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Synthesis and pharmacological characterization of constrained analogues of Vestipitant as in vitro potent and orally active NK_1 receptor antagonists

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ABSTRACT

A focused exploration targeting conformationally restricted analogues of Vestipitant, resulted in the discovery of novel, in vitro potent NK_1 antagonists. In particular, two of the compounds reported exhibited a good pharmacokinetic (PK) profile and produced anxiolytic-like effects in the gerbil foot tapping (GFT) in vivo model.

The neurokinin-1 (NK_1[a]) receptor is a 7TM G-protein coupled receptor (GCPR) known to be implicated in the pathophysiology of a range of pathological conditions, namely: inflammatory bowel disease, pain, migraine, movement disorders, schizophrenia, sleep disorders, emesis, nausea, anxiety and depression.[1] In particular, the NK_1 receptor antagonists MK-869[2] and CP-122,721,[3] shown in Figure 1, were proven to possess antidepressant activity in clinical trials performed by Merck and Pfizer, respectively, whereas GR205171 showed anxiolytic-type activity in social phobia.[4]

Moreover, MK-869 (Aprepitant)[5] is currently on the market for the treatment of post-operative and chemotherapy-induced nausea and vomiting.

As a consequence of their large therapeutic potential, the identification of novel non-peptide, potent and brain penetrant NK_1 receptor antagonists is still perceived within the medicinal chemistry community as an important target to achieve. Recently, we reported the preparation and the pre-clinical profile of a novel series of phenylpiperazine derivatives as potent drug-like NK_1 receptor antagonists.[6] In particular, Vestipitant (Fig. 1) showed appropriate pharmacokinetics and excellent anxiolytic-like behaviour in the gerbil foot tapping (GFT) in vivo model, after oral administration. As part of a wider exploration performed within this chemical series, and with the aim of expanding our knowledge in terms of the pharmacophoric requirements of the arylpiperazine template, we sought to introduce some elements of rigidity within this template (Fig. 2).

This was achieved either by introducing a conformational constrain, tethering the nitrogen atom to the adjacent arylpiperazine

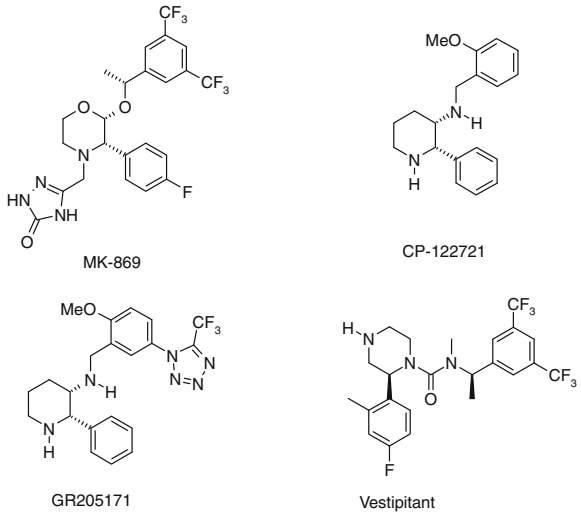

Figure 1. Some examples of potent NK_1 antagonists described in the literature.

Abbreviations: NK, neurokinin; 7-TM, 7-transmembrane; SP, substance P; GPCR, G-roteincoupled receptor; CNS, central nervous system; SAR, structure–activity relationship; CDI, arbonyldiimidazole; THF, tetrahydrofuran; DMF, dimethylformamide; DCM, dichloromethane; BOC, *tert*-butoxycarbonyl; TEA, triethylamine; MsCl, mesyl chloride; TFA, trifluoroacetic acid; CHO, Chinese Hamster Ovary; FLIPR, fluorescence imaging plate reader; GFT, gerbil foot tapping.

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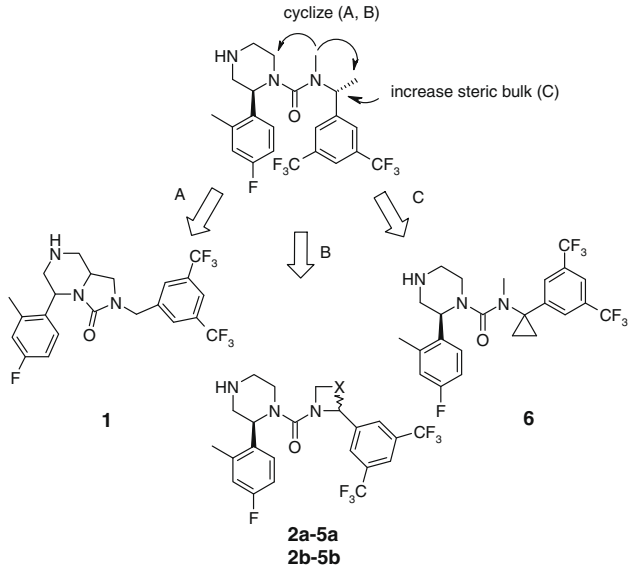
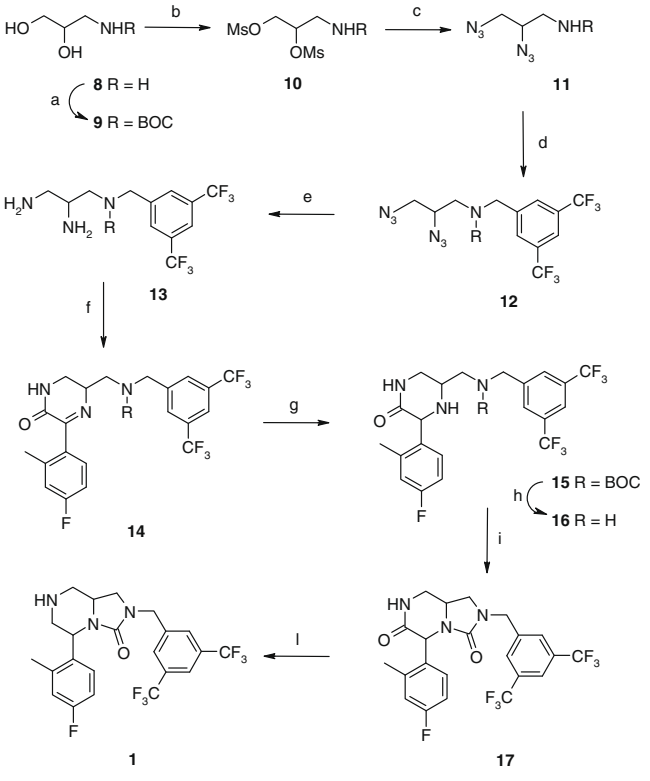


Figure 2. Constrained analogues of Vestipitant.

nucleus (A) forming appropriate five- or six-membered rings (B) or, as an alternative, making the benzylic position quaternary (C). Herein, we describe the synthesis of the novel compounds designed, along with their structure activity relationship (SAR). In addition, the pharmacokinetics and in vivo activity in the GFT model of some selected compounds, are also reported.

Compound **1** was prepared as shown in Scheme 1. Commercially available 1-amino glycerol **8** was transformed into the azido derivative **11** in high overall yield by sequential protection of the primary amino group as BOC, mesylation reaction and nucleophilic substitution with NaN_3 in DMF at 80 °C. Then, the alkylation reaction of the nitrogen with NaH and 3,5-bis-trifluoromethylbenzylamine gave compound **12** which was reduced to give intermediate **13**. The following reaction afforded the lactam **14** which was transformed into the imidazolinone **17**, as a single cis diastereoisomer, by sequential imine reduction and removal of the *N*-BOC protecting group followed by a cyclization reaction in the presence of CDI. Finally, the chemoselective reduction of the lactam function afforded the target compound **1**.

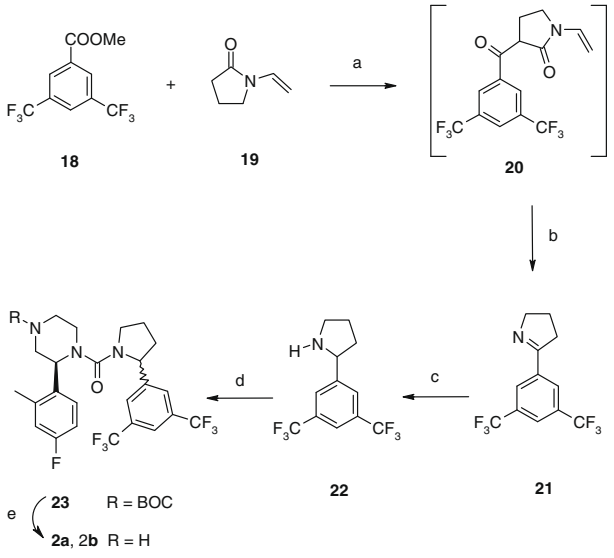


Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, EtOH, 1 h, rt, 100%; (b) DCM, TEA, 0 °C, MsCl, rt, 30′, 87%; (c) DMF, NaN_3, 80 °C, 3 h, 82%; (d) NaH, DMF, 3,5-bis-trifluoromethylbenzylamine, 0 °C, 1 h, 100%; (e) Pd/C 10%, MeOH, H_2 (1 atm), 24 h, 97%; (f) EtOH, ethyl(4-fluoro-2-methylphenyl)(oxo) acetate, reflux, 24 h, 60%; (g) Pd/C 10%, MeOH, H_2 (1 atm), 3 h, 67%; (h) MeOH/HCl 37%, reflux, 3 h, 45%; (i) CDI, DMF, 100 °C, 3 h, 76%; (l) BH_3THF, 2 h, rt, then HCl, 26%.

Preparation of the five-membered cyclic diastereoisomers **2a** and **2b** is shown in Scheme 2. Condensation between the commercially available ester **18** and the *N*-vinyl lactam **19** followed by one-pot deprotection, lactam ring opening and rearrangement of intermediate **20**, afforded the cyclic imine **21**, which was reduced to form the corresponding amine **22** and then transformed into the urea **23** as a mixture of diastereoisomers. Removal of the *N*-BOC protecting group and separation of the two diastereoisomers gave the final compounds **2a** and **2b**. The synthesis of the corresponding six-membered cyclized compounds **3a**, **3b** and **4a**, **4b** is shown in Scheme 3. The commercially available carboxylic acid derivative **24** was allylated at the benzylic position. Then, the following Curtius-type rearrangement afforded a mixture of the urea intermediates **26a** and **26b**.

After smooth allylation of the urea nitrogen atom the diastereoisomers **27a** and **27b** were separated by flash chromatography. Then, the ring closing metathesis reaction was carried out and the desired cyclized compounds **28a** and **28b**, were smoothly obtained from **27a** and **27b**, respectively.

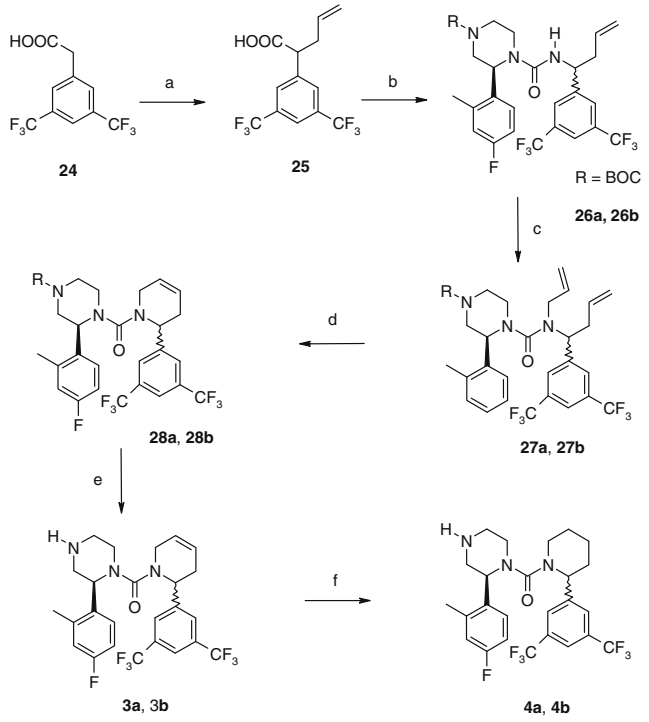
Subsequent removal of the *N*-BOC protecting group afforded the compounds **3a** and **3b**. Finally, hydrogenation of the double bond gave title compounds **4a** and **4b**. The preparation of the bis-piperazine derivatives **5a** and **5b** is shown in Scheme 4. The commercially available bromide **29** was smoothly acylated to give the oxalate derivative **30**, which was treated with ethylenediamine to afford **31**. After sequential reduction of the imine function and the lactam, the 2-arylpiperazine intermediate **33** was obtained. The less hindered amino atom was chemoselectively protected as BOC. Ureas **35a** and **35b** were then formed in the presence of the



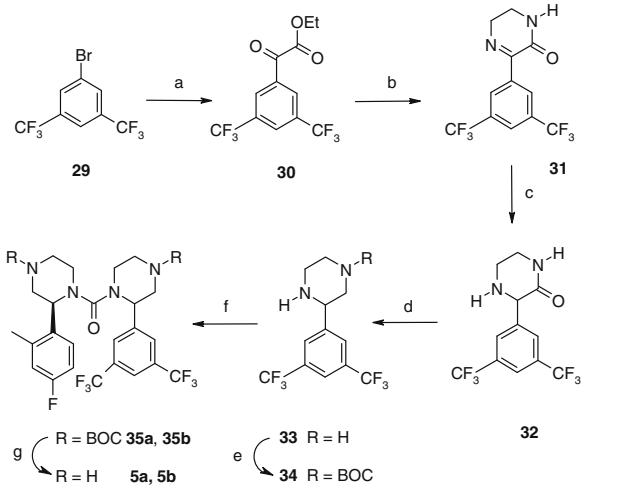
Scheme 2. Reagents and conditions: (a) NaH, toluene, reflux, 12 h; (b) (i) HCl 6 N, reflux, THF, 4 h; (ii) KOH; $y = 5\%$; (c) $NaBH_4$, MeOH, 0 °C, 1 h, $y = 85\%$; (d) TEA, triphosgene, MeCN, 1,1-dimethylethyl (3*S*)-3-(4-fluoro-2-methylphenyl)-1-piperazinecarboxylate (**7**), rt, 2 h, reflux, 5 h, $y = 56\%$; (e) MeOH, HCl, reflux, 2 h, $y = 100\%$.

chiral intermediate **7** and final removal of the *N*-BOC protecting group followed by separation of the diastereoisomers gave the final compounds **5a** and **5b**.

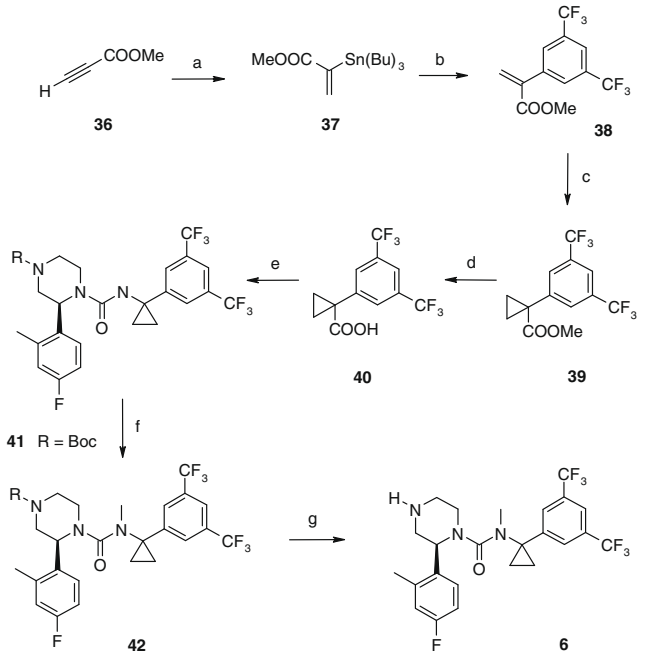
The synthesis of compound **6** is reported in Scheme 5. Hydrostannylation reaction of the commercially available methyl 2-propynoate **36** afforded intermediate **37**, which was transformed into the vinylester derivative **38** by Stille-type coupling with commercially available 1-bromo-3,5-bis(trifluoromethyl)benzene. Then the key cyclopropanation reaction was performed according to Corey's conditions.[7] Basic hydrolysis of the ester **39**, followed by Curtius-type rearrangement in the presence of the chiral arylpiperazine derivative,[8] gave intermediate **41**. Sequential methylation of the urea nitrogen atom and removal of the *N*-BOC protecting group afforded the final compound **6**.



Scheme 3. Reagents and conditions: (a) BuLi, DIPEA, allyl bromide, THF, −78 °C, *y* = 69%; (b) **7**, TEA, $(PhO)_2P(O)N_3$, toluene, 3 h, rt, reflux, 1 h, *y* = 37%; (c) THF, allyl iodide, *t*-BuONa, rt, 3 h, *y* = 58%; (d) DCM, rt, 3 h, Grubb's catalyst ((benzylidene)dichlorobis(tricyclohexylphoshine)ruthenium), *y* = 67% (diast. 1), *y* = 62% (diast. 2); (e) (i) TFA/DCM, 30′, rt; (ii) H_2O, K_2CO_3; (iii) HCl 1 M, Et_2O, 30′, rt, *y* = 58% (diast. 1), *y* = 59% (diast. 2); (f) (i) H_2, Pd/C 10%, 1 Atm, rt, 3 h; (ii) TFA/DCM, rt, 1 h; (iii) K_2CO_3/H_2O; (iv) HCl 1 M, Et_2O; *y* = 32% (diast. 1), *y* = 25% (diast. 2).



Scheme 4. Reagents and conditions: (a) (i) Mg, I_2, Et_2O, reflux, 3 h; (ii) diethyl oxalate, −60 °C, rt, *y* = 63%; (b) $NH_2CH_2CH_2NH_2$, Na_2SO_4, toluene, reflux, 16 h, *y* = 32%; (c) H_2, Pd/C, MeOH, 1 atm, rt, 3 h, *y* = 100%; (d) (i) BH_3 THF, reflux, 4 h, (ii) HCl Et_2O, 45 °C, 1 h, *y* = 67%; (e) $(Boc)_2O$, TEA, 0 °C, 60′, *y* = 100%; (f) triphosgene, **7**, TEA, rt, 1 h, *y* = 40%; (g) TFA, DCM, H_2O, K_2CO_3, HCl Et_2O, *y* = 48% (diast. 1), *y* = 30% (diast. 2).



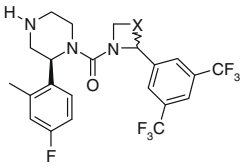
Scheme 5. Reagents and conditions: (a) Bu_3SnH, $Pd(PPh_3)_2Cl_2$, THF, rt, 10′, *y* = 46%; (b) DMF, 1-bromo-3,5-bis(trifluoromethyl)benzene, CuI, Pd-tetrakis, *y* = 21%; (c) $(Me)_3SOI$, NaH 60%, DMF, rt, 1 h, *y* = 16%; (d) LiOH, MeOH, reflux, 2 h, *y* = 100%; (e) $(PhO)_2P(O)N_3$, TEA, **7**, toluene, rt, 3 h, then 100 °C, 1 h, *y* = 56%; (f) MeI, *t*-BuONa, THF, rt, 1 h, *y* = 100%; (g) MeOH, HCl 37%, reflux, 15′, *y* = 54%.

The in vitro affinities of the compounds described above are shown in Table 1. Compound **1** (cyclization A, Fig. 2) exhibited a significant drop of affinity with respect to Vestipitant, most likely as a consequence of the inappropriate spatial orientation of the urea carbonyl group which, as observed from X-ray analysis of Vestipitant,[6] should lie out of the plane of the piperazine ring. Conversely, cyclization of type B (Fig. 2) enabled us to identify **3b** as a novel compound retaining good in vitro affinity compared to Vestipitant (pK_i = 9.3 and 9.6, respectively), while the corresponding saturated analogue **4b** and the five-membered ring derivative **2b** were found to be less active (pK_i = 8.2 and 7.2, respectively). Noteworthy, the introduction of an amino group within the saturated six-membered ring was well tolerated (compound **5b**, pK_i = 9.1); this result was particularly significant as for the first time it was shown that the presence of an additional basic heteroatom on the right hand side of the template can be tolerated, suggesting the possible presence of an additional pharmacophoric point.

Diastereoisomers **3a**, **4a** and **5a** exhibited reduced in vitro affinity with respect to **3b**, **4b** and **5b** (pK_i = 7.7, 7.5 and 7.0, respectively).

Modelling studies using compound **1**, the 2*S*,6*R*,-diastereoisomer of compound **3a**/**3b** (IUPAC name: (2*S*)-1-{[(6*R*)-6-[3,5-bis(trifluoromethyl)phenyl]-3,6-dihydro-1(2*H*)-pyridinyl]carbonyl}-2-(4-fluoro-2-methylphenyl)piperazine) and the 2*S*,2*S*-diastereoisomer of the compound **5a**/**5b** (IUPAC name: (2*S*)-2-[3,5-bis(trifluoromethyl)phenyl]-1-{[(2*S*)-2-(4-fluoro-2-methylphenyl)-1-

Table 1
In vitro affinity, measured by inhibition of the binding of [^{3}H]-substance P ([^{3}H]-SP) to membranes obtained from Chinese Hamster Ovary cells transfected with human NK_1 receptors (*h*-NK_1 CHO)[9]



Compound	X	pK_i[a]	ID_{50}[b]
Vestipitant	—	9.6	0.014
1	—	7.3	
2[a]	$(CH_2)_2$	7.2	
2b	$(CH_2)_2$	8.2	
3a	$CH_2CH{=}CH$	7.7	
3b	$CH_2CH{=}CH$	9.3	
4a	$(CH_2)_3$	7.5	
4b	$(CH_2)_3$	8.9	
5a	$(CH_2)_2NH$	7.0	
5b	$(CH_2)_2NH$	9.1	0.4
6	—	9.3	0.2

[a] pK_i values are the mean of at least two experiments performed in duplicate. The difference between the averaged pK_i values was always lower than 0.3.
[b] ID_{50} is the compound dose causing 50% inhibition of the tapping behaviour elicited by the icv injection of GR73632 (substance P), 3 pmol/5 μL to gerbils. ID_{50} values are expressed in mg/kg after oral or sc administration one hour before the test.

piperazinyl]carbonyl}piperazine), resulted in the identification of good shape and pharmacophoric 3D alignments.[10] As shown in Figure 3A these conformationally locked analogues are able to project their common pharmacophoric features (i.e., the positive ionizable group corresponding to the secondary amine, the two hydrophobic moieties mapped by the two phenyl rings and a hydrogen bond-acceptor feature mapped by the carbonyl moiety) into the same spatial area mapped by Vestipitant. Furthermore, the accessory six-membered rings could be accommodated in a common binding pocket. However, from this analysis, it cannot be ruled out that they can lie in a different pocket as shown by the 3D alignment of the 2*S*,2*R*-diastereoisomer of compound **5a**/**5b** (IUPAC name: (2*R*)-2-[3,5-bis(trifluoromethyl)phenyl]-1-{[(2*S*)-2-(4-fluoro-2-me thylphenyl)-1-piperazinyl]carbonyl}piperazine, Figure 3B), making the association of the relative

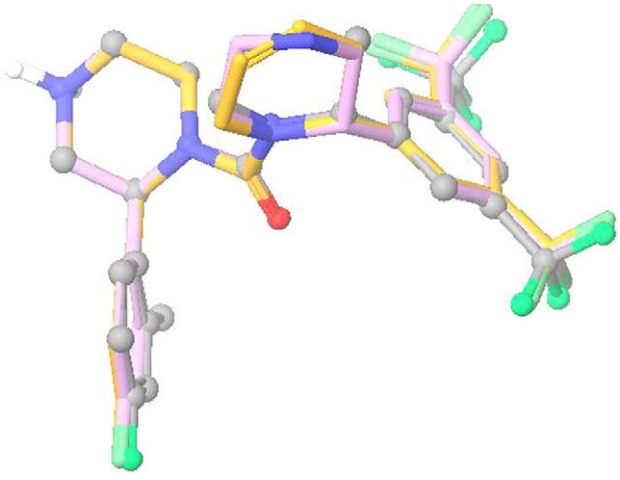


Figure 3A. Shape and pharmacophoric superimposition of Vestipitant (ball&stick, colour by atom type), the 2*S*,6*R*-diastereoisomer of compound **3a**/**3b** (stick, yellow) and the 2*S*,2*S*-diastereoisomer of compound **5a**/**5b** (stick, pink).

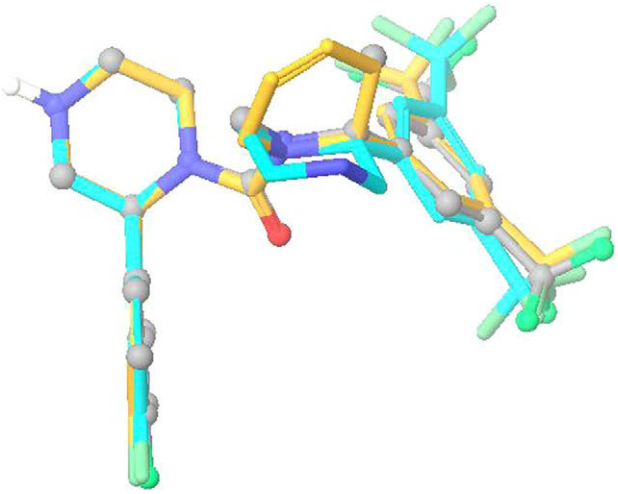


Figure 3B. Shape and pharmacophoric overlay of Vestipitant (ball&stick, colour by atom type), the 2*S*,6*R*-diastereoisomer of compound **3a**/**3b** (stick, yellow) and the 2*S*,2*R*-diastereoisomer of compound **5a**/**5b** (stick, light blue).

stereochemistry of the two stereogenic centres and the in vitro affinity to the NK_1 receptor binding site not obvious. The slight drop in potency exhibited by the five-membered ring derivatives, exemplified by compound **2a**/**2b** (Table 1), might be related to the suboptimal orientation of their pendant phenyl rings.

Finally, compound **6**, in which a quaternary centre was introduced at the benzylic position, retained good potency compared to Vestipitant (pK_i 9.3 and 9.6, respectively). Two of the most in vitro potent compounds, **5b** and **6**, were endowed with good in vivo PK profile in the rat.[11]

Compound **5b**, in rat, 5 min after administration, exhibited acceptable brain penetration (B/P = 0.9). In addition a C_{max} = 32 ng/mL and AUC(0–8 h) = 145 ng h/mL were observed at 1 mg/Kg, po. Compound **6**, instead, displayed increased brain penetration (B/P = 4.0) and comparable exposure in plasma (C_{max} = 29 ng/mL; AUC(0–8 h) = 108 ng h/mL) after oral administration at 1 mg/kg. When compound **5b** was given sc, 1 h prior to icv infusion of the NK_1 agonist GR73632,[12] a dose dependent inhibition of foot tapping in gerbils was observed, with an estimated ID_{50} of 0.4 mg/kg. Following the same protocol, compound **6** caused a dose dependent inhibition of foot tapping in gerbils with an estimated ID_{50} of 0.2 mg/kg.

In conclusion, the design and the exploration of some conformationally constrained analogues of Vestipitant enabled the identification of novel in vitro and in vivo potent and brain penetrant NK_1 receptor antagonists, exhibiting good drug-like properties. In particular, compounds **5b** and **6** were shown to be active in vivo in the GFT model. Furthermore, these findings contributed to a better understanding of the pharmacophoric and structural requirements of these series of NK_1 antagonists.

Acknowledgements

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10. Conformational analysis of Vestipitant, of the 2*S*,1*R*-diastereoisomer of compound **3a**/**3b** and of the 2*S*,1*S* and the 2*S*,1*R*-diastereoisomers of compound **5a**/**5b** was carried out with BatchMinV9.6 (Schrodinger LLC) and the following parameters: 1000 steps/rotatable bond, OPLS_2005 FF (Force Field), implicit water model, 10,000 minimization steps with PRCG (energy minimizer). Conformations lying within 3 kcal/mol energy window were selected. Shape and pharmacophoric 3D alignments were performed with ROCS 2.3.1 (OpenEye Scientific Software Inc.) coupled to colour force field (ImplicitMillsDean). The 3D alignments generated were ranked according to their combo score and visually inspected within Maestro (Schrodinger LLC).
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