 (br d, J = 9.1 Hz, 2H), 4.40 (t, J = 5.3 Hz, 1H), 3.50 (q, J = 5.9 Hz, 2H), 3.29–3.22 (m, 4H), 3.21–3.05 (m, 8H), 2.44–2.35 (m, 6H). HPLC purity 99.2%.

7.3.47. 1-(4-(4-Chlorophenyl)piperazine-1-carbonyl)piperidine-4-carboxylic acid (55)

A solution of crude **71** (300 mg, 1.16 mmol) in DCM (9 mL) was cooled to 0 °C and treated dropwise with an ice-cold solution of methyl piperidine-4-carboxylate (328 mg, 2.29 mmol) and DIPEA (0.40 mL, 2,29 mmol) in DCM (3 mL). The mixture was stirred at 20 °C for 16 h, then diluted with DCM, washed with $NaHCO_3$, dried and evaporated. Chromatography on silica gel, melting with EtOAc/DCM (1:1), gave crude methyl 1-(4-(4-chlorophenyl)piperazine-1-carbonyl)piperidine-4-carboxylate (**72**) (217 mg, 70%) which was used directly. A solution of ester **72** in MeOH (2 mL) and 1 N aqueous NaOH (0.5 mL) was heated under reflux for 1 h, then diluted with water and concentrated to remove the MeOH. The concentrate was filtered and acidified with AcOH to give **55** (91%), mp (EtOAc/pet ether) 192–194 °C. ^{1}H NMR ($CDCl_3$) δ 12.23 (s, 1H), 7.24 (br d, J = 9.0 Hz, 2H), 6.95 (br d, J = 9.1 Hz, 2H), 3.61–3.51 (m, 2H), 3.30–3.21 (m, 4H), 3.16–3.07 (m, 4H), 2.87–2.76 (m, 2H), 2.46–2.36 (m, 1H), 1.86–1.74 (m, 2H), 1.56–1.42 (m, 2H). HPLC purity 99.1%.

7.3.48. 1-(4-(4-(4-Chlorophenyl)piperazine-1-carbonyl)piperazin-1-yl)-2-hydroxyethanone (56)

A solution of crude **71** (295 mg, 1.14 mmol) was cooled to 0 °C and added dropwise to a solution of *tert*-butyl piperazine-1-carboxylate (**62**) (335 mg, 1.80 mmol) and DIPEA (0.52 mL, 2 equiv) in DCM (2 mL). The mixture was stirred at 20 °C for 2 h, then diluted with DCM, washed with aqueous Na_2CO_3, dried and evaporated to give *tert*-butyl 4-(4-(4-chlorophenyl)piperazine-1-carbonyl)piperazine-1-carboxylate (**73**) (292 mg, 48%), which was used directly. ^{1}H NMR (DMSO) δ 7.24 (d, J = 9.0 Hz, 2H), 6.97 (d, J = 9.0 Hz, 2H), 3.37–2.26 (m, 8H), 3.18–3.10 (m, 8H), 1.41, s, 9H).

A solution of **73** (225 mg, 0.55 mmol) in DCM (10 mL) and TFA (10 mL) was stirred at 20 °C for 3 h, then evaporated. The residue was diluted with DCM, washed with 4 N NH_4OH and evaporated, and the residue from this was extracted with boiling hexane, which cooling gave (4-(4-chlorophenyl)piperazin-1-yl)(piperazin-1-yl)methanone (**74**) (88%), which was used directly. ^{1}H NMR (DMSO) δ 7.24 (d, J = 9.0 Hz, 2H), 6.95 (s, J = 9.0 Hz, 2H), 3,21 (m, 4H), 3.15–3.06 (m, 8H), 2.69–2.62 (m, 4H).

A solution of **74** (85 mg. 0.28 mmol) and DIPEA (72 µL, 1.5 equiv) in DCM (2 mL) was treated at 0 °C with a solution of 3-chloro-3-oxopropyl acetate (45 mg, 1.2 equiv) in DCM (1 mL). The mixture was stirred at 20 °C for 10 min, then diluted with DCM, washed with aqueous $NaHCO_3$ and water, dried and evaporated. The residue was triturated with iPr_2O to give crude 3-(4-((4-(4-chlorophenyl)piperazin-1-yl)methyl)piperazin-1-yl)-3-oxopropyl acetate (**75**) (82 mg, 73%), which was used directly. ^{1}H NMR (DMSO) δ 7.23 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 3.41 (br s, 4H), 3.36 (br s, 4H), 3.30–3.24 (m, 4H), 3.21–3.11 (m, 4H), 3.03–3.09 (m, 4H), 2.06 (s, 3H).

A solution of crude **75** (40 mg, 0.90 mmol) in MeOH (6 mL) was treated with a solution of Cs_2CO_3 (50 mg, 1.5 equiv) in water (1 mL). The mixture was stirred at 20 °C for 15 min, then diluted with water, concentrated to remove the MeOH and extracted with EtOAc. Evaporation of the organic layer gave **56** (92%), mp (EtOAc/pet ether) 150–152 °C. ^{1}H NMR ($CDCl_3$) δ 7.24 (br d, J = 9.0 Hz, 2H), 6.96 (br d, J = 9.1 Hz, 2H), 4.60 (t, J = 5.5 Hz, 1H), 4.09 (d, J = 5.5 Hz, 2H), 3.53–3.43 (m, 2H), 3.39–3.23 (m, 6H) after D_2O. HPLC purity 98.1%.

7.3.49. (4-(4-Chlorophenyl)piperazin-1-yl)(3,4-dihydroisoquinolin-2(1*H*)-yl)methanone (57)

Solutions of **70** (75 mg, 0.38 mmol) and DIPEA (0.13 mL) in DCM (3 mL) and 3,4-dihydroisoquinoline-2(1*H*)-carbonyl chloride (**76**) (82 mg, 1.1 equiv) in DCM (1 mL) were mixed and stirred at 20 °C for 30 min, then diluted with DCM and water and the organic layer was evaporated to give **57** (84%), mp (EtOAc/pet ether) 153–154 °C. ^{1}H NMR ($CDCl_3$) δ 7.24 (br d, J = 9.0 Hz, 2H), 7.18–7.12 (m, 4H), 6.97 (br d, J = 9.1 Hz, 2H), 4.41 (s, 2H), 3.46 (t, J = 5.9 Hz, 2H), 3.36–3.29 m (m, 4H), 3.20–3.13 (m, 4H), 2.84 (t, J = 5.8 Hz, 2H). HPLC purity 98.8%.

7.3.50. Compounds of Table 3 (Scheme 3). 4-((4-(4-Chlorophenyl)piperazin-1-yl)sulfonyl)morpholine (58)

A solution of morpholine hydrochloride (6.0 g, 49 mmol) in $CHCl_3$ (20 mL) and SO_2Cl_2 (80 mL) was heated under reflux for 6 h, then evaporated under reduced pressure. The residue was dissolved in DCM, diluted with pet ether, filtered through Celite and evaporated to give crude morpholine-4-sulfonyl chloride[23] (**77**). A solution of 1-(4-chlorophenyl)piperazine (**70**) (48 mg, 0.24 mmol) in DCM (1 mL) was treated with crude **77** (50 mg, 0.27 mmol) in DCM 3 mL) and DIPEA (0.l mL). The mixture was stirred for 2 h and diluted with DCM, washed with dilute aqueous Na_2CO_3 and evaporated. The residue was dissolved in EtOAc, filtered through Celite, evaporated and triturated with iPr_2O to give **58** (77%), mp (EtOAc/pet ether) 152–153 °C. ^{1}H NMR ($CDCl_3$) δ 7.26 (br d, J = 9.0 Hz, 2H), 6.97 (br d, J = 9.1 Hz, 2H), 3.66–3.58 (m, 4H), 3.33–3.22 (m, 4H) after D_2O, 3.20–3.11 (m, 8H). HPLC purity 98.2%.

7.3.51. (4-(4-Chlorophenyl)piperazin-1-yl)(morpholino)methanethione (59)

A solution of **70** (90 mg, 0.46 mmol) and DIPEA (0.16 mL, 0.92 mmol) in DCM (3 mL) was cooled to 0 °C and treated with morpholine-4-carbothioyl chloride[24] (**78**) (83 mg, 0.5 mmol). The mixture was stirred at 20 °C for 8 h, then diluted with DCM and water and the organic layer was evaporated. The residue was dissolved in EtOAc and filtered through silica gel to give **59** (76%), mp (EtOAc/pet ether) 157–160 °C. ^{1}H NMR ($CDCl_3$) δ 7.25 (br d, J = 9.0 Hz, 2H), 6.96 (br d, J = 9.1 Hz, 2H), 3.67–3.60 (m, 8H), 3.57–3.51 (m, 4H), 3.24–3.18 (m, 4H). HPLC purity 99.8%.

7.3.52. (4-(4-Chlorophenyl)-1,4-diazepan-1-yl)(morpholino)methanone (60)

A solution of 1-(4-chlorophenyl)-1,4-diazepane[10] (**79**) (80 mg, 0.38 mmol) and DIPEA (0.13 mL, 2 equiv) in DCM (3 mL) was cooled to 0 °C and treated with morpholine-4-carbonyl chloride (**63**) (68 mg, 0.45 mmol) at 20 °C for 1 h. The mixture was washed with aqueous Na_2CO_3 and the organic layer was evaporated. The residue was dissolved in EtOAc and filtered through a column of silica gel to give **60** (73%), mp (hexane) 70–73 °C. ^{1}H NMR ($CDCl_3$) δ 7.13 (br d, J = 9.1 Hz, 2H), 6.72 (br d, J = 9.1 Hz, 2H), 3.61 (t, J = 5.5 Hz, 2H), 3.55–3.42 (m, 8H), 3.21 (t, J = 5.7 Hz, 2 H), 2.91 (t, J = 4.7 Hz, 2H), 1.93–1.83 (m, 2H). HPLC purity 99.8%.

7.3.53. 5-(4-Chlorophenyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)(morpholino)methanone (61)

Similar reaction of 2-(4-chlorophenyl)- 2,5-diazabicyclo[2.2.1]heptane[11] (**80**) (50 mg, 0.24 mmol) with morpholine-4-carbonyl chloride (**63**) gave **61** (77%), mp (EtOAc/heptanes) 181–182 °C. ^{1}H NMR ($CDCl_3$) δ 7.16 (br d, J = 9.0 Hz, 2H), 6.59 (br d, J = 9.0 Hz, 2H), 4.49 (s, 1H), 4.34 (s, 1H), 3.57–3.44 (m, 6H), 3.23–3.03 (m, 6H), 1.86 (dd, J = 26.4, 9.5 Hz, 2H). HPLC purity 99.9%.

7.4. Molecular modelling

Protein structures were prepared for docking by the addition of hydrogens using SYBYL-X2.1 (Tripos, St Louis), and the side chains of His117, His194 and His314 were adjusted to improve hydrogen bonding. All crystallographic waters were stripped prior to docking. Ligand preparation involved the generation of a single low energy conformation using the SYBYL protoplex and stereoplex modules followed by energy minimisation using Gastieger Marsili charge set with the Tripos forcefield for 1000 steps of Steep Descents followed by Conjugate gradients with the gradient convergence set at 0.05) using a dielectric constant of 1 and the distance dependent dielectric function. Docking was performed using GOLDv5.2 with the ChemPLP scoring function and a 10 Å cavity centred on **24** crystallised in the AKR1C3 active site. The grid spacing for cavity detection and flood-fill steps was set at 0.25, and ligands were constrained to the position of **24** using the heavy atoms of the scaffold represented by **9** and a constraint weight set at 20. All ligand flexibility terms were used along with the flip option where available. Diverse solutions was turned on and used at default values and each ligand was subjected to 20 genetic algorithm runs, and 20 predicted poses per compound were kept. The top ranked poses were then used. Atom types were automatically assigned.

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