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ARTICLE TYPE

Discovery of a series of 2-(pyridinyl)pyrimidines as potent antagonists of GPR40

Michael J. Waring,*[a] David J. Baker, Stuart N. L. Bennett, Alexander G. Dossetter,[b] Mark Fenwick, Rob Garcia, Jennie Georgsson, Sam D. Groombridge, Susan Loxham, Philip A. MacFaul, Katie G. Maskill, David Morgan, Jenny Morrell, Helen Pointon, Graeme R. Robb, David M. Smith, Stephen Stokes and Gary Wilkinson

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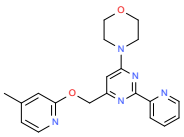
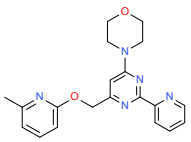
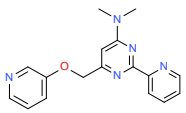
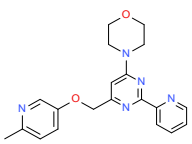
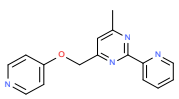
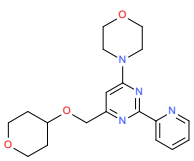
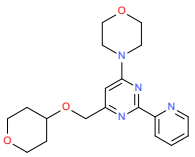
Additional SAR for phenyl ring replacements

	pIC_{50}	$logD_{7.4}$	pIC_{50}- $logD_{7.4}$
	5.5	2.8	2.7
	5.4	3.0	2.4
	<4.4	1.6	<2.8
	<4.4	1.7	<2.7
	<4.4	-0.2	<4.6
	<4.4	1.3	<2.8
	5.2	2.7	2.6

GPR40 assay

HEK293s FLPIN h-GPR40 Clone 2C8 cells were grown in DMEM Glutamax-I medium with 10% FBS and hygromycin (200 µg/mL) in 5% CO_2 and 95% humidity at 37 °C. Cryo-preserved cells were used for screening. The cells were thawed at 37 °C and resuspended in DMEM with 30% FCS (1 mL cells / 11.5 mL medium) and centrifuged at 1000 rpm for 5 mins. The cell pellet was resuspended in assay buffer to a concentration of 2.5×10^6 cells/mL. The cell suspension (8 µL / well) was dispensed in 384-well plates, giving 10,000 cells / well.

To reduce edge effects, assay plates were pre-warmed in the incubator at 37 °C for 2 hours before start of assay. The cell suspension and stimulation buffer were kept at 37 °C.

100 nL / well of compound solution was dispensed into low-volume 384-well plates with 16 compounds / plate at 10 separate concentrations / compound (final concentrations 37.5, 9.375, 2.34, 0.586, 0.147, 0.0366, 0.00915, 0.00289, 0.000572, 0.000143 µM) leaving columns 21 to 24 blank. 4 µl of the cell suspension (10,000 cells / well) was added to the plate and incubated for 15 min at 37 °C.

Elaidic acid buffer {4 µL, made up of elaidic acid solution (17.5 µL, 20mM) in DMSO (82.5 µL), added to stimulation buffer [4.9 mL, in turn made up of $CaCl_2$ (1M, 1 mL), $MgCl_2$ (1M, 0.5 mL), NaCl (2M, 73 mL), KCl (1M, 4.2 mL) LiCl (1M, 10 mL), Hepes (1M, 10 mL), glucose (0.99 g) diluted to 1 L by addition of distilled water and pH adjusted to 7.4 by addition of NaOH solution (5M)]} was added to columns 1-22 and 2% DMSO control buffer [4 µL, made up of DMSO (100 µL) addded to stimulation buffer (4.9 mL)] was added to columns 23 and 24. The resulting plates were incubated for 2 hrs at 37 °C. 4 µL lysis buffer with IPone d2 (CisBio) was added to each well, followed

by 4 µl lysis buffer with anti-IPone Tb cryptate (CisBio) and the plates incubated for a further 1 hour at room temperature. Fluorescence at 615 and 665 nm was then determined. The ratio of these emissions was used to calculate the magnitude of the FRET signal and the corresponding % inhibition determined by comparison with a calibration curve. Concentration response data were then used to determine IC_{50} values.

$LogD_{7.4}$ measurements

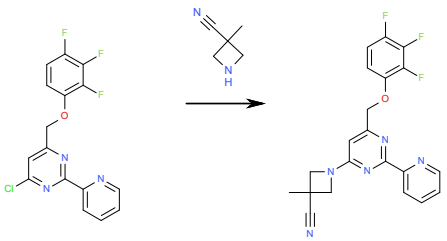
All quoted logD7.4 values refer to measured data. The protocols for the experimental method, along with those for other quoted ADMET data are described in:

D. Buttar, N. Colclough, S. Gerhardt *et al*. *Bioorg. Med. Chem.* 2010, **18**, 7486.

M. H. Bridgland-Taylor, A. C. Hargreaves, A. Easter *et al*. *J. Pharmacological and Toxicological Methods*, 2006, **54**, 189.

Synthesis of compound 39

3-methyl-1-[2-(2-pyridyl)-6-[(2,3,4-trifluorophenoxy)methyl]pyrimidin-4-yl]azetidine-3-carbonitrile



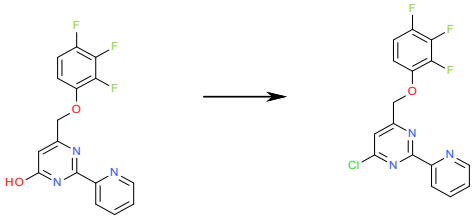
3-Methylazetidine-3-carbonitrile 2,2,2-trifluoroacetate (2.1 g, 10 mmol) was added in one portion to 4-chloro-2-(pyridin-2-yl)-6-((2,3,4-trifluorophenoxy)methyl)pyrimidine (3.2 g, 9.1 mmol) and potassium carbonate (3.8 g, 27 mmol) in dioxane (40 mL) at 20 °C under nitrogen. The resulting suspension was heated to 65 °C and stirred for 24 hours. The reaction mixture was filtered, the solids washed with dioxane and the resulting solution was evaporated and redissolved in dichloromethane (50 mL). The resulting solution was washed with water (2 × 20 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and evaporated. The resulting oil was triturated with diethyl ether to afford the crude solid product. This was recrystallised from absolute ethanol (40 mL) with crystallisation at 40 °C for 16 hours before cooling to 20 °C. The crystalline product was collected by filtration and washed with cold ethanol and ether. The resulting solid was dissolved in dichloromethane (20 mL) and treated with decolourising charcoal (0.3 g) for 30 minutes before filtering and evaporating. The resulting residue was recrystallised from ethanol (27 mL) with crystallisation at 55 °C for 1 hour before cooling to 20 °C for 2 hours. The crystalline product was collected by filtration and washed with cold ethanol and ether to afford the product as a colourless crystalline solid (2.2 g, 52%).

Mpt 173 °C

δ^H (400 MHz): 1.70 (3H, s), 4.13 (2H, d), 4.48 (2H, d) 5.22 (2H, s), 6.56 (1H, s), 7.11-7.19 (1H, m), 7.24-7.33 (1H, m), 7.48-7.52(1H, m), 7.90-7.95 (1H, m), 8.25-8.29 (1H, m), 8.69-8.73 (1H, m)

m/z (ESI^+) 412 ($M+H^+$)

4-chloro-2-(pyridin-2-yl)-6-((2,3,4-trifluorophenoxy)methyl)pyrimidine

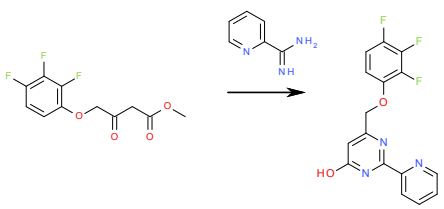


2-(pyridin-2-yl)-6-((2,3,4-trifluorophenoxy)methyl)pyrimidin-4-ol (3.4 g, 10 mmol) was added portionwise to phosphorous oxychloride (9.5 mL, 10 mmol) over 5 minutes under nitrogen. The resulting mixture was heated to 100 °C and stirred for 1 hour. The resulting solution was then cooled to ambient temperature and evaporated. The resulting oil was azetroped with toluene (anhydrous 2 × 3 mL) and triturated with diethyl ether. The resulting solid was collected by filtration, washed with ether and redissolved in dichloromethane (75 mL) and washed with saturated sodium hydrogen carbonate solution (25 mL). The organic phase was separated, washed with water (25 mL) and brine (25 mL), dried ($MgSO_4$) and evaporated to afford the crude product, which was used without further purification (3.3 g, 91%).

δ^H (400 MHz): 5.45 (2H, s), 7.21-7.29 (1H, m), 7.30-7.40 (1H, m), 7.64-7.70 (1H, m), 7.82 (1H, s), 8.05-8.11 (1H, m), 8.40-8.44 (1H, m), 8.83-8.88 (1H, m)

m/z (ESI^+) 352 ($M+H^+$)

2-(pyridin-2-yl)-6-((2,3,4-trifluorophenoxy)methyl)pyrimidin-4-ol



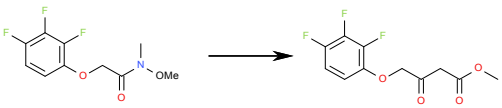
Picolinimidamide hydrochloride (4.7 g, 30 mmol) was added to methyl 3-oxo-4-(2,3,4-trifluorophenoxy)butanoate (7.8 g, 30 mmol)and sodium carbonate (9.5 g, 89 mmol) in acetonitrile (100 mL) at 25 °C. The resulting mixture was stirred at 75 °C for 1 hour. The solution was evaporated, diluted with water (75 mL) and stirred for 30 minutes. The resulting precipitate was collected by filtration. The filtrate was suspended in citric acid solution (1 M, 20 mL) and stirred for 30 minutes. The solid was collected by filtration, washed with water (3 × 20 mL) and dried

under vacuum at 55 °C to afford the product, which was used without further purification (4.5 g, 45%).

δ^H (400 MHz): 5.18 (2H, s), 6.43 (1H, s), 7.11-7.20 (1H, m), 7.22-7.31 (1H, m), 7.63-7.68 (1H, m), 8.03-8.09 (1H, m), 8.25-8.30 (1H, m), 8.73-8.79 (1H, m), 12.18 (1H, s)

m/z (ESI^+) 334 ($M+H^+$)

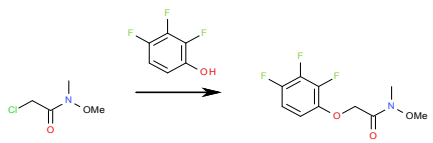
Methyl 3-oxo-4-(2,3,4-trifluorophenoxy)butanoate



Lithium hexamethyldisilylamide (1M in tetrahydrofuran, 88 mL, 88 mmol) was added dropwise to methyl acetate (7.0 mL, 88 mmol) in tetrahydrofuran (300 mL) at -78 °C under nitrogen and stirred for 15 minutes. N-methoxy-N-methyl-2-(2,3,4-trifluorophenoxy)acetamide (11 g, 44 mmol) in tetrahydrofuran (100 mL) was added dropwise over 15 minutes, maintaining the internal temperature below -65 °C. The mixture was stirred at -78 °C for 30 minutes and then neutralised by the addition of glacial acetic acid (12 mL) and then saturated ammonium chloride solution (75 mL). Ethyl acetate (250 mL) and water (100 mL) were added and the organic phase was separated, washed with water (50 mL) and brine (200 mL), dried (MgSO4) and evaporated to afford the product, which was used without further purification (9.0 g, 78%).

Complex NMR spectrum resulting form a mixture of keto and enol forms.

N-methoxy-N-methyl-2-(2,3,4-trifluorophenoxy)acetamide

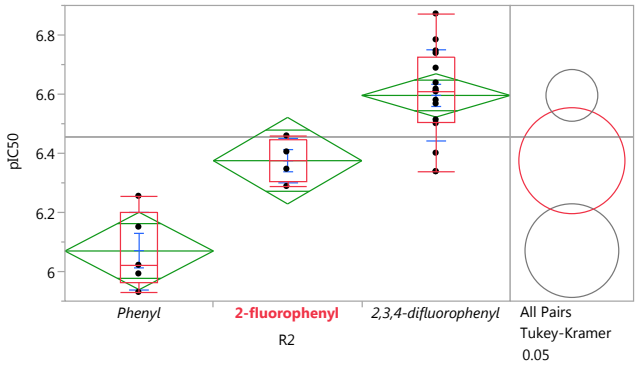


Potassium carbonate (8.7 g, 63 mmol) was added in one portion to 2-chloro-N-methoxymethylacetamide (4.3 g, 31 mmol) and 2,3,4-trifluorophenol (3.3 g 22 mmol) in acetone (50 mL) under nitrogen. The resulting mixture was stirred at reflux for 48 hours. The mixture was filtered through Celite® and evaporated. The resulting oil was dissolved in ethyl acetate (100 mL) and treated with charcoal (2.5 g) for 30 minutes. The mixture was filtered and evaporated. The resulting solid was purified by flash column chromatography, eluting with 50 to 100% ethyl acetate in heptane, to afford the product as a colourless solid (4.8 g, 62%).

δ^H (400 MHz): 3.11 (3H, s), 3.72 (3H, s), 5.05, 2H, s), 6.92-7.01 (1H, m) 7.16-7.26 (1H, m)

Experimental details of other compounds described are available on request.

Statistical comparison of pIC50 values for phenyl (2), 2-fluorophenyl (18) and 2,3,4-trifluorophenyl (24) derivatives



	n	Mean	Std dev	Std err
Phenyl (**2**)	5	6.1	0.13	0.058
2-fluorophenyl (**18**)	4	6.4	0.074	0.037
2,3,4-difluorophenyl (**24**)	16	6.6	0.15	0.038

Molecular Modelling

The GPR40 crystal structure (PDB=4PHU) was prepared using the LigX protocol in the MOE software package using the Amber10:EHT forcefield. All heavy atoms were tethered (force constant = 1000), with buffers set to zero. Atoms further than 8Å from the ligand were fixed.

3D-RISM analysis was performed using mostly default parameters for binding, but including hydrophobe in the calculation at 50 mM. Analysis was performed with the aid of the optional Solvent Analysis – Site panel.

Docking was performed using the default Induced Fit protocol with initial pose selection guided by the pharmacophore described in the main text. The induced fit allows protein side-chains within 6A of the ligand to move during refinement of the complex. Ligands were prepared by hand with charges being assigned by the forcefield. Post-docking minimisation was used to further optimise the ligand and its interactions with the surrounding protein.

Secondary pharmacology profile of 39

39 was screened against 144 protein targets in the Cerep panel (www.**cerep**.fr). Only 7 targets showed measurable activity and only pig Na+/K+ ATPase had activity sub 1 µM. Full details are listed below.

Protein target	pIC_{50} / pEC_{50}
Na+/K+ ATPase pig inhibition	6.1
TSPO human binding	5.3
GABA rat binding	5.2
AT Gpig binding	5.2
OPRk1 rat binding	5.0

NK1 human binding	4.8
CaV-L rat extract binding	4.3
D2 human binding	<4.0
a1B human binding	<4.0
a2C human binding	<4.0
Ang2 AT1 human binding	<4.0
EtA human binding	<4.0
GABAa1b2g2 human binding	<4.0
H1 human binding	<4.0
H2 human binding	<4.0
GR human Cyto binding	<4.0
DOP2 human binding	<4.0
b1 human antagonism	<4.0
D1 human antagonism	<4.0
Ang2 AT1 human agonism	<4.0
Ang2 AT1 human antagonism	<4.0
DAT human binding	<4.0
D3 human binding	<4.0
a1A human agonism	<4.0
a1A human antagonism	<4.0
b1 human agonism	<4.0
5HT2B human binding	<4.0
A1 human binding	<4.0
a1A human binding	<4.0
CB1 human binding	<4.0
ACHE human inhibition	<4.0
A2A human binding	<4.0
PDE3 human inhibition	<4.0
PDE4D human inhibition	<4.0
5-HT1A human binding	<4.0
5-HT1D rat binding	<4.0
5-HT2C human binding	<4.0
5HT3 human binding	<4.0
5HT1B rat binding	<4.0
5HT4 human binding	<4.0
D3 human agonism	<4.0
D3 human antagonism	<4.0
MR2 human antagonism	<4.0
SST4 human agonism	<4.0
SST4 human antagonism	<4.0
SST4 human binding	<4.0
H2 human antagonism	<4.0
OPRk1 rat agonism	<4.0
OPRk1 rat antagonism	<4.0
M1 human agonism	<4.0
M1 human antagonism	<4.0
H1 human agonism	<4.0
H1 human antagonism	<4.0
H2 human agonism	<4.0
CB1 human agonism	<4.0
a2A human binding	<4.0
CB1 human antagonism	<4.0
b1 human binding	<4.0
D1 human binding	<4.0
5HT2B human antagonism	<4.0
D1 human agonism	<4.0
M2 human agonism	<4.0
M2 human binding	<4.0
OPRm1 human binding	<4.0
NAchA1 human binding	<4.0
NAchA4 human binding	<4.0
NMDA rat binding	<4.0
NET human binding	<4.0
a1B human agonism	<4.0
M1 human binding	<4.0
M5 human binding	<4.0
MR2 human binding	<4.0
a1B human antagonism	<4.0
M2 human antagonism	<4.0
OPRm1 human agonism	<4.0
OPRm1 human antagonism	<4.0
5HT2B human agonism	<4.0
5HT4 human agonism	<4.0
5HT4 human antagonism	<4.0
5-HT7 human antagonism	<4.0
BK2 human agonism	<4.0
BK2 human antagonism	<4.0
b2 human agonism	<4.0
b2 human antagonism	<4.0
NAchA7 human binding	<4.0
NMDA rat binding	<4.0
Sigma1 human binding	<4.0
CatS human inhibition	<4.0
PPARgamma human binding	<4.0
5-HT2C human agonism	<4.0
ROCK2 human inhibition	<4.0

5HT7 human binding	<4.0
GABAB human binding	<4.0
Ghre human bindinging	<4.0
COX1 human inhibition	<4.0
cKIT human inhibition	<4.0
GSK3b human inhibition	<4.0
PDE6 bovine inhibition	<4.0
PDK1 human inhibition	<4.0
ROCK1 human inhibition	<4.0
MAO-B human inhibition	<4.0
MMP2 human inhibition	<4.0
PDE10A1 human inhibition	<4.0
ALK4 human inhibition	<4.0
FGFR1 human inhibition	<4.0
A2A rat agonism	<4.0
A2A rat antagonism	<4.0
MR2 human agonism	<4.0
EGFR kinase human inhibition	<4.0
MAP3K7 human inhibition	<4.0
TRKA human inhibition	<4.0
eNOS human inhibition	<4.0
NK1 human agonism	<4.0
NK1 human antagonism	<4.0
TXA2 sy human inhibition	<4.0
5-HT2C human antagonism	<4.0
BK2 human binding	<4.0
ECE1 human inhibition	<4.0
b2 human binding	<4.0
KDR human inhibition	<4.0
SET human binding	<4.0
INSR human inhibition	<4.0
COX2 human inhibition	<4.0
RARa human binding SPA	<4.0
Ghre human agonism	<4.0
Ghre human antagonism	<4.0
AurKA human inhibition	<4.0
CaV-L rat extract binding	<4.0
PI3Ka human inhibition	<4.0
a2A human agonism	<4.5
D2 human agonism	<4.5
D2 human antagonism	<4.5
5HT1B hamster agonism	<4.5
5-HT1D rat antagonism	<4.5
5-HT1A human agonism	<4.5
5-HT1D rat agonism	<4.5
a2A human antagonism	<4.5
DOP2 rat agonism	<4.5
DOP2 rat antagonism	<4.5
src human inhibition	<4.5
GABAB human agonism	<4.5
GABAB human antagonism	<4.5
5HT1B Ham antagonism	<5.0
5-HT1A human antagonism	<5.0