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Identification of a new series of non-peptidic NK₃ receptor antagonists

Karsten Juhl^{a,*}, Tore Hansen^{a,†}, Jan Kehler^a, Nikolay A. Khanzhin^a, Morten B. Nørgaard^a, Thomas Ruhland^a, Dorrit B. Larsen^a, Klaus G. Jensen^a, Björn Steiniger-Brach^b, Søren M. Nielsen^b, Klaus B. Simonsen^a

^a Discovery Chemistry and DMPK, H. Lundbeck A/S, Ottiliavej 9, DK 2500 Valby, Denmark

^b Discovery Pharmacology Research, H. Lundbeck, Ottiliavej 9, DK 2500 Valby, Denmark

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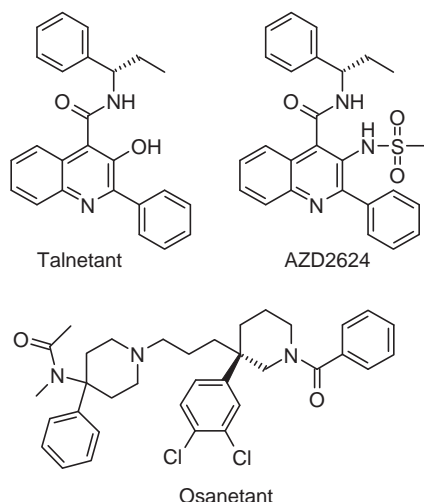
Senktide induced hypoactivity

ABSTRACT

The identification and structure–activity relationships of 2-aminomethyl-1-aryl cyclopropane carboxamides as novel NK₃ receptor antagonists are reported. The compound series was optimized to give analogues with low nanomolar binding to the NK₃ receptor and brain exposure, leading to activity in vivo in the senktide-induced hypoactivity model in gerbils.

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The NK₃ receptor is one of three known neurokinin receptors. The NK₃ receptor is expressed both centrally and in some peripheral organs.^{1–3} The expression in the central nervous system (CNS) and the modulatory effect on various monoaminergic transmitter systems⁴ make NK₃ receptor antagonists interesting as potential therapeutic agents for the treatment of CNS diseases such as schizophrenia,⁵ Parkinsons disease,⁶ panic attacks and major depressive disorder.⁷



Three non-peptide based NK₃ receptor antagonists have entered the clinical trials: Talnetant and AZD2624 from a common 2-aryl-4-carboxamide-quinoline core structure and Osanetant from a piperidine amide series. All three compounds have been withdrawn from further clinical development.

As a result of an NK₃-focused screening campaign including compounds from a previous neurokinin project, we identified a new structural class of non-peptidic NK₃ receptor antagonists. Specifically, we found lead compound **1a** and structurally related analogues that derived from a tri-substituted cyclopropane scaffold. This scaffold is capable of arranging three distinct functionalities (a carboxamide, an aryl and an aminomethyl) in a well-defined orientation. Compound **1a** displays high affinity for the NK₃ receptor (K_i = 5.9 nM) and selectivity towards the NK₁ and NK₂ receptors (K_i = 130 nM and K_i = 88 nM, respectively).⁸

The synthesis of **1a** (and derivatives) commenced from a diazo-transfer from *p*-acetamidobenzenesulfonyl azide (*p*-ABSA) to the phenyl acetic acid allyl ester **2** (see Fig. 1). The resulting diazo compound **3** was converted through intramolecular cyclopropanation to the racemic lactone **4**. The racemate was resolved either through chiral chromatography separation of the two enantiomers (1*S*,5*R*)-**4** and (1*R*,5*S*)-**4** or through a series of chemical modifications involving (a) lewis acid mediated lactone opening with (*R*)- α -methyl benzyl amine, (b) separation of the resulting two diastereomers **5a** and **5b**, (c) acid-mediated conversion of **5a** and **5b** to the enantiomeric pure lactones. Alternatively, analogues of (1*S*,5*R*)-**4** were synthesized from (*R*)-(-)-epichlorohydrin and the appropriate benzyl cyanide.⁹ The synthesis continued by opening the lactone ring with various benzyl amines mediated by AlCl₃. Conversion of the alcohol **6** to the bromide and subsequent treatment with an amine completed the synthesis of **1a**.

* Corresponding author. Tel.: +45 36434041; fax: +45 36438237.

E-mail address: kaju@lundbeck.com (K. Juhl).

[†] Present address: University of Oslo, Department of Chemistry, Sem Sælands vei 26, 0315 Oslo, Norway.

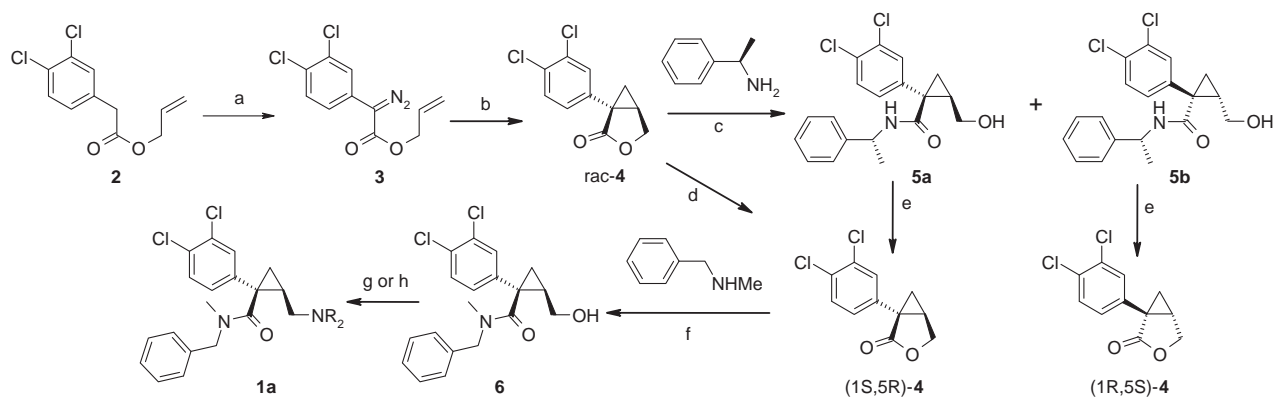


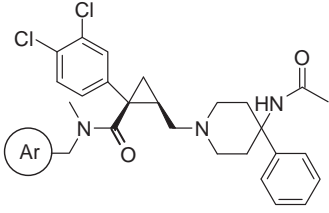
Figure 1. Reagents and conditions: (a) *p*-ABSA (1.2 equiv), DBU (1.3 equiv) MeCN, 0 °C to rt, 20 h; (b) Rh₂(Oct)₄ (0.005 equiv), DCM, rt, 4 h; (c) AlCl₃ (2 equiv), DCE, column chromatography; (d) chiral HPLC separation; (e) H₂SO₄; (f) benzyl amine, AlCl₃ (2 equiv), DCE, 30 min; **4**, 60 min; (g) (i) PBr₃ (3 equiv), DCM, 3 h (ii) amine, Et(iPr)₃N, MeCN, 85 °C, 3 h or (i) IBX, 1,2-dichloroethane, (ii) amine, Na(CN)BH₃.

The variation of the carboxamide group was the most synthetically feasible approach to systematically analyze the structure–activity relationships (SAR) of this part of the scaffold. In parallel manner, we varied the *N*-methyl-*N*-benzyl amide moiety while keeping the remainder of the molecule unchanged as shown in Table 1. The results indicate that the NK₃ receptor affinities are not dramatically influenced by substituents on the *N*-benzyl moiety. A screen of other amides with substituents different from *N*-methyl and *N*-benzyl was also performed. However, this led to compounds

with lower affinity for the NK₃ receptor (data not shown). On the basis of these SAR, the *N*-benzyl-*N*-methyl and *N*-4-fluorobenzyl-*N*-methyl amide moieties were selected to be the optimal substitution patterns. Furthermore, the data in Table 1 indicate a relatively high degree of stereoselectivity in the cyclopropane scaffold with the (1*S*,2*R*) configuration giving superior affinity for the NK₃ receptor. Therefore the (1*S*,2*R*) isomer was chosen for further SAR studies.

The aryl group directly bond to the cyclopropane ring is most sterically restricted and we speculated that substituents in this part of the scaffold would likely effect receptor affinity. Therefore our next design iteration was devoted to vary the substitution pattern of this aromatic ring. (Table 2).¹⁰ In this study, it was found that the original 3,4-dichloro substitution pattern was superior. While compounds (e.g., **7**, **8** and **9**) with either neutral or electron-withdrawing substituents maintain NK₃ receptor affinity, more polar substituents such

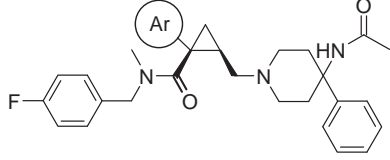
Table 1
SAR of *N*-benzyl amide analogues



#	Stereo-chemistry	Ar	hNK ₃ R K _i (nM) ^a
1a	(1 <i>S</i> , 2 <i>R</i>)	C ₆ H ₅	5.9
1b	(1 <i>R</i> , 2 <i>S</i>)	C ₆ H ₅	83
7	<i>rac</i>	2-Cl-C ₆ H ₄	20
8	<i>rac</i>	2-F-C ₆ H ₄	18
9	<i>rac</i>	2-Me-C ₆ H ₄	5.3
10	<i>rac</i>	2-OMe-C ₆ H ₄	14
11	<i>rac</i>	3-F-C ₆ H ₄	12
12	<i>rac</i>	3-Cl-C ₆ H ₄	24
13	<i>rac</i>	3-OMe-C ₆ H ₄	33
14	<i>rac</i>	3-CF ₃ -C ₆ H ₄	50
15	(1 <i>S</i> , 2 <i>R</i>)	4-F-C ₆ H ₄	5.8
16	<i>rac</i>	4-Cl-C ₆ H ₄	11
17	<i>rac</i>	4-Me-C ₆ H ₄	7.5
18	<i>rac</i>	4-OMe-C ₆ H ₄	15
19	<i>rac</i>	4-CF ₃ -C ₆ H ₄	4.6
20	<i>rac</i>	4-SMe-C ₆ H ₄	28
21	<i>rac</i>	2,3-Cl ₂ -C ₆ H ₃	24
22	<i>rac</i>	2,4-Cl ₂ -C ₆ H ₃	16
23	<i>rac</i>	3,4-Cl ₂ -C ₆ H ₃	13
24	<i>rac</i>	3,5-Cl ₂ -C ₆ H ₃	23
25	<i>rac</i>	2,4-OMe ₂ -C ₆ H ₃	5.9
26	<i>rac</i>	3,4-OMe ₂ -C ₆ H ₃	67
27	<i>rac</i>	3,5-OMe ₂ -C ₆ H ₃	32
28	<i>rac</i>	2-Thiophenyl	70
29	<i>rac</i>	3-Thiophenyl	14

^a Displacement of ³H-labelled Lu AE93103 from the cloned hNK₃ receptor expressed in BHK cells. Data are means (*n* = 2 or more).

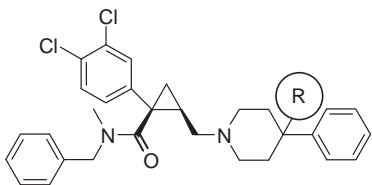
Table 2
SAR of aromatic ring on cyclopropane core



#	Ar	hNK ₃ R K _i (nM) ^a
30	4-F-C ₆ H ₄	67
31	4-Cl-C ₆ H ₄	36
32	4-Me-C ₆ H ₄	37
33	4-OMe-C ₆ H ₄	350
34	4-CF ₃ -C ₆ H ₄	1000
35	4-CN-C ₆ H ₄	270
36	3-F-C ₆ H ₄	140
37	3-Cl-C ₆ H ₄	32
38	3-Me-C ₆ H ₄	40
39	3-OMe-C ₆ H ₄	56
40	3-CF ₃ -C ₆ H ₄	210
41	3-CN-C ₆ H ₄	1900
42	3,4-F ₂ -C ₆ H ₃	47
43	3,4-Cl ₂ -C ₆ H ₃	5.8
44	3,5-Cl ₂ -C ₆ H ₃	90
45	3,4-Me ₂ -C ₆ H ₃	7.3
46	3,4-OCH ₂ O-C ₆ H ₃	140
47	2-Thiophenyl	93
48	3-Thiophenyl	110

^a Displacement of ³H-labelled Lu AE93103 from the cloned hNK₃ receptor expressed in BHK cells. Data are means (*n* = 2 or more).

Table 3
SAR of phenyl piperidine analogues



#	R	hNK ₃ R K _i ^a (nM)	Intrinsic clearance ^b (L/min)	Caco-2 Papp ^c (× 10 ⁻⁶ cm/s)	Caco-2 efflux ratio ^c
1a		5.9	3.5	5.6	2.6
49		4.2	19	0.8	12
50		6.1	>20	0.3	17
51		1.5	6.3	0.4	>100
52		2.8	>20	NA	NA
53		5.6	6.8	0.6	42
54		12	2	0.6	85
55		3.6	6.9	1.3	13
56		7.8	15	0.1	120

^a Displacement of ³H-labelled Lu AE93103 from the cloned hNK₃ receptor expressed in BHK cells. Data are means (n = 2 or more).

^b See Ref. 9.

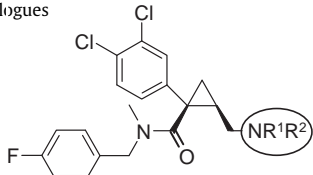
^c See Ref. 10.

as a cyano group, resulted in compounds (e.g., **35** and **41**) with lower affinity.

However, in contrast to binding, the ADME profile of compound **1a** was found to be poor. The intrinsic clearance in human liver microsomes¹¹ was high relative to the human liver-blood flow (1.4 L/min). Furthermore, a high efflux ratio and low permeability were found in the Caco-2 assay¹² (Table 3). These results are indications of poor systemic exposure and the results from the Caco-2 assay also imply poor blood–brain barrier penetration. The first attempt to improve the ADME profile of the lead series, was to vary the amide functionality located in the phenyl-piperidine-fragment. As shown in Table 3, several non-basic nitrogen containing functional groups were tolerated at the 4-position of the piperidine ring. However, only in one case the clearance was improved; a urea group rather than an acetamide group gave an intrinsic clearance of 2 L/min, but for this compound an even higher efflux ratio in the Caco-2 assay was found (**54**, Table 3).

We speculated that the efflux issue of the compound series might derive from the phenyl ring in the piperidine fragment. This prompted us to explore the SAR and ADME properties of these molecules with a selection of amines that did not carry aromatic substituents. As shown in Table 4, high affinity for the NK₃ receptor was maintained with a series of N-substituted piperazines. More

Table 4
SAR of piperazine analogues



#	NR ¹ R ²	hNK ₃ R K _i ^a (nM)	Intrinsic clearance ^b (L/min)	Caco-2 Papp ^c (× 10 ⁻⁶ cm/s)	Caco-2 efflux ratio ^c
57		3.3	16	35	1.5
58		3.7	4.3	NA	NA
59		1.8	12	27	1.3
60		17	8.5	28	1.1
61		7.3	10	2.9	4.2
62		12	5.6	5.7	3.5
63		7.6	>20	25	1.5
64		5.8	>20	NA	NA
65		8.7	15	NA	NA
66		1.9	12	NA	NA
67		150	0.9	5.4	2.7
68		140	0.9	11	1.5

^a Displacement of ³H-labelled Lu AE93103 from the cloned hNK₃ receptor expressed in BHK cells. Data are means (n = 2 or more).

^b See Ref. 9.

^c See Ref. 10.

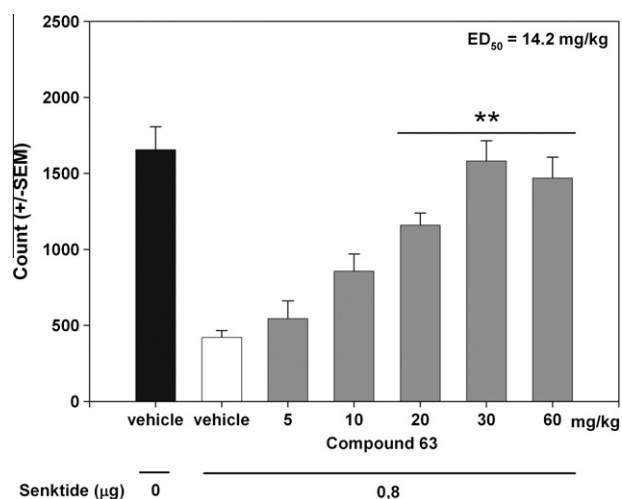


Figure 2. Senktide induced hypoactivity (gerbils). Compound **63** was administered sc, 60 min prior to senktide. Senktide was administered icv, directly before behavioural analysis was started. Data shown are the mean activity counts over a period of 30 min after senktide administration. **p < 0.01 versus vehicle/senktide treatment. n = 8–16 per group.

importantly, the Caco-2 permeability and efflux were significantly improved and the efflux ratio approached unity ($P_{app} > 10$ and efflux ratio < 2 are indicative of good permeability, and that no efflux is involved). However, the stability of the compounds in human liver microsomes was not improved.

With the aim to improve the metabolic stability, a broader variation of amine substituents NR_2 on the structure shown in Table 4 was also performed. The best results from that variation came from small aliphatic amines such as compounds **67** and **68** (Table 4). These compounds have a low intrinsic clearance and a relatively small Caco-2 efflux ratio. However, the affinity for the NK_3 receptor could not be maintained.

We selected compound **63** for in vivo PK studies in rat. Intrinsic clearance in rat microsomes was 210 mL/min and correlated well with high in vivo clearance of 108 mL/min/kg. Oral bioavailability was $< 5\%$ which is most likely explained by first-pass metabolism, since the Caco-2 data suggested high permeability and low efflux ratio. Compound **63** was also selected for in vivo efficacy in the senktide-induced hypoactivity assay in gerbils. The selection of this particular compound was based on its low Caco-2 efflux ratio and high permeability leading to expectation of good CNS penetration as well as on the high NK_3 receptor affinity. Compound **63** was also found to display selectivity towards the NK_1 and NK_2 receptors ($K_i = 340$ nM and $K_i = 75$ nM, respectively).⁸ In this model, central administration of the selective NK_3 receptor agonist senktide induces a reduction of explorative behavior (hypoactivity), which lasts approximately 30 min.¹³ The reversal of this functional read out of NK_3 receptor agonism indicates central mediated in vivo activity of the test compound. Compound **63** was administered sc in order to avoid expected first-pass metabolism, 60 min prior to senktide administration and fully reversed senktide-induced hypoactivity with an $ED_{50} = 14.2$ mg/kg (Fig. 2). Subsequent to the behavioral test, that is, 90 min after drug administration, four animals of each dose group were sacrificed for plasma and brain sampling. Compound **63** was found to enter well into the brain, with an average brain/plasma ratio of 1.9.

In this Letter, we have described the discovery of a series of tri-substituted cyclopropanes as novel non-peptidic NK_3 receptor antagonists. Extensive SAR analysis and targeted optimization of the ADME profile resulted in compounds with good blood–brain

barrier penetration as verified by in vivo activity in the senktide-induced hypoactivity model. However, to advance this compound series further, an improvement in metabolic stability needs to be obtained.

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11. The stability of compounds in liver microsomes is determined by the $t_{1/2}$ method, that is, the disappearance of 1 μ M drug over time was measured by LCMS. Using 0.5 mg/ml of microsomal protein (liver microsomes from several donors pooled to obtain an average enzyme content) in a NADPH generating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate and 0.4 U/ml glucose 6-phosphate dehydrogenase), 3.3 mM $MgCl_2$, 0.1 M potassium phosphate buffer (pH 7.4), in a total volume of 100 μ L, and stopping the incubations at time points 0, 5, 15, 30 and 60 min with 1:1 v/v acetonitrile. $t_{1/2}$ is subsequently scaled to the metabolic competence of a whole liver using 45 mg microsome/g liver, 20 g liver/kg and standard body weight 70 kg.
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