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4,4-Disubstituted Cyclohexylamine NK_1 Receptor Antagonists II

Laura C. Cooper,[a,*] Emma J. Carlson,[b] Jose L. Castro,[a] Gary G. Chicchi,[c] Kevin Dinnell,[a] Jerry Di Salvo,[c] Jason M. Elliott,[a] Gregory J. Hollingworth,[a] Marc M. Kurtz,[c] Mark P. Ridgill,[a] Wayne Rycroft,[b] Kwei-Lan Tsao[c] and Christopher J. Swain[a]

[a]*Department of Medicinal Chemistry, Merck Sharp & Dohme Research Laboratories, The Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK*

[b]*Department of Pharmacology, Merck Sharp & Dohme Research Laboratories, The Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK*

[c]*Department of Biochemistry, Merck Research Laboratories, Rahway, NJ 07065, USA*

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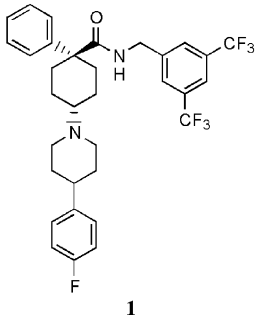
Abstract—A series of novel 4,4-disubstituted cyclohexylamines as NK_1 receptor antagonists is described: modifications to the amine moiety retain NK_1 receptor binding affinity whilst disrupting I_{Kr} affinity.

The mammalian tachykinin Substance P (SP) has been implicated in numerous conditions[1–3] including cystitis, depression and cytotoxin-induced emesis. This wide range of therapeutic indications has led to the development of various potent antagonists[3–5] of the receptor to which SP preferentially binds, neurokinin-1 (NK_1).



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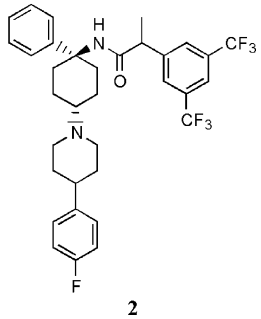

2

The lead compound **1** for the cyclohexyl series of NK_1 receptor antagonists was identified during targeted screening. In its favour were high affinity in the in vitro NK_1 receptor binding assay (hNK_1 IC_{50} 0.34 nM) and high oral bioavailability (70% in rat). However, it was poorly brain penetrant in vivo: a convenient in vivo assay for CNS penetration is inhibition of the foot-tapping exhibited by gerbils after central infusion of an NK_1 receptor-selective agonist (GR 73632).[6] The inhibition of foot-tapping after systemic administration of the test compounds provides an indication of brain penetration and central duration of action. Compound **1** was moderately active in this assay when administered immediately following pre-treatment (ID_{50} 1.9 mg/kg iv) but had short duration of action, giving only 15% inhibition of foot-tapping at 3 mg/kg iv after a 2 h pre-treatment time. Another major issue was that it showed very high affinity for the I_{Kr} channel (29 nM). Initial work concentrated on optimizing the amide 'linker' to provide improved central activity and duration of action.[7] The best combination of NK_1 receptor binding affinity (IC_{50} 0.63 nM) and in vivo properties (ID_{50} 1.8 mg/kg iv after a 24 h pre-treatment) was obtained by reversing the amide and introducing an α-methyl substituent to give compound **2**. However, the I_{Kr} affinity remained unacceptably high at 43 nM. This paper describes how compound **2** was modified in order to reduce binding to the I_{Kr} channel whilst retaining good NK_1 receptor binding affinity, brain penetration and duration of action.

Modelling studies on compound **1** and the high affinity I_{Kr} blocker dofetilide **3**[8] (Fig. 1) provided the working hypothesis that it is the amine portion of **1** which plays a key role in binding to the I_{Kr} channel. It was hoped that judicious modification of this moiety would reduce

*Corresponding author. Tel.: +44-1279-440433; fax: +44-1279-440390; e-mail: laura_cooper@merck.com

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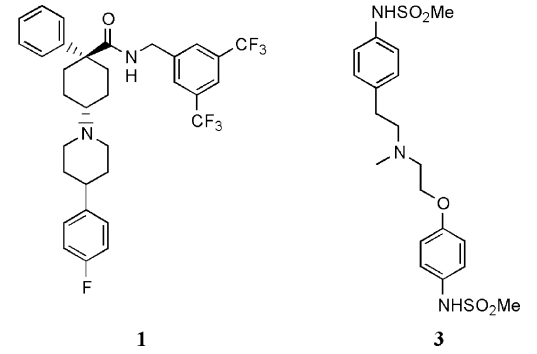
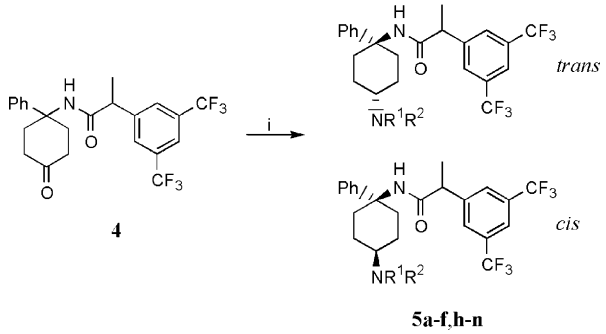
I_{Kr} affinity whilst retaining NK_1 receptor binding affinity. To this end, many analogues of **2** were made.

The standard procedure for preparation of analogues of compound **2** was reductive amination of the cyclohexanone **4**[7] with a range of secondary amines, giving mixtures of *cis*- and *trans*- isomers **5** (Scheme 1). In general, the *trans*-isomers (isolated by chromatography if possible) showed higher affinity.[7] Compound **5g** was made according to Scheme 2: reductive amination of the ketoacid **6** with 1,4-dioxa-8-azaspiro[4.5]decane gave an inseparable mixture of *cis*- and *trans*-isomers. The *trans*-isomer could be selectively esterified with methanol, allowing the insoluble *cis*-acid to be removed by filtration. Hydrolysis gave pure *trans*-acid **7** which was subjected to a Curtius rearrangement to give the *trans*-amine **8**. This was then coupled with the acid chloride derived from α-methyl-3,5-bis(trifluoromethyl)phenylacetic acid to give **5g**.



1 **3**

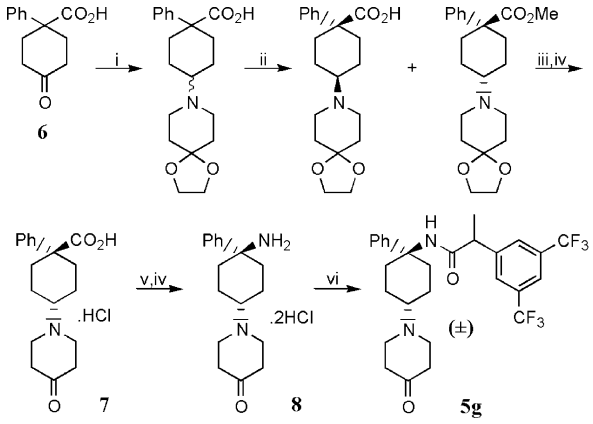
Figure 1. Overlay of **1** (black) with dofetilide **3** (grey).



4 **5a-f,h-n**

Scheme 1. Reagents and conditions: (i) R^1R^2NH, $NaCNBH_3$, $ZnCl_2$, MeOH (rt, 16 h) or R^1R^2NH, $NaBH(OAc)_3$, DCE (rt, 16 h).

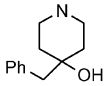
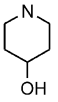
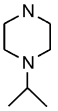
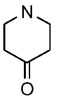
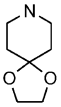
The in vitro NK_1 and I_{Kr} binding results for a selection of compounds **5a–h** are shown in Table 1. In cases where the compound was tested as a mixture, it was assumed that most of the activity resides in the *trans*-



6 **7** **8** **5g**

Scheme 2. (i) 1,4-Dioxa-8-azaspiro[4.5]decane, $NaBH(OAc)_3$, DCE (rt, 16 h); (ii) AcCl, MeOH (reflux, 24 h); (iii) separation of *trans*-ester and *cis*-acid; (iv) HCl, water (reflux, 16 h); (v) diphenylphosphorylazide, Et_3N, toluene (90 °C, 1.5 h); (vi) (±)-3,5-$(CF_3)_2C_6H_3CH(Me)COCl$, Et_3N, DCM (rt, 16 h).

Table 1. NK_1 and I_{Kr} activities for compounds **5a–h**

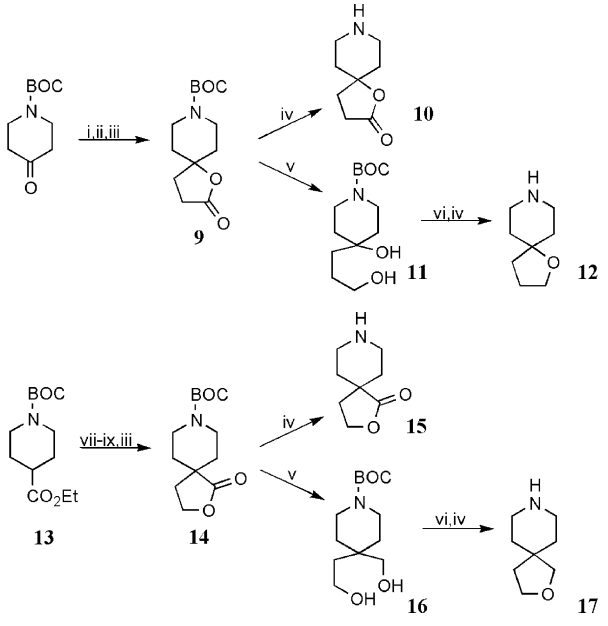
Compd	NR^1R^2	Stereochemistry	hNK_1 IC_{50} (nM)[a]	hI_{Kr} K_i (nM)[b]
5a		*trans*:*cis* 1:2.5	22±3	1900±230[c]
5b		*trans*:*cis* 1:1.4	2.4±0.20	1200±180[c]
5c		*trans*:*cis* 1:1	1.2±0.8	460±97[c]
5d		*trans*:*cis* 9:1	0.33±0.09	260±120[d]
5e		*trans*	0.85±0.21	1400±420[c]
5f		*trans*	0.39±0.07	1200±360[c]
5g		*trans*	1.6±0.4	4000±2200[d]
5h		*trans*	0.30±0.10	1000±130[d]

[a]Displacement of [^{125}I]-labelled substance P from the cloned hNK_1 receptor expressed in CHO cells. Data are mean±SD ($n=3$).[9]
[b]Displacement of labelled MK-499 from the cloned channel expressed in HEK cells. Data are mean±SD.[10]
[c]$n=3$.
[d]$n=4$.

isomer. It can be seen that good NK_1 receptor binding is generally achieved, most compounds having an IC_{50} below 3 nM. However, there is great variability in I_{Kr} affinity. Compound **5h** has sub-nanomolar NK_1 receptor binding affinity and I_{Kr} affinity greater than 1 μM but is poor in the gerbil foot-tapping assay, giving an ID_{50} of 5.4 mg/kg iv after a 24 h pre-treatment. This is possibly due to the instability of the acetal group in vivo and it was thought that replacement of one of the oxygen atoms with carbon would make the compound less metabolically labile. Accordingly, a series of spiroethers was synthesized.

The synthesis of the spirocyclic amines is shown in Scheme 3. Reaction of *N*-BOC-4-piperidone with the anion of ethyl propiolate followed by reduction, cyclization and deprotection afforded spiro-lactone **10**. Alkylation of the enolate derived from ester **13** with allyl bromide followed by ozonolysis with reductive work-up, cyclization and deprotection gave the isomeric spiro-lactone **15**. Reduction of the lactones **9** and **14** to the diols **11** and **16** followed by cyclization under Mitsonobu conditions and amine deprotection gave the spiro-tetrahydrofurans **12** and **17** respectively. Reductive amination using the four amines, followed by separation of isomers, gave the *trans*-compounds **5i–l**.

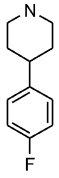
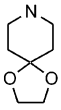
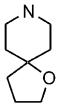
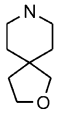
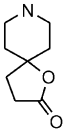
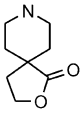
The in vitro NK_1 and I_{Kr} binding results and the in vivo gerbil foot-tapping results for compounds **5i–l** are shown in Table 2. It can be seen that both ethers but only one lactone, **5l**, have excellent NK_1 receptor binding affinity. The ethers, **5i** and **5j**, were tested in the gerbil foot-tapping assay and the results show that their in vivo activities are better than that of the original lead **1** (15% inhibition at 3 mg/kg iv after a 2 h pre-treatment time) and comparable with that of compound **2**. The activity is maintained after 24 h, showing that duration is improved compared to **5h**. **5j** is also active when dosed orally (ID_{50} 2.2 mg/kg po following a 24 h pre-treatment). Most importantly, the I_{Kr} affinities of all four compounds have been markedly reduced compared to the lead compound **2**.



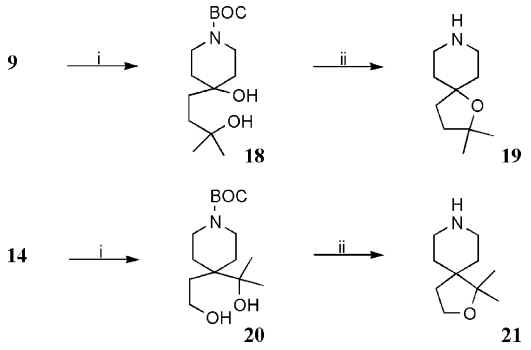
Scheme 3. (i) *n*BuLi, ethyl propiolate, THF (−78 °C, 1 h); (ii) H_2, Pd/C, EtOH (rt, 45 psi, 1.5 h); (iii) *p*TsOH, toluene (reflux, 3 h); (iv) AcCl, MeOH (0 °C to rt, 16 h); (v) DIBAL-H, DCM (−78 to 0 °C, 5 h); (vi) PPh_3, diethyl azodicarboxylate, THF (0 °C to rt, 16 h); (vii) KHMDS, allyl bromide, THF (−78 °C, 1 h); (viii) O_3, MeOH, DCM (−78 °C); (ix) $NaBH_4$, MeOH, DCM (−78 °C to rt, 3 h).

In an attempt to improve brain penetration even further by increasing the lipophilicity, some alkylated analogues were synthesized (Scheme 4). Reaction of the spiro-lactones **9** and **14** with excess methyl Grignard reagent gave the dimethyl compounds **18** and **20** which underwent cyclization and deprotection in one step using trifluoroacetic acid to give **19** and **21** (Scheme 4). Reductive amination as in Scheme 1, followed by isomer separation, gave the *trans*-compounds **5m** and **5n**.

Table 2. NK_1, I_{Kr} and gerbil activities for compounds **2** and **5h–l**

Compd	NR^1R^2	hNK_1 IC_{50} (nM)[a]	hI_{Kr} K_i (nM)[e]	Gerbil FT ID_{50} (mg/kg iv)[i]		
				$t=0$ h	$t=2$ h	$t=24$ h
2		0.63 ± 0.45[b]	43 ± 30[f]	1.7	0.6	1.8
5h		0.30 ± 0.10	1000 ± 130[g]			5.4
5i		0.22 ± 0.04[c]	730 ± 150[h]		0.7	3.1
5j		0.34 ± 0.10[d]	710 ± 130[f]	1.4	1.1	1.6
5k		2.1 ± 0.4[c]	400 ± 110[f]			
5l		0.42 ± 0.09[c]	490 ± 250[f]			

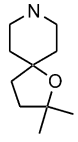
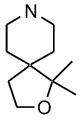
[a]Displacement of [^{125}I]-labelled substance P from the cloned hNK_1 receptor expressed in CHO cells. Data are mean ± SD.[9]
[b]$n=4$.
[c]$n=3$.
[d]$n=5$.
[e]Displacement of labelled MK-499 from the cloned channel expressed in HEK cells. Data are mean ± SD.[10]
[f]$n=3$.
[g]$n=4$.
[h]$n=6$.
[i]Inhibition of GR 73632-induced foot-tapping in gerbils.[6]



Scheme 4. (i) MeMgCl, THF (0 °C to rt, 16 h); (ii) TFA, DCM (rt, 16 h).

The in vitro and in vivo results for compounds **5m** and **5n** are shown in Table 3. Both compounds retain excellent NK_1 receptor binding affinity and the improvement already seen in I_{Kr} affinity is maintained in **5m** and increased in **5n**. Both compounds show good activity in vivo following a 2 h pre-treatment and **5m** maintains this at 24 h, however duration of action is much reduced in **5n** which gave only 7.5% inhibition of foot-tapping after a 24 h pre-treatment time. This is disappointing, especially in light of its much reduced I_{Kr} affinity.

Table 3. NK_1, I_{Kr} and gerbil activities for compounds **5m** and **5n**

Compd	NR^1R^2	hNK_1 IC_{50} (nM)[a]	hI_{Kr} K_i (nM)[b]	Gerbil FT ID_{50} (mg/kg iv)[e]	
				$t=2$ h	$t=24$ h
5m		0.35±0.15	950±170[c]	1.0	72% @ 3
5n		0.26±0.08	2200±1300[d]	1.1	7.5% @ 3

[a]Displacement of [^{125}I]-labelled substance P from the cloned hNK_1 receptor expressed in CHO cells. Data are mean±SD ($n=3$).[9]
[b]Displacement of labelled MK-499 from the cloned channel expressed in HEK cells. Data are mean±SD.[10]
[c]$n=4$.
[d]$n=3$.
[e]Inhibition of GR 73632-induced foot-tapping in gerbils.[6]

In conclusion, we have investigated substitution of the amine portion of the lead compound **2** and found that a wide range of large lipophilic amines is tolerated by the NK_1 receptor although the changes in I_{Kr} affinity are unpredictable. Three compounds, **5i**, **5j** and **5m**, achieved the objectives of retaining NK_1 receptor affinity and in vivo activity while significantly reducing I_{Kr} affinity.

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10. I_{Kr} affinity is measured using the MK-499 (L-706000)[11] binding assay which is performed on membrane preparations from human embryonic kidney (HEK) cells constitutively expressing hERG. The membranes are prepared by homogenization of the HEK cells in Tris–EDTA buffer (50 mM Tris, 1 mM EDTA, pH 7.5) and centrifugation at 45,000*g* for 20 min at 4 °C. The pellet containing membrane is resuspended in Tris–EDTA and stored at −70 °C. In the binding assay, hERG membranes are diluted into assay buffer (60 mM KCl, 71.5 mM NaCl, 1 mM $CaCl_2$, 10 mM Hepes, pH 7.4) to a final concentration of 4.16 μg per 400 μL well volume. Radiolabelled MK-499 (^{35}S-L-706000; specific activity ≈1000 Ci/mmol) is then added to a final concentration of 50 pM and 400 μL of membrane-ligand mixture is added per well to 96-well assay blocks containing 4 μL of 100× stocks of tested drugs or 100% DMSO (maximum binding control) or 100 μM cold MK-499 (non-specific binding control). Membranes are incubated at room temperature for 75 min, filtered through GF/B Unifilters presoaked in 0.1% BSA and washed 5×500 μL with wash buffer at 4 °C (10 mM Hepes, 131.5 mM NaCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, pH 7.4). Filters are dried at room temperature, 50 μL Microscint-20 is added to each well, and Unifilters are counted for 1 min in Topcount. Dose-inhibition curves and inflection points are determined by curve-fitting the equation: response = [(max−min)/(1 + ([I]/IP)^s)] + min, where I is the concentration of inhibitor, max is the maximum response (DMSO control), min is the minimum response (excess, cold MK-499 control), IP is the inflection point, and s is the slope. Generally, the inflection point is similar to the concentration of drug that inhibits 50% of radiotracer binding.
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