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Original article

Investigation of SAR requirements of SR 142801 through an indexed combinatorial library in solution

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Abstract – To rapidly gain information on structure-activity relationship (SAR) requirements of the human neurokinin 3 (hNK-3) receptor antagonist SR 142801, an indexed combinatorial library was synthesised in solution and screened on the hNK-3 receptor. SAR considerations drawn from binding affinity of combinatorial mixtures were confirmed through the synthesis and biological evaluation of some individual compounds. © 1999 Éditions scientifiques et médicales Elsevier SAS

tachykinins / neurokinin 3 receptor antagonists / combinatorial chemistry / indexed libraries / structure-activity relationships

1. Introduction

The neurokinin 3 (NK-3) is one of the three receptors for the family of peptides named tachykinins or neurokinins and belongs to the G protein-coupled receptor superfamily [1]. In 1996, our group described the identification of potent and selective non-peptide NK-3 receptor antagonists featuring the 2-phenyl-4-quinolinecarboxamide framework, including SB 223412 [2, 3]; this is one of the three main chemical classes of non-peptide NK-3 receptor antagonists reported to date [4, 5]. The other two are the peptide-derived structures reported by Parke-Davis (PD 161182) [6] and the 3,4-dichlorophenylpiperidines which were first described by Sanofi (exemplified by SR 142801, *figure 1*) [7] and subsequently by Merck Sharp and Dohme [8]. Due to our involvement in the NK-3 area, we were interested in investigating the SAR requirements of the 3,4-dichlorophenylpiperidine NK-3 receptor antagonists, only marginally described in the literature [8]. For this reason, the synthesis of a small library of analogues of SR 142801 was planned via a combinatorial chemistry approach.

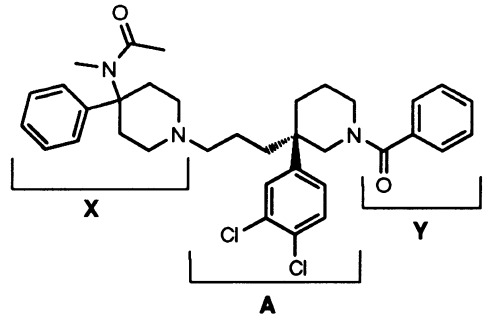

Figure 1. Structure of SR 142801.

Since the 3-(3,4-dichlorophenyl)-3-propylpiperidine framework A *(figure 1)* of SR 142801 appears to be a key feature of this class of NK-3 receptor antagonists, able to drive their selectivity for the NK-3 receptor with respect to the NK-1 and NK-2 [4, 8], we decided to keep this

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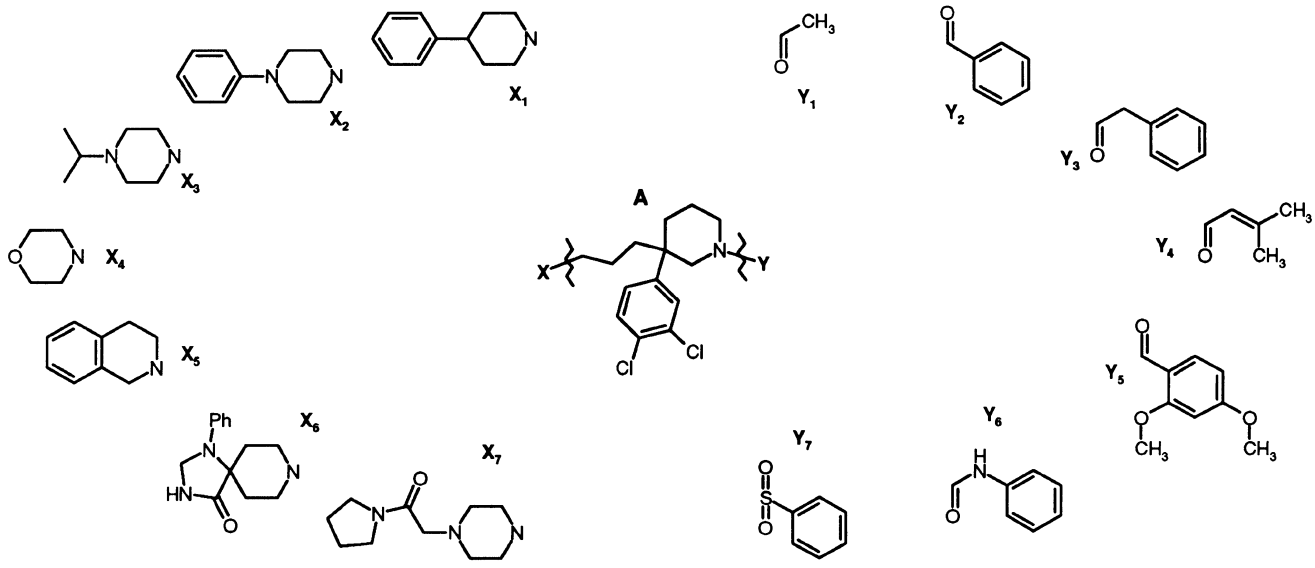


Figure 2. Library design.

moiety fixed and focus our attention on variations in the basic head X and the acylating group Y.

In designing the library, 7 amines ($\mathbf{X_1}$–$\mathbf{X_7}$) and 7 acylating groups ($\mathbf{Y_1}$–$\mathbf{Y_7}$) were selected, on the basis of commercial availability of corresponding reagents, to give a total of 49 compounds ($\mathbf{X_{1–7}AY_{1–7}}$), as all the possible combinations of X and Y substituents *(figure 2)*. To simplify the synthetic pathway, it was planned to work on racemic mixtures.

Solution phase combinatorial chemistry was the technique of choice for the synthesis of the library because a suitable point of attachment to the resin could not be found, ruling out a solid phase approach. Moreover, since in the synthetic scheme the "combinatorial reaction" was a well consolidated amide coupling at the last step (*figures 4* and *5*), and since the number of compounds to synthesise was small (49), we reasoned that pools of compounds could easily be obtained by using mixtures of reactants, without risk of getting too many by-products. In particular, to help the structural determination of active components of the library, the strategy of indexed (or orthogonal) combinatorial libraries was utilised [9–11].

2. Chemistry

2.1. Synthesis of intermediate compounds

In *figure 3*, the synthesis of intermediate compounds is described. Racemic aminol **1** was obtained according to literature procedure [12], protected at the nitrogen and activated to nucleophilic substitution by converting the hydroxy group into the mesylate and this, in turn, into the more reactive iodo derivative **2** [13]. Displacement of iodine with the 7 amines $\mathbf{X_1}$–$\mathbf{X_7}$ (CH_3CN, K_2CO_3, 90 °C, 18 h) afforded the desired intermediate compounds $\mathbf{X_1A}$–$\mathbf{X_7A}$, which were purified by flash column chromatography.

2.2. Synthesis of the library: set 1

As depicted in *figure 4*, each intermediate compound was subsequently deprotected (20% TFA/CH_2Cl_2, r.t., 18 h) and reacted with a stoichiometric and equimolar mixture of the 7 acylating agents $\mathbf{Y_1}$–$\mathbf{Y_7}$, (CH_2Cl_2, K_2CO_3, r.t., 18 h). Simple washing of the reaction mixtures with H_2O and evaporation to dryness afforded the first set (set 1) of desired mixtures (or sublibraries) $\mathbf{X_1AY_{1–7}}$–$\mathbf{X_7AY_{1–7}}$ in satisfactory to high yields (60–80%) [14a] and high purity (95–98%, LC/UV) [14b]. Peak identity was assessed by LC/MS and all expected molecular weights were detected in each mixture.

It is worth noting that the seven sublibraries of set 1 contain all the desired 49 compounds; each sublibrary has the X substitution fixed and different from one another, while containing all possible Y substituents. Since the Y portion is common to all the sublibraries, binding affinity data on this set will clarify the SAR related to the X group.

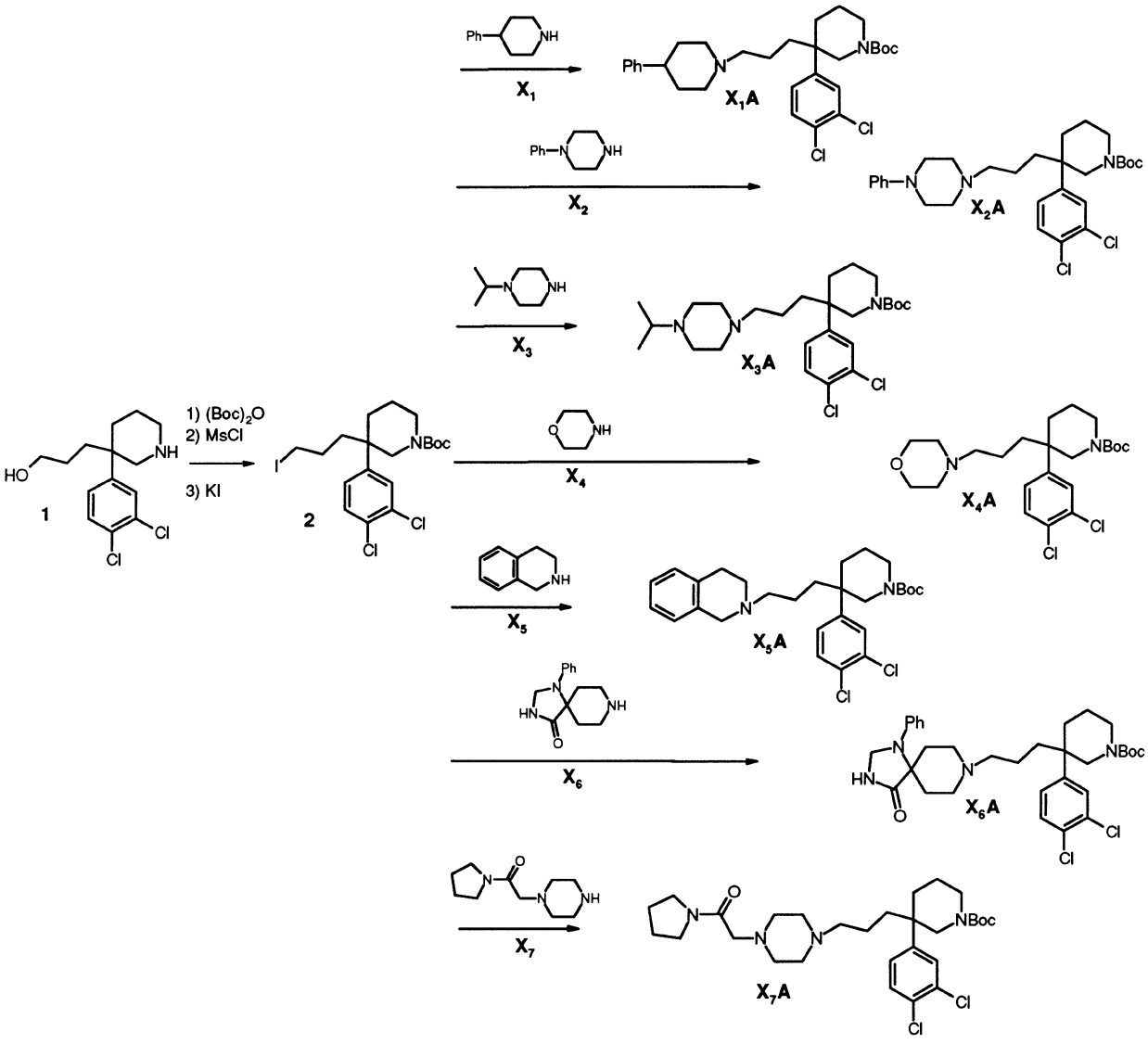


Figure 3. Synthesis of intermediates.

2.3. Synthesis of the library: set 2

In *figure 5*, the synthesis of the second set (set 2) of sublibraries is described. An equimolar amount of each of the intermediate compounds $\mathbf{X_1A}$–$\mathbf{X_7A}$ *(figure 3)* was mixed into the same reaction vessel and Boc-deprotected; the mixture was then split into seven equal portions ($\mathbf{X_{1-7}A}$), and each portion was reacted with a stoichiometric amount of acyl chloride/phenylisocyanate/phenylsulfonyl chloride $\mathbf{Y_1}$–$\mathbf{Y_7}$ (CH_2Cl_2, K_2CO_3, r.t., 18 h). Again, simple washing of the reaction mixtures with H_2O and evaporation to dryness produced the desired sublibraries $\mathbf{X_{1-7}AY_1}$–$\mathbf{X_{1-7}AY_7}$ in high yields (74–95%) [14a] and high purity (90–98%, LC/UV, except for $\mathbf{X_{1-7}AY_4}$: 83%) [14b]. All expected molecular weights were detected in each mixture by LC/MS.

The seven sublibraries of set 2 contain the same 49 compounds of set 1, but in a different combination: in this case, the Y substituent is fixed and peculiar to any sublibrary, while all possible X substitutions are common to all. Binding affinity data on this second set will clarify

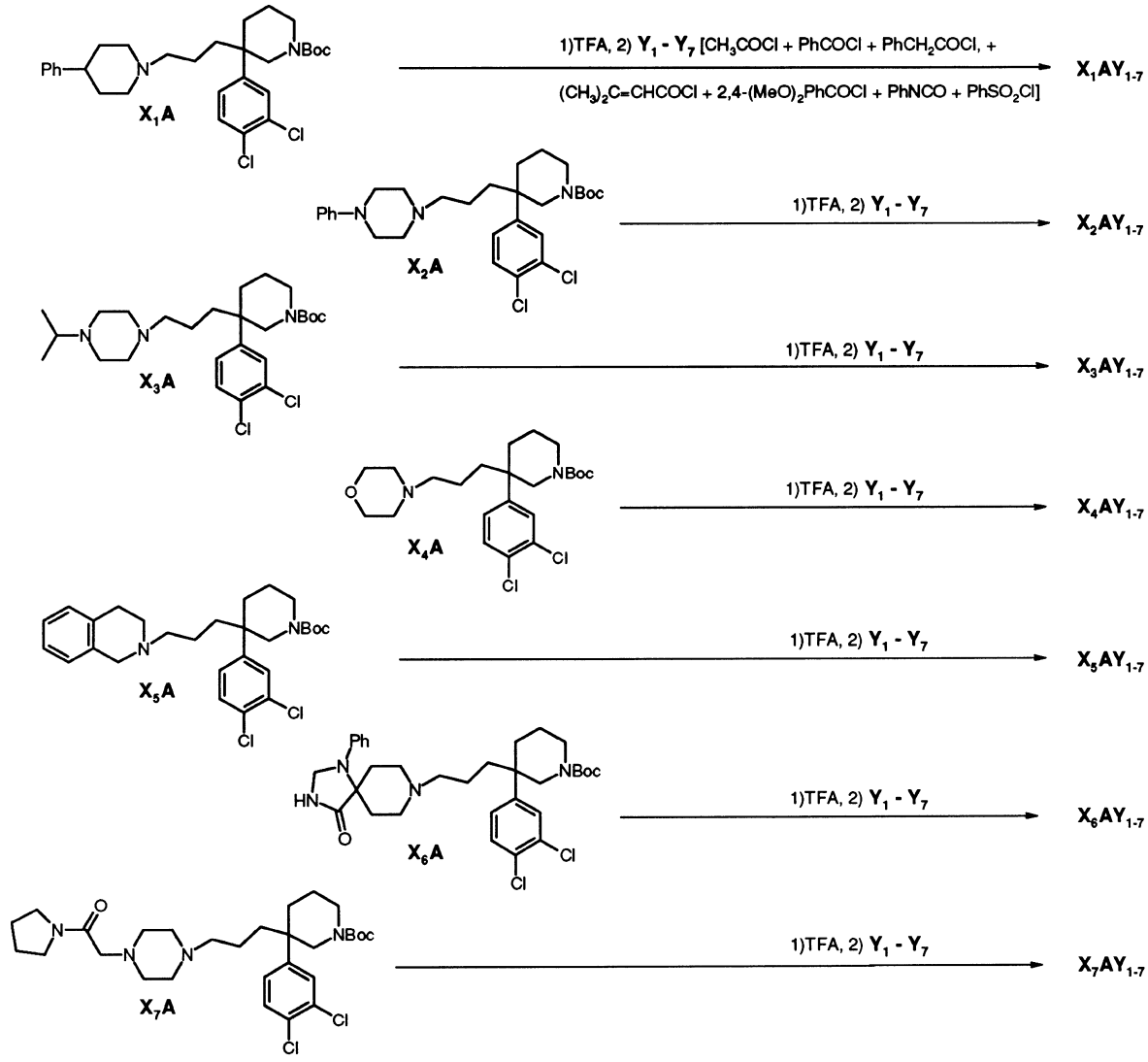


Figure 4. Synthesis of the library: set 1.

the SAR related to the Y group. By combining information coming from the two sets of sublibraries, the best X (set 1) and the best Y (set 2) substituents should be identified, thus allowing structural determination of the most active compounds of the whole library.

2.4. Synthesis of individual compounds

To prove the validity of the technique and the predictions made from binding affinity data of mixtures, the six individual compounds reported in *table II* were synthesised, deprotecting the appropriate intermediate compound (20% TFA/CH_2Cl_2, r.t., 18 h) and reacting it with the appropriate acylating agent (CH_2Cl_2, K_2CO_3, r.t., 18 h).

3. Pharmacology

Receptor binding assays were performed with crude membranes from CHO cells expressing the hNK-3 receptor as detailed previously [3, 15]. For NK-3 receptor competition binding studies, [^{125}I]-[MePhe^7]-NKB binding to hNK-3-CHO membranes was performed using the

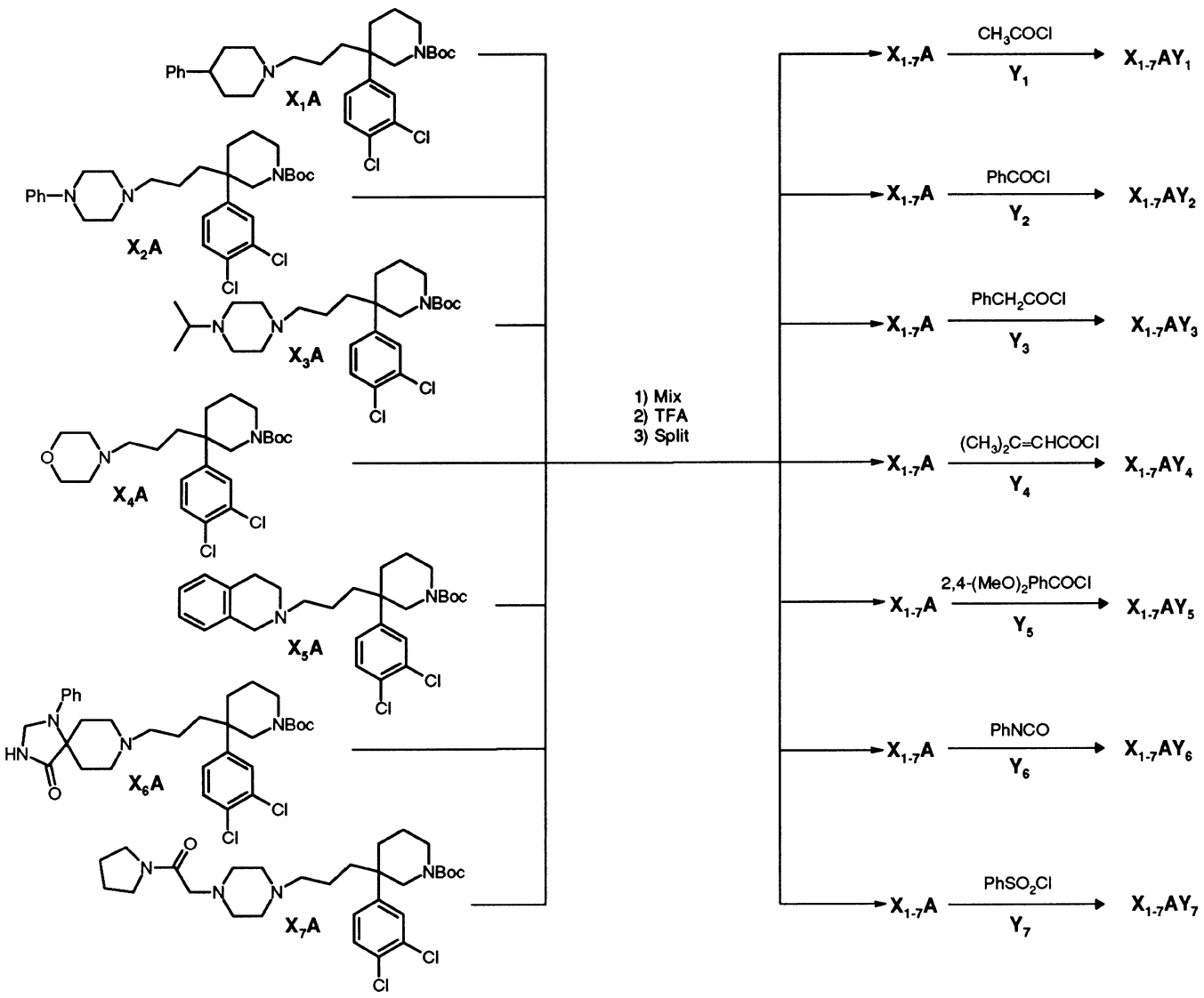


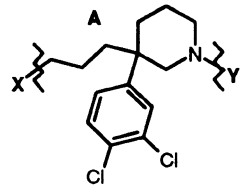
Figure 5. Synthesis of the library: set 2.

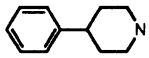
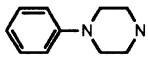
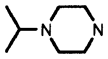
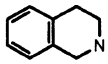
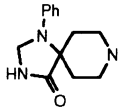
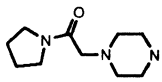
procedure of Sadowski and co-workers [16]. Specific binding was determined by subtracting total binding from non-specific binding, which was assessed as the binding in the presence of 0.5 μM cold [MePhe^7]-NKB. Percent inhibition of specific binding was determined for each concentration of the compounds and the IC_{50}, defined as the concentration required to inhibit 50% of the specific binding, obtained from concentration-response curves. Values reported in *tables I and II* are the apparent inhibition constant (K_i), which was calculated from the IC_{50} as described by Cheng and Prusoff [17].

4. Results and Discussion

Human NK-3 receptor binding affinity data for the 14 sublibraries synthesised are reported in *table I*. Results of sublibraries of set 1 revealed that pyrrolidinocarbonylmethylpiperazine (X_7, K_i = 113 nM) should be slightly more active than phenylpiperidine (X_1, K_i = 160 nM) which, in turn, should be more active than phenylpiperazine (X_2, K_i = 232 nM). A strongly reduced affinity is forseen for isopropylpiperazine (X_3, K_i = 743 nM) and morpholine (X_4, K_i = 1235 nM).

Table I. Binding affinities of library compounds.

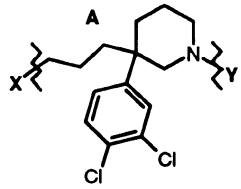


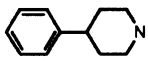
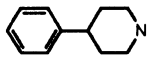
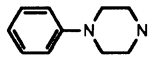
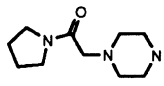
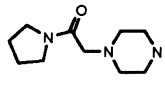
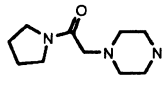
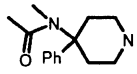
Set 1				Set 2			
X		Y	hNK-3 K_i (nM) [a,b]	X	Y		hNK-3 K_i (nM)[a,b]
X_1		$Y_{1\text{-}7}$	160	$X_{1\text{-}7}$	Y_1	CH_3CO	9163
X_2		$Y_{1\text{-}7}$	232	$X_{1\text{-}7}$	Y_2	PhCO	242
X_3		$Y_{1\text{-}7}$	743	$X_{1\text{-}7}$	Y_3	$PhCH_2CO$	394
X_4		$Y_{1\text{-}7}$	1235	$X_{1\text{-}7}$	Y_4	$(CH_3)_2C{=}CHCO$	503
X_5		$Y_{1\text{-}7}$	525	$X_{1\text{-}7}$	Y_5	$2,4\text{-}(MeO)_2PhCO$	1835
X_6		$Y_{1\text{-}7}$	440	$X_{1\text{-}7}$	Y_6	PhNHCO	1497
X_7		$Y_{1\text{-}7}$	113	$X_{1\text{-}7}$	Y_7	$PhSO_2$	570

[a]Inhibition of [^{125}I]MePhe^7-NKB binding in hNK-3-CHO cell membranes. [b]single determination (n = 1).

As far as the Y substitution is concerned (set 2), benzoyl (Y_2, K_i = 242 nM), also present in SR 142801, appears to be the best substituent. Increasing the distance of the phenyl ring from the carbonyl with a methylene spacer (Y_3, K_i = 394 nM) slightly decreases the binding affinity, while substitution at the phenyl ring with two methoxy groups (Y_5, K_i = 1 835 nM) or replacement of the phenyl with a methyl (Y_1, K_i = 9 163 nM) resulted in a marked loss of activity. Dimethylacryloyl (Y_4, K_i = 503 nM) and phenylsulfonyl (Y_7, K_i = 570 nM), although less potent than the benzoyl (Y_2, K_i = 242 nM), maintain a certain activity, while the urea (Y_6, K_i = 1497 nM) does not appear to be a good replacement for the benzamide. To test the reliability of the predictions made from mixtures, six individual compounds (some predicted to be the most active and some predicted to have medium activity) were synthesised and screened *(table II)*. The rank order of potency of individual compounds was found to be in good agreement with the prediction, $\mathbf{X_7AY_2}$ being the most active (hNK-3 binding affinity, K_i = 35.4 ± 10.4 nM) and $\mathbf{X_1AY_5}$ the least active (hNK-3 binding affinity, K_i = 513 ± 99.5 nM) amongst individual compounds prepared. It is worth noting that the best individual compound synthesised, $\mathbf{X_7AY_2}$, appears to

Table II. Binding affinities of individual compounds.



Individual compounds			
X		Y	hNK-3 K_i (nM)[a,b]
X_1		PhCO	46.2 ± 9.2
X_1		2,4-$(MeO)_2$PhCO	513 ± 99.5
X_2		PhCO	50.9 ± 9.2
X_7		PhCO	35.4 ± 10.4
X_7		$PhCH_2CO$	289 ± 68.0
X_7		$PhSO_2$	175 ± 50.2
		PhCO SR 142801[c,d]	1.2 ± 0.3

[a]Inhibition of [^{125}I]MePhe^7-NKB binding in hNK-3-CHO cell membranes. [b]mean ± SEM for three determinations (n = 3). [c]in house data. [d]SR 142801 is a single enantiomer [18].

have an hNK-3 binding affinity about 30 times lower than SR 142801 (hNK-3 binding affinity, K_i = 1.2 ± 0.3 nM) [18]. Since $\mathbf{X_7AY_2}$ differs from SR 142801 only for the basic head X, this SAR study outlines the importance of the X substitution in conferring high binding affinity to the 3,4-dichlorophenylpiperidine NK-3 receptor antagonists. Additional work is needed to understand the requirements of this portion of the molecule and X_7 could be a good starting point for further chemical modifications in order to obtain novel 3,4-dichlorophenylpiperidine NK-3 receptor antagonists with an improved biological profile compared to SR 142801 [19].

5. Conclusions

In conclusion, an indexed combinatorial library of analogues of SR 142801 was synthesised in solution and screened for its hNK-3 receptor binding affinity. Synthesis and biological evaluation of some individual compounds proved the reliability of this very simple, accessible and rather under-utilised technique and allowed us to gain information on SAR requirements of 3,4-dichlorophenylpiperidine NK-3 receptor antagonists, significantly decreasing the number of reactions and samples to test.

6. Experimental protocols

6.1. Chemistry

Melting points were determined with a Büchi 530 hot stage apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker ARX 300 spectrometer at 303 K unless otherwise indicated. Chemical shifts were recorded in parts per million (δ) downfield from tetramethylsilane (TMS); NMR spectral data are reported as a list. IR spectra were recorded in Nujol mull or neat on sodium chloride disks or in KBr with a Perkin-Elmer 1420 spectrophotometer; mass spectra were obtained on a Finnigan MAT TSQ-700 spectrometer. Silica gel used for flash column chromatography was Kiesegel 60 (230–400 mesh) (E. Merck AG, Darmstadt, Germany). All evaporations were performed at reduced pressure. Combustion elemental analyses were performed by Redox s.*n*.c., Milan, Italy and analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values. All reagents utilised in *figures 3–5* are commercially available compounds and were used without further purification. Racemic aminol **1** and SR 142801 were synthesised according to Giardina et al. [12] and to Chen et al. [13].

6.2. N-tert-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-(3-iodopropyl)piperidine **2**

Racemic aminol **1** [12] (4.7 g, 16.3 mmol) and di-*tert*-butyl dicarbonate (4.3 g, 19.6 mmol) were dissolved, under nitrogen atmosphere, in dry CH_2Cl_2 (75 mL). The reaction mixture was stirred at room temperature for 8 h and left standing overnight. The solvent was evaporated to dryness and the crude material was purified by flash column chromatography, eluting with a mixture of

CH_2Cl_2/MeOH 98:2, to obtain 5.4 g (85%) of N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-(3-hydroxypropyl)piperidine as a pale yellow oil. This compound (5.4 g, 13.9 mmol) was dissolved in CH_2Cl_2 (50 mL); triethylamine (TEA) (2.2 mL, 15.8 mmol) was added and the solution was cooled to 0 °C. Methanesulfonyl chloride (1.2 mL, 15.4 mmol), dissolved in CH_2Cl_2 (7 mL), was added dropwise and the reaction mixture was allowed to reach room temperature and stirred for additional 2 h. The reaction was quenched with water and the extracted organic layer was washed with 10% citric acid, 5% $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and evaporated to dryness to yield 6.0 g (93%) of N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-(3-methanesulfonyloxypropyl)piperidine as a pale yellow oil. This compound (6.0 g, 12.9 mmol) was dissolved in acetone (120 mL) and KI (3.5 g, 20.9 mmol) was added to the solution which was refluxed for 16 h. Insoluble material was filtered off and the filtrate was evaporated to dryness to yield 6.4 g (100%) of the title compound as a red oil, which was used in the following reactions without further purification. IR (neat) 2 940, 2 885, 1 692, 1 470, 1 428 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.44 (d, 1H), 7.38 (d, 1H), 7.17 (dd, 1H), 3.91 (d, 1H), 3.58–3.47 (m, 1H), 3.31 (d, 1H), 3.31–3.20 (m, 1H), 3.01 (t, 2H), 2.07–1.98 (m, 1H), 1.80–1.40 (m, 7H), 1.49 (s, 9H).

6.3. General procedure for the synthesis of intermediate compounds $\mathbf{X_1A}$–$\mathbf{X_7A}$

K_2CO_3 (0.303 g, 2.2 mmol) and the appropriate amine (1.1 eq.) were added to a solution of N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-(3-iodopropyl) piperidine **2** (0.99 g, 1.98 mmol) in CH_3CN (7 mL). The reaction mixture was heated to 80 °C under magnetic stirring for 18 h; then it was filtered and the filtrate was evaporated to dryness. The crude solid obtained was dissolved in EtOAc and washed with H_2O. The organic layer was dried over $MgSO_4$, filtered and evaporated to dryness to yield the title compounds which were purified by flash column chromatography. Yields after chromatography were in the range: 75–96%. Spectroscopic data for compounds $\mathbf{X_1A}$–$\mathbf{X_7A}$ are reported below.

6.3.1. N-tert-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperidin-1-yl)propyl]piperidine $\mathbf{X_1A}$

IR (Nujol) 2 926, 1 690, 1 674, 1 604, 1 554 cm^{-1}. ^{1}H-NMR ($CDCl_3$–343 K) δ 7.47 (d, 1H), 7.37 (d, 1H), 7.30–7.14 (m, 6H), 4.00 (d, 1H), 3.56 (dt, 1H), 3.26 (d, 1H), 3.26–3.18 (m, 1H), 2.90–2.83 (m, 2H), 2.50–2.40 (m, 1H), 2.21 (t, 2H), 2.09–1.91 (m, 3H), 1.80–1.13 (m, 11H), 1.49 (s, 9H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 531 (MH^+).

6.3.2. N-tert-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperazin-1-yl)propyl]piperidine $\mathbf{X_2A}$

IR (KBr) 2 942, 2 816, 1 692, 1 602, 1 554, 1 502 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.44 (d, 1H), 7.38 (d, 1H), 7.24 (dd, 2H), 7.18 (d br, 1H), 6.90 (d, 2H), 6.84 (dd, 1H), 4.05 (m br, 1H), 3.60 (m, br, 1H), 3.20–3.10 (m, 6H), 2.43 (m, 4H), 2.21 (t, 2H), 2.05 (m br, 1H), 1.75–1.10 (m, 7H), 1.46 (s, 9H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 532 (MH^+), 554 (MNa^+).

6.3.3. N-tert-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(4-isopropylpiperazin-1-yl)propyl]piperidine $\mathbf{X_3A}$

IR (neat) 2 942, 2 812, 1 690, 1 554, 1 470 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.42 (d, 1H), 7.31 (d, 1H), 7.15 (d br, 1H), 4.05 (m br, 1H), 3.60 (m br, 1H), 3.13 (d, 1H), 3.12 (m, 1H), 2.64 (m, 1H), 2.50 (m, 4H), 2.35 (m, 4H), 2.20 (t, 2H), 2.05 (m, 1H), 1.70–1.00 (m, 7H), 1.45 (s, 9H), 1.00 (d, 6H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 498 (MH^+).

6.3.4. N-tert-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(morpholin-4-yl)propyl]piperidine $\mathbf{X_4A}$

IR (neat) 2 944, 2 858, 2 810, 1 690, 1 590, 1 554 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.44 (d, 1H), 7.35 (d, 1H), 7.14 (d br, 1H), 4.02 (m br, 1H), 3.63 (m br, 4H), 3.59 (m br, 1H), 3.13 (d, 1H), 3.12 (m, 1H), 2.30 (m, 4H), 2.18 (t, 2H), 2.05 (m br, 1H), 1.71–1.00 (m, 7H), 1.46 (s, 9H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 457 (MH^+), 479 (MNa^+).

6.3.5. N-tert-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(1,2,3,4-tetrahydroisoquinolin-2-yl)propyl]piperidine $\mathbf{X_5A}$

IR (neat) 2 932, 1 692, 1 554 cm^{-1}. ^{1}H-NMR ($CDCl_3$–343 K) δ 7.48 (d, 1H), 7.36 (d, 1H), 7.20 (dd, 1H), 7.10–7.03 (m, 3H), 6.94 (m, 1H), 4.01 (d, 1H), 3.58 (dt, 1H), 3.47 (s, 2H), 3.25 (d, 1H), 3.20 (ddd, 1H), 2.83 (t, 2H), 2.59 (t, 2H), 2.34 (t, 2H), 2.09–2.00 (m, 1H), 1.78–1.13 (m, 7H), 1.49 (s, 9H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 503 (MH^+).

6.3.6. N-tert-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(1-phenyl-1,3,8-triazaspiro[4,5]decan-4-on-8-yl)propyl] piperidine **X_6A**

IR (neat) 2 938, 2 856, 1 708, 1 690, 1 602, 1 502 cm^{-1}. ^{1}H-NMR ($CDCl_3$–343 K) δ 7.46 (d, 1H), 7.36 (d, 1H), 7.29 (dd, 2H), 7.19 (dd, 1H), 6.99–6.89 (m, 3H), 5.61 (s br, 1H), 4.70 (s, 2H), 3.90 (d br, 1H), 3.56 (m, 1H), 3.29–3.19 (m, 2H), 2.75–2.42 (m, 6H), 2.23 (m, 2H), 2.05 (m, 1H), 1.78–1.05 (m, 9H), 1.47 (s, 9H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 601 (MH^+), 545.

6.3.7. N-tert-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-{3-[4-(pyrrolidinocarbonylmethyl)piperazin-1-yl]propyl} piperidine **X_7A**

IR (neat) 2 944, 2 874, 2 812, 1 690, 1 642, 1 554 cm^{-1}. ^{1}H-NMR ($CDCl_3$–343 K) δ 7.44 (d, 1H), 7.36 (d, 1H), 7.18 (dd, 1H), 3.90 (d, 1H), 3.55 (dt, 1H), 3.54–3.43 (m, 4H), 3.23 (d, 1H), 3.20 (ddd, 1H), 3.09 (s, 2H), 2.55 (m, 4H), 2.36 (m, 4H), 2.19 (t, 2H), 2.08–1.99 (m, 1H), 1.98–1.80 (m, 4H), 1.78–1.00 (m, 7H), 1.49 (s, 9H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 567 (MH^+).

6.4. General procedure for the synthesis of library (set 1) compounds **$X_1AY_{1\text{-}7}$**–**$X_7AY_{1\text{-}7}$**

6.4.1 Synthesis of sublibrary **$X_1AY_{1\text{–}7}$**

The synthesis of sublibrary **$X_1AY_{1\text{–}7}$** is reported as an example of the synthesis of all sublibraries of the first set of the library. N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperidin-1-yl)propyl]piperidine **X_1A** (0.301 g, 0.55 mmol) was dissolved in dry CH_2Cl_2 (8 mL); TFA (2 mL) was added and the reaction mixture was stirred at room temperature overnight. After evaporation to dryness, the residue was taken up with CH_2Cl_2, washed with 5% $NaHCO_3$, dried over $MgSO_4$, filtered and evaporated to dryness, to obtain 3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperidin-1-yl)propyl]piperidine. This compound was dissolved in dry CH_2Cl_2 (10 mL), and K_2CO_3 (165.3 mg, 1.2 mmol) was added; then a solution of acetyl chloride (564 μL, 7.9 mmol), benzoyl chloride (918 μL, 7.9 mmol), phenylacetylchloride (1 047 μL, 7.9 mmol), dimethylacryloyl chloride (880 μL, 7.9 mmol), 2,4-dimethoxybenzoyl chloride (1 585 mg, 7.9 mmol), phenylisocianate (859 μL, 7.9 mmol) and phenylsulfonyl chloride (1 013 μL, 7.9 mmol), brought to 100 mL with dry CH_2Cl_2, was prepared and 1 mL of this solution was added to the reaction mixture, which was stirred at room temperature overnight. The reaction was quenched with H_2O (5 mL) and the extracted organic layer was washed with H_2O, dried over $MgSO_4$, filtered and evaporated to dryness to obtain 0.233 g (78%) of the title sublibrary. All seven compounds forming the sublibrary were characterised by LC/MS.

Purity of the seven sublibraries of set 1, calculated as the sum of the areas of the seven peaks of the LC/UV chromatogram attributed by LC/MS to the seven compounds forming each sublibrary, is reported below:

$X_1AY_{1\text{–}7}$ 98%; **$X_2AY_{1\text{–}7}$** 98%; **$X_3AY_{1\text{–}7}$** 95%; **$X_4AY_{1\text{–}7}$** 98%; **$X_5AY_{1\text{–}7}$** 97%; **$X_6AY_{1\text{–}7}$** 98%; **$X_7AY_{1\text{–}7}$** 97%.

6.5. General procedure for the synthesis of library (set 2) compounds **$X_{1\text{–}7}AY_1$**–**$X_{1\text{–}7}AY_7$**

6.5.1. Synthesis of sublibrary **$X_{1\text{–}7}AY_1$**

The synthesis of sublibrary **$X_{1\text{–}7}AY_1$** is reported as an example of the synthesis of all sublibraries of the second set of the library. N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperidin-1-yl)propyl] piperidine **X_1A** (116 mg, 0.217 mmol), N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperazin-1-yl)propyl]piperidine **X_2A** (116 mg, 0.217 mmol), N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(4-isopropylpiperazin-1-yl)propyl]piperidine **X_3A** (108 mg, 0.217 mmol), N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(morpholin-4-yl)propyl] piperidine **X_4A** (100 mg, 0.217 mmol), N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(1,2,3,4-tetrahydroisoquinolin-2-yl)propyl]piperidine **X_5A** (110 mg, 0.217 mmol), N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(1-phenyl-1,3,8-triazaspiro[4,5] decan-4-on-8-yl)propyl]piperidine **X_6A** (131 mg, 0.217 mmol) and N-*tert*-butoxycarbonyl-3-(3,4-dichlorophenyl)-3-{3-[4-(pyrrolidinocarbonylmethyl)piperazin-1-yl]propyl}piperidine **X_7A** (124 mg, 0.217 mmol) were dissolved, under magnetic stirring, in CH_2Cl_2 (36 mL); TFA (9 mL) was added and the reaction mixture was stirred at room temperature overnight. After evaporation to dryness, the crude material was taken up with CH_2Cl_2 and washed with 5% $NaHCO_3$, dried over $MgSO_4$, filtered and evaporated to dryness to yield 0.826 g of a mixture of Boc-deprotected **X_1A**–**X_7A**. This mixture was split into seven equal portions (one for each sublibrary) and K_2CO_3 (65 mg, 0.47 mmol) and acetyl chloride (15.49 μL, 0.217 mmol) were added to one of these portions, dissolved in CH_2Cl_2 (15 mL). After stirring overnight, the reaction was quenched with H_2O (5 mL), the organic layer was separated, washed with H_2O, dried over $MgSO_4$, filtered and evaporated to dryness to yield 75 mg (74%) of the title sublibrary. All seven compounds forming the sublibrary were characterised by LC/MS.

Purity of the seven sublibraries of set 2, calculated as the sum of the areas of the seven peaks of the LC/UV chromatogram attributed by LC/MS to the seven compounds forming each sublibrary, is reported below:

$\mathbf{X_{1-7}AY_1}$ 98%; $\mathbf{X_{1-7}AY_2}$ 94%; $\mathbf{X_{1-7}AY_3}$ 98%; $\mathbf{X_{1-7}AY_4}$ 83%; $\mathbf{X_{1-7}AY_5}$ 98%; $\mathbf{X_{1-7}AY_6}$ 94%; $\mathbf{X_{1-7}AY_7}$ 98%.

6.6. Synthesis of individual compounds reported in table II

6.6.1. Synthesis of N-benzoyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperidin-1-yl)propyl]piperidine hydrochloride $\mathbf{X_1AY_2}$

The synthesis of this compound is reported as a general procedure for the synthesis of all six individual compounds prepared. N-*tert*-Butoxycarbonyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperidin-1-yl)propyl] piperidine $\mathbf{X_1A}$ (0.23 g, 0.43 mmol) was dissolved in CH_2Cl_2 (8 mL); TFA (2.2 mL), dissolved in CH_2Cl_2 (2 mL), was added, and the reaction was stirred at room temperature overnight. After evaporation to dryness, the crude material was taken up with CH_2Cl_2 and washed with 5% $NaHCO_3$, dried over $MgSO_4$, filtered and evaporated to dryness to yield crude 3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperidin-1-yl)propyl] piperidine. Half of this crude material (≈ 0.21 mmol) was dissolved in CH_2Cl_2 (5 mL), K_2CO_3 (80 mg, 0.58 mmol) and benzoyl chloride (24.4 μL, 0.21 mmol) were added and the reaction was stirred at room temperature overnight and then quenched with H_2O (2 mL). The organic layer was separated, washed with H_2O, dried over $MgSO_4$, filtered and evaporated to dryness to yield 140 mg (61%) of N-benzoyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperidin-1-yl)propyl]piperidine as a yellow oil. This compound was transformed into its hydrochloride by dissolving in MeOH and treating with HCl/Et_2O. Evaporation to dryness and trituration with Et_2O afforded the title compound as a solid. IR (KBr) 3 425, 2 937, 2 532, 1 622, 1 444 cm^{-1}. ^{1}H-NMR ($CDCl_3$–333 K) δ 7.40–7.10 (m, 13H), 4.25 (m, 2H), 3.58–3.30 (m, 4H), 2.90–2.81 (m, 2H), 2.45 (m, 1H), 2.22 (t, 2H), 2.20–2.09 (m, 1H), 2.00–1.55 (m, 9H), 1.35–1.10 (m, 2H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 535 (MH^+). Anal. $C_{32}H_{37}Cl_3N_2O$ (C, H, N, Cl).

6.6.2. N-(2,4-dimethoxy)benzoyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperidin-1-yl)propyl]piperidine hydrochloride $\mathbf{X_1AY_5}$

IR (KBr) 3 447, 2 939, 2 668, 1 607, 1 467 cm^{-1}. ^{1}H-NMR ($CDCl_3$–333 K) δ 7.50–7.20 (m, 9H), 6.50–6.40 (m, 2H), 4.25 (m, 2H), 3.80 (s, 6H), 3.70–3.10 (m, 4H), 2.90–2.81 (m, 2H), 2.45 (m, 1H), 2.20 (m, 2H), 2.15–2.00 (m, 1H), 2.00–1.60 (m, 9H), 1.30–1.10 (m, 2H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 595 (MH^+). Anal. $C_{34}H_{41}Cl_3N_2O_3$ (C, H, N, Cl).

6.6.3. N-benzoyl-3-(3,4-dichlorophenyl)-3-[3-(4-phenylpiperazin-1-yl)propyl]piperidine dihydrochloride $\mathbf{X_2AY_2}$

IR (KBr) 3 447, 2 947, 2 403, 1 614, 1 440 cm^{-1}. ^{1}H-NMR ($CDCl_3$–333 K) δ 7.42–7.20 (m, 10H), 6.90 (d, 2H), 6.80 (dd, 1H), 4.25 (m, 1H), 3.53 (d, 1H), 3.53–3.30 (m, 2H), 3.15 (m, 4H), 2.45 (m, 4H), 2.24 (t, 2H), 2.18–2.08 (m, 1H), 1.88 (ddd, 1H), 1.79–1.42 (m, 4H), 1.40–1.10 (m, 2H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 536 (MH^+). Anal. $C_{31}H_{37}Cl_4N_3O$ (C, H, N, Cl).

6.6.4. N-benzoyl-3-(3,4-dichlorophenyl)-3-{3-[4-(pyrrolidinocarbonylmethyl)piperazin-1-yl]propyl}piperidine dihydrochloride $\mathbf{X_7AY_2}$

IR (KBr) 3 436, 2 954, 2 437, 1 662, 1 618, 1 438 cm^{-1}. ^{1}H-NMR ($CDCl_3$–333K) δ 7.40–7.10 (m, 8H), 4.40 (m, 1H), 3.52–3.30 (m, 7H), 3.09 (s, 2H), 2.51 (m, 4H), 2.31 (m, 4H), 2.18 (t, 2H), 2.15–2.05 (m, 1H), 1.95–1.80 (m, 5H), 1.70–1.40 (m, 4H), 1.35–1.05 (m, 2H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 571 (MH^+). Anal. $C_{31}H_{42}Cl_4N_4O_2$ (C, H, N, Cl).

6.6.5. N-phenylacetyl-3-(3,4-dichlorophenyl)-3-{3-[4-(pyrrolidinocarbonylmethyl)piperazin-1-yl]propyl}piperidine dihydrochloride $\mathbf{X_7AY_3}$

IR (KBr) 3 435, 2 954, 2 546, 1 653, 1 630, 1 451 cm^{-1}. ^{1}H-NMR ($CDCl_3$–333 K) δ 7.40–7.05 (m, 8H), 4.40 (m, 1H), 3.70 (s, 2H), 3.49 (m, 5H), 3.23 (m, 2H), 3.09 (s, 2H), 2.52 (m, 4H), 2.31 (m, 4H), 2.11 (t, 2H), 2.10–2.00 (m, 1H), 1.98–1.75 (m, 4H), 1.75–1.00 (m, 7H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 585 (MH^+). Anal. $C_{32}H_{44}Cl_4N_4O_2$ (C, H, N, Cl).

6.6.6. N-benzenesulfonyl-3-(3,4-dichlorophenyl)-3-{3-[4-(pyrrolidinocarbonylmethyl)piperazin-1-yl]propyl} piperidine dihydrochloride $\mathbf{X_7AY_7}$

IR (KBr) 3 427, 2 943, 2 582, 1 658, 1 469, 1 340, 1 162 cm^{-1}. ^{1}H-NMR ($CDCl_3$–333 K) δ 7.78 (m, 2H), 7.55 (m, 3H), 7.40 (m, 2H), 7.21 (m, 1H), 3.47 (m, 5H), 3.10 (m, 1H), 3.10 (s, 2H), 2.79 (m, 2H), 2.51 (m, 4H), 2.32 (m, 4H), 2.17 (t, 2H), 2.00–1.50 (m, 10H), 1.30–1.10 (m, 2H). ESI-MS (positive, solvent: methanol, spray 4.5 keV, skimmer: 60 eV, capillary 220 °C) *m/z* 607 (MH^+). Anal. $C_{30}H_{42}Cl_4N_4O_3S$ (C, H, N, Cl).

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[18] Being SR 142801 a single enantiomer about 2-fold more potent than the corresponding racemate (in-house data), the real difference in binding affinity for the hNK-3 receptor between X7AY2 and (±) SR 142801 is about 15-fold.

[19] In house data demonstrate that SR 142801 has high systemic plasma clearance (36.9 ± 10.6 mL/min/kg) and low oral bioavailability (11 ± 2%) in rats; in addition, it showed significant interactions with some isoenzymes of P450 family (specifically, P2D6 and P3A4).