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Structure–activity relationships of 1-alkyl-5-(3,4-dichlorophenyl)-5-{2-[3-(substituted)-1-azetidinyl]-ethyl}-2-piperidones. Part 2: Improving oral absorption

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Abstract—A series of piperidone analogues of **1b**–**q**, seeking replacements for the polar sulfamide moiety in clinical candidate UK-224,671 **1a**, possessing reduced H-bonding potential as a strategy to improve oral absorption, were prepared. These studies led to the successful identification of **1n**, which demonstrated equivalent pharmacology and metabolic stability to **1a**, and greatly improved oral absorption as assessed in rat PK studies.

1. Introduction

Tachykinins and their receptors have been postulated to play a significant role in the pathophysiology of a wide variety of diseases including gastrointestinal disorders,[1] emesis,[2] chronic pain,[3] depression[4] and asthma.[5] Our own long-standing efforts in this field have focused on investigating the role of neurokinin-2 (NK_2) antagonists in urological disorders.[6] In an earlier communication from these laboratories,[6c] we reported the discovery of UK-224,671 **1a**, (Fig. 1), as a potent selective antagonist of the NK_2 receptor. Progression of this compound into human clinical trials, however, revealed the compound to possess poor oral bioavailability,[7] as a consequence of poor oral absorption.[8]

In this communication, we report a successful strategy to improve the oral absorption of this piperidone series while retaining high functional potency against the human NK_2 receptor.

Our strategy (Fig. 1) focused on replacement of the polar (high H-bond count), primary sulfamide. In tandem, we sought to identify alternative, metabolically stable replacements for the *N*-cyclopropylmethyl piperidone substituent, which could confer greater potency. These studies led to the successful identification of **1n**, which possessed both equivalent pharmacology and metabolic stability to **1a** and greatly improved oral absorption as assessed in rat PK studies.

2. Chemistry

Test compounds **1a**–**f**, **h**–**n**, and **p**–**q** were prepared from homochiral (*S*)-piperidone (**2**)[9] by the general method in Scheme 1, as described previously.[9,10] Alkylation of piperidone (**2**) with the appropriate alkyl halide or tosylate as leaving group, followed by acid-mediated deprotection of the dioxolane group, yielded the aldehyde. Reductive amination using $NaBH(OAc)_3$ and the appropriate 3-substituted azetidine gave targets **1a**–**f**, **h**–**n**, and **p**–**q**.

Test compounds **1g** and **o** were also prepared by the methods previously described[10] as shown in Scheme 2. Treatment of ester (**3**)[10] with amine (**4**)[11] at 100 °C gave the amide (**5**). Conversion of the primary alcohol to the mesylate and intramolecular displacement with the

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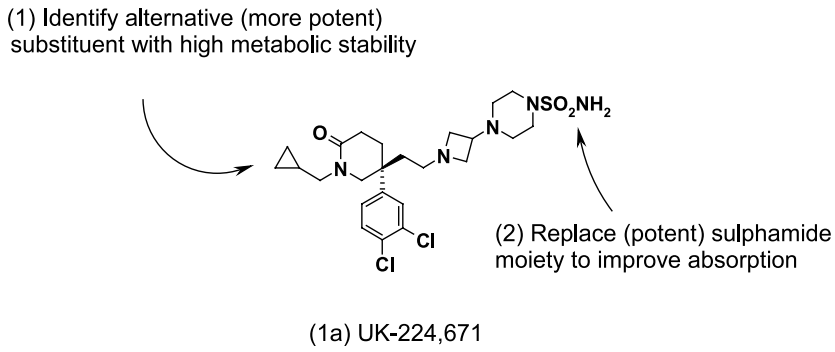
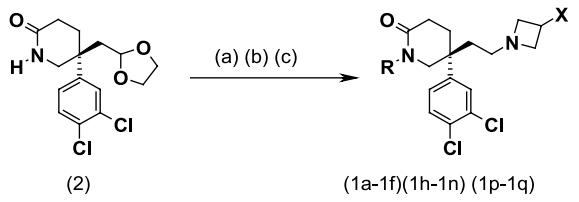
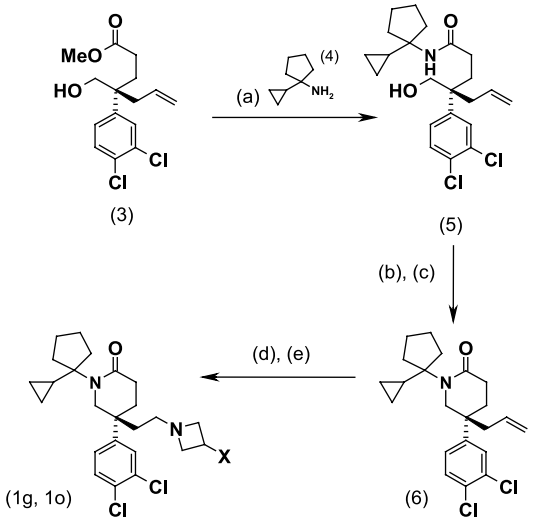


Figure 1. Revised medicinal chemistry strategy to improve oral absorption in piperidone series (**1**).



Scheme 1. Synthesis of 1-alkyl-5-(3,4-dichlorophenyl)-5-{2-[3-(substituted)-1-azetidinyl]-ethyl}-2-piperidones **1a**–**f**, **h**–**n**, **p**,**q**. Reagents and conditions: (a) R–X (X = Br or –OTs), KOH, DMSO, 16 h, rt; (b) 5 N HCl, THF, reflux, 4 h; (c) 3-(substituted)azetidine, $NaBH(OAc)_3$, AcOH, NEt_3, THF, rt.



Scheme 2. Synthesis of 1-alkyl-5-(3,4-dichlorophenyl)-5-{2-[3-(substituted)-1-azetidinyl]-ethyl}-2-piperidones **1g** and **o**. Reagents and conditions: (a) (**4**), 100 °C, 16 h; (b) MsCl, CH_2Cl_2, NEt_3, rt; (c) NaH, THF, reflux; (d) O_3, MeOH, Me_2S; (e) 3-(*N*-morpholino)azetidine (**1g**) or 3-(4-fluoropiperidinyl)azetidine (**1o**), $NaBH(OAc)_3$, AcOH, NEt_3, THF, rt.

amide anion, generated using sodium hydride, gave piperidone (**6**). Ozonolysis of the terminal olefin and reductive amination of the resulting aldehyde with appropriate 3-substituted azetidine in the presence of $NaBH(OAc)_3$ gave target compounds **1g** and **o**.

The synthesis of all 3-substituted azetidines has been reported previously.[9,10]

3. Results and discussion

All binding, functional and human liver microsomal stability screening were performed using the methods previously reported.[6c]

Previous communications from these laboratories described the discovery of potent NK_2 antagonist, UK-224,671.[6c] Progression of this compound into human clinical trials revealed the compound to possess long terminal half-life (28 h) and low clearance (2–3 ml/min/kg).[7] Oral bioavailability in human, however, was found to be very low (5–10%), indicative of very poor absorption, likely caused by a combination of intrinsically poor permeability and transporter-mediated (P–gp) efflux.[8]

Our medicinal chemistry objectives in seeking a clinical replacement for UK-224,671 were therefore to (1) retain the excellent potency and selectivity; (2) retain high metabolic stability, as assessed in human liver microsomes (HLM, $T_{1/2} > 120$ min), as a strategy to minimise first-pass in vivo metabolism; and (3) significantly improve intrinsic transcellular permeability, as assessed in a Caco-2[12] model for passive GI transit (target: Caco-2 >20%/h), to minimise any potential impact of P–gp affinity in this series.

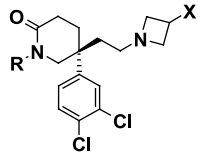
We made a decision to remain in the piperidone series from which UK-224,671 originated, as this template offered excellent potency, general selectivity and metabolic stability. Much work has been published on physicochemical descriptors for oral absorption[13] and preliminary assessment of this series (MW 500–550 kDa, relatively low H-bond donor (HBD) and acceptor count, moderate, positive, c log *P*) did not suggest that its physicochemistry should be incompatible with oral absorption. However, while we did not regard the HBD count in **1a** as excessively high, (HBD 2), we felt this, combined with the exposed nature of the polar primary sulfamide moiety, high topological polar surface area (TPSA) (98 $Å^2$), and relatively high MW (545), might explain the origin of the poor permeability observed in man.

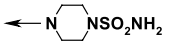
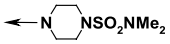
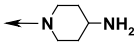
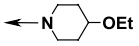
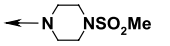
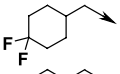
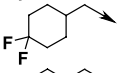
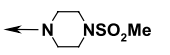
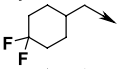
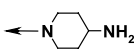
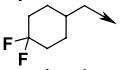
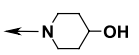
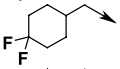
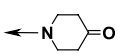
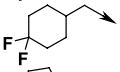
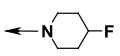
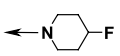
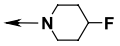
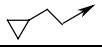
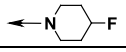
We, therefore, targeted modulation of TPSA and HBD count in **1a** to improve intrinsic permeability as the principal focus of our design programme, recognising that a

likely consequence of this strategy would also be to increase lipophilicity, again, consistent with our goal of improving permeability.

We were reassured that high permeability, coupled to good HLM stability, could be achieved in this series by morpholine analogue **1c** (Table 1) (HBD 0, MW 466 kDa, TPSA 36 Å^2), which showed greatly improved permeability in the Caco-2 assay [A–B, 12%/h], relative to **1a** (A–B 1%/h), while retaining good stability in the HLM assay ($T_{1/2}$ 120 min). Unfortunately, **1c**, demonstrated low potency in h-NK$_2$ binding studies (pIC$_{50}$

Table 1. Human, NK$_2$ binding, functional (RPA) rabbit pulmonary artery, human liver microsomal half-life and Caco-2 flux permeability data for 1-alkyl-5-(3,4-dichlorophenyl)-5-{2-[3-(substituted)-1-azetidinyl]-ethyl}-2-piperidones **1a**–**1q**



Compound[a]	R	X	NK$_2$ (pIC$_{50}$)[b]	RPA (pA_2)[b]	c log P (log D, pH 7.4)	HLM ($T_{1/2}$, min)	Molecular weight (kDa)	TPSA (Å^2)	HBD	Caco-2 (%/h) (A–B/B–A)
UK-224,671[c] **1a**			8.4	8.9	2.2 (1.7)	>120	545	98	2	1/18
1b			7.8	8.0	2.3	NT	573	76	0	NT
1c			7.1	8.7	2.5 (2.3)	120	466	36	0	12/18
1d			8.2	8.7	1.4	NT	480	47	2	2/3 [f]
1e			7.8	8.2	2.4	NT	509	36	0	34 ER = 1
1f			8.1	8.5	2.2	<10	544	73	0	10 ER < 2
1g			9.5[d]	9.6	4.1	< 1	521	36	0	NT
1h			9.1	9.0	4.2	14	509	36	0	12/16
1i			8.1	8.9	3.9	80	545	36	0	33/35
1j			8.9	9.0	3.3	NT	622	73	0	6/12
1k			9.0	9.7	2.5 (0.1)	NT	558	53	2	< 1
1l			8.9	10.1	2.4 (2.2)	NT	559	47	1	2/12
1m			8.5	9.7	3.8	NT	557	44	0	NT
1n[e]			9.4	9.2	4.1	>120	561	27	0	>35 ER = 1
1o			10.0[d]	NT	4.7	2	537	27	0	60/60
1p			8.0	8.6	3.1	85	482	27	0	60/60
1q			8.6	8.8	3.6	NT	497	27	0	NT

[a] All compounds are single (*S*)-enantiomers.
[b] All assay determination $\geqslant n = 2$ (each experiment performed in triplicate). NT = not tested; ER = efflux ratio (ratio of apical-basolateral (A–B):basolateral-apical (B–A)).
[c] pA_2 (human bladder) = 8.8.
[d] pK_i.
[e] pA_2 (human bladder) = 8.0. human NK$_1$ binding (IM9 cells) IC$_{50}$ > 10 μM.
[f] P_{app} value (10^6 cm/s). This value is indicative of very poor transcellular permeability.

7.1). Introduction of polar, 4-aminopiperidine functionality **1d** gave a >10-fold increase in binding potency (h-NK_2 pIC_{50} 8.2) relative to **1c** (h-NK_2 pIC_{50} 7.1); however, the compound was poorly fluxed in the Caco-2 assay. 4-Ethoxypiperidine analogue **1e**, which possessed no HBD functionality, showed excellent Caco-2 flux (A–B, 34%/h). Similarly, piperazine sulfonamide **1f**, which also possessed no HBD groups, displayed significantly higher permeability in the Caco-2 assay than **1d** (A–B, 10%/h) despite both higher TPSA (73 $Å^2$ vs 47 $Å^2$) and MW (544 kDa vs 480 kDa) than **1d**.

These data suggested that minimising HBD count, in particular, was key to achieving good permeability in this series. Building on these observations, simple dimethylation (**1b**) (Table 1) of the primary sulfamide in **1a** as a strategy to remove the two HBD groups was undertaken. Unfortunately, this resulted in an unacceptable drop in potency in both the human NK_2 binding assay (pIC_{50} 7.8) and the rabbit pulmonary artery (RPA) functional screen (pA_2 8.0).

Increasing the lipophilicity of the piperidone N-substituent in the 3-(*N*-morpholino)azetidine series as a strategy to increase potency,[6c] **1g** and **h**, did result in an increase in activity against the human NK_2 receptor (pK_i 9.5 and pIC_{50} 9.1, respectively). However, in both instances, these piperidone N-substitutions led to a reduction in metabolic stability, relative to the *N*-cyclopropylmethyl analogue **1c** (**1g**, HLM $T_{1/2} < 1$ min; **1h**, HLM, $T_{1/2}$ 14 min).

Metabolic route profiling in this series revealed that CYP3A4-mediated oxidation at the 4-position of the *N*-cyclohexyl ring in **1h** was a likely major metabolic pathway. Blocking this metabolically vulnerable site by difluorination (**1i**) resulted in a significant improvement in both metabolic stability relative to **1h** (**1h,** $T_{1/2}$ 14 min; **1i**, $T_{1/2}$ 80 min) and a further improvement in permeability in the Caco-2 assay (A–B, 33%/h). Unfortunately, this 4,4,-difluoro substitution resulted in a 10-fold reduction in potency in the h-NK_2 binding assay (**1i**, pIC_{50} 8.1; **1h**, pIC_{50} 9.1). As a strategy to further improve potency in this series, we then evaluated a range of alternative 3-azetidine substituents **1j–n**, all designed to possess lower HBD count and/or TPSA than **1a**. Piperazine sulfonamide (**1j**) was found to be potent in the NK_2 binding assay (pIC_{50} 8.9) and functionally in the RPA screen (pA_2 9.0). Encouragingly, **1j** like its *N*-cyclopropylmethyl analogue **1f** displayed encouraging Caco-2 permeability (A–B 6%/h), despite its relatively high MW (622 kDa) and TPSA (73 $Å^2$).

4-Amino piperidine (**1k**) and 4-hydroxypiperidine (**1l**) were potent in the RPA assay; however, both suffered from poor permeability in the Caco-2 assay (A–B, 1%/h and 2%/h, respectively), consistent with the emerging SAR that any HBD functionality in this region was generally not compatible with high permeability in the Caco-2 assay. Simple oxidation of the primary alcohol in **1l** to the ketone **1m** was also found to retain potency.

Excitingly, replacing the 4-hydroxy group in **1l** with a 4-fluoro substituent **1n** gave a compound with excellent potency (pIC_{50} 9.4, RPA pA_2 9.2, human bladder pA_2 8.0), coupled with high permeability in the Caco-2 assay (A–B, >35%/h). In addition, **1n** retained high metabolic stability in the HLM assay ($T_{1/2} > 120$ min), the high metabolic stability of **1n** being particularly noteworthy, given its significantly increased lipophilicity (c log *P* 4.1) relative to **1a** (c log *P*, 2.3). Similarly, very high permeability was observed with 4-fluoropiperidine analogues **1o** and **p** (A–B 60%/h), although with highly variable metabolic stabilities (HLM; **1o**, $T_{1/2}$ 2 min; **1p**, $T_{1/2}$ 85 min). Attempts to further improve the potency and stability profile through incorporating *N*-cyclopropyl ethyl substitution (**1q**) were also investigated; however, this compound did not possess our overall target profile.

Analogue **1n** was progressed into rat pharmacokinetic studies (Table 2) to assess whether the in vitro permeability improvement over **1a** seen in the Caco-2 assay translated into improved absorption in vivo. Excitingly, **1n** exhibited greatly improved oral absorption (>80%) relative to prototype clinical candidate **1a** (<20%), consistent with the observed excellent Caco-2 permeability. This, coupled to its high metabolic stability in HLM studies, equivalent to **1a** which showed low clearance in human, made **1n** highly attractive. Following broader profiling, **1n**, UK-290,795 was nominated for clinical development.

In summary, a detailed analysis of the origin of the poor human pharmacokinetics observed with UK-224,671 led to a revised medicinal chemistry design strategy, targeting compounds with reduced HBD potential and reduced TPSA in this high MW series, to improve transcellular permeability. Metabolic route profiling of potent, highly permeable, but metabolically vulnerable *N*-cyclohexylmethyl analogue (**1h**) led to the design of *N*-(4,4-difluorocyclohexyl)methyl substitution, to block CYP3A4-mediated oxidative metabolism, leading to potent compounds with significantly enhanced metabolic stability.

Exploration of the 3-azetidine substituent SAR led to the discovery that while HBD groups in this region conferred potency, the presence of even a single HBD generally compromised permeability in this series.

Table 2. Comparative Caco-2 permeability, HLM stability and oral rat PK data for UK-224,671 **1a** and UK-290,795 **1n**

Compound	Caco-2 (%/h) (A–B/B–A)	Rat PK (oral absorption) (%)[a]	HLM ($T_{1/2}$, min)	Human in vivo clearance (ml/min/kg)
UK-224,671 (**1a**)	1/18	<20[b]	>120	<3[b]
UK-290,795 (**1n**)	>35 (ER = 1)	>80	>120	—

[a] UK-224,671 (10 mg/kg), UK-290,795 (20 mg/kg).
[b] See Ref. 7.

HBD score generally proved a better predictor of permeability than TPSA values in this series. Poor permeability was successfully overcome by incorporation of a 4-fluoro-substituent (**1n**) in place of 4-amino- or 4-hydroxy-functionality on the piperidine ring. Analogue **1n** displayed excellent potency and metabolic stability. Evaluation of **1n** in rat PK showed this compound to possess greatly enhanced oral absorption relative to UK-224,671.

Our continuing studies in this area will be the subject of future communications from these laboratories.

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