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Bioorganic & Medicinal Chemistry Letters 17 (2007) 890–894
Bioorganic & Medicinal Chemistry Letters

Design and synthesis of 3,5-disubstituted benzamide analogues of DNK333 as dual NK_1/NK_2 receptor probes

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Received 16 October 2006; revised 16 November 2006; accepted 20 November 2006
Available online 1 December 2006

Abstract—*N*-[(*R*,*R*)-(*E*)-(3,4-dichlorobenzyl)-3-(2-oxoazepan-3-yl)carbamoyl]allyl-*N*-methyl-3,5-bis(trifluoromethyl)benzamide (DNK333, **1b**) has been reported to be a potent and balanced dual neurokinin (tachykinin) receptor antagonist. A recent clinical trial using DNK333 has shown that it blocks the NKA-induced bronchoconstriction in patients with asthma. A series of six analogues **3**–**8** derived from modification of 3,5-bis(trifluoromethyl)benzamide moiety of DNK333 has been synthesized to serve as the dual NK_1/NK_2 receptor probes. The 3,5-dinitro substituted benzamide compound **3** was found to possess potent and balanced dual NK_1/NK_2 receptor antagonist activities ($pK_b = 8.4$ for the NK_1 receptors, $pK_b = 7.87$ for the NK_2 receptors) in the functional assay using guinea pig trachea. Furthermore, SAR analysis suggests that steric, electronic, and lipophilic characteristics of substituents in the benzamide region of DNK333 have a crucial effect on both the NK_1 and NK_2 receptor antagonist activities.

Neurokinins, also known as tachykinins, are a family of small peptides that are widely distributed throughout the central and peripheral nervous system, wherein they act as neurotransmitters and neuromodulators. The three tachykinin receptors, NK_1, NK_2, and NK_3, constitute a group of homologous receptors within the family of G protein-coupled receptors.[1,2] The neuropeptides substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) act as full agonists on the three neurokinin receptors, although they exhibit preferential binding to the NK_1, NK_2, and NK_3 receptors, respectively. These peptides have been proposed to be involved in a number of pathological conditions such as pain, arthritis, migraine, emesis, cancer, anxiety, depression, schizophrenia, and asthma.[3,4] There is mounting evidence that neurokinins play an important role in airway disease induction and progression via the activation of NK_1 and NK_2 receptors.[5–8] A number of studies suggest that neurokinin receptor antagonists, especially dual NK_1/NK_2 antagonists, may represent a new treatment option for asthma and other airway diseases, particularly since lung tissue from asthma patients has been shown to overexpress NK_1 and NK_2 receptors.[8,9] Within the lungs, release of neuropeptides substance P and neurokinin A from sensory nerves as well as from inflammatory cells results in an inflammatory response characterized by bronchoconstriction, microvascular leakage, and mucus hypersecretion, which are the typical pathological characteristics of asthma and chronic bronchitis. Substance P primarily causes microvascular leakage and mucus hypersecretion, and NKA predominantly produces hyper-responsiveness. These effects are mediated through specific receptors, namely NK_1 and NK_2 receptors. Thus, designed multiple ligand (DML) approach[10] has emerged as an attractive strategy for the treatment of asthma and other airway diseases by simultaneous and balanced blockade of NK_1 and NK_2 receptors.[11–18] DNK333 **1b** is an example of a designed multiple ligand derived from the structural modification of CGP49823 **1a**, an NK_1 receptor-selective antagonist (Fig. 1).[17,18] DNK333 (IC_{50} $NK_1 = 4.8$ nM, IC_{50} $NK_2 = 5.5$ nM in cloned human NK_1 and NK_2 receptors, respectively) has been shown to inhibit bronchoconstriction induced by NK_1 and NK_2 receptor agonists in guinea pigs and squirrel monkeys in preclinical investigations. In patients with mild asthma, DNK333 has been shown to block NKA-induced bronchoconstriction, thus further establishing the potential of dual NK_1/NK_2 receptor antagonists as anti-asthma agents.[19]

Keywords: Neurokinin receptor antagonists; Asthma.
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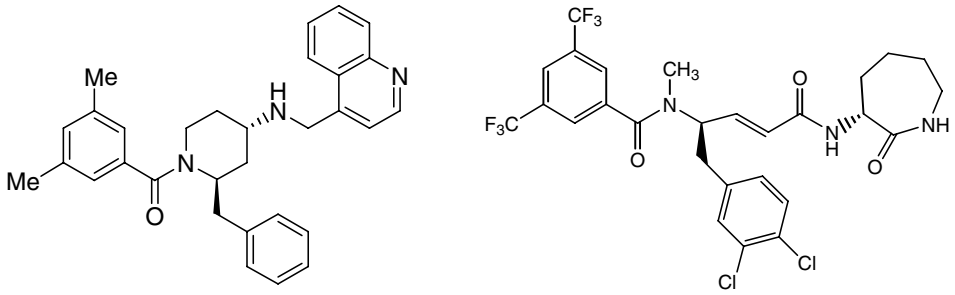
doi:10.1016/j.bmcl.2006.11.064



CGP49823 **1a**

IC_{50} NK_1 = 12 nM
IC_{50} NK_2 > 1000 nM

DNK333 **1b**

IC_{50} NK_1 = 4.8 nM
IC_{50} NK_2 > 5.5 nM

Figure 1. NK_1-selective ligand CGP49823 and dual NK_1/NK_2 ligand DNK333.

A series of compounds **3**–**8** derived from DNK333, which retained the structural characteristics important for NK_1 and NK_2 receptor antagonist activities, was designed to further explore the SAR and to obtain dual NK_1/NK_2 receptor probes.[17,18,20] Previous SAR studies on DNK333 **1b** indicate that *R*,*R*-stereochemistry is important. Furthermore, the 3,4-dichlorophenyl ring and the caprolactam ring were found to be optimal for balanced dual NK_1/NK_2 receptor antagonist activity. No SAR studies investigating modification of the 3,5-bis(trifluoromethyl) substituted phenyl ring have been reported. The aim of this project was to investigate modification of this region of DNK333, with the goal of obtaining potential electrophilic affinity labels for neurokinin receptors.

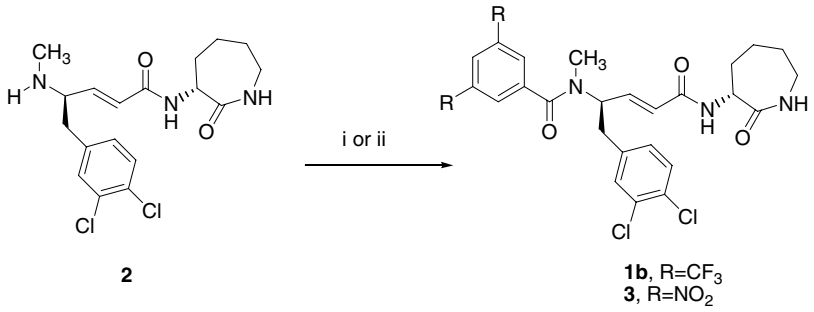
In the target compounds, the 3,5-bis(trifluoromethyl) substituents have been replaced by other groups. 3,5-Diisothiocyanate and 3,5-dibromoacetamide analogues (**5**, **8**) were designed to serve as the electrophilic affinity labels, and to cover a range of reactivities and chemical selectivities. These ligands have the potential to label NK_1, NK_2, or both NK_1 and NK_2 receptors depending on the presence of the nucleophilic groups on these receptors in the region where the benzamide group binds. Furthermore, presence of two electrophilic groups in each of these ligands increases the possibility of covalently labeling these receptors. The synthetic intermediates, 3,5-dinitro analogue **3** and 3,5-diamino analogue **4**, and the target compound 3,5-diacetamide analogue **7** were chosen to serve as the nonelectrophilic controls and to help understand the SAR of the benzamide region of DNK333.

Compound **2** (*R*, *R*-isomer) was prepared starting from commercially available Boc-D-3,4-dichlorophenylalanine in five steps according to the procedure reported by Gerspacher and coworkers.[20] Acylation of the secondary amine with either 3,5-bis(trifluoromethyl)benzoyl chloride or 3,5-dinitrobenzoyl chloride led to the 3,5-bis(trifluoromethyl) derivative **1b** (DNK333) or the 3,5-dinitro derivative **3**, respectively (Scheme 1).

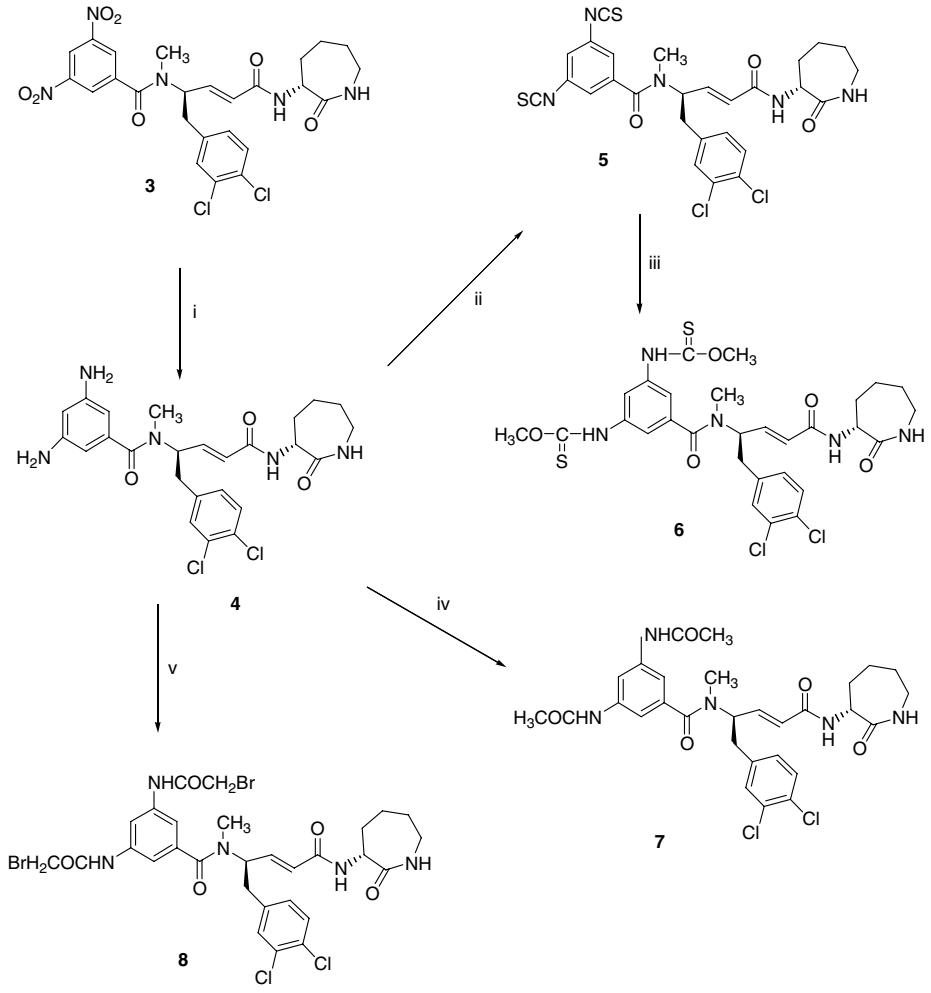
The 3,5-diaminobenzamide derivative **4** was prepared in high yields by reduction of the corresponding 3,5-dinitro derivative **3** with hydrazine monoformate and zinc dust at room temperature[21] which was then reacted with different reagents to provide target compounds **5**–**8** as shown in Scheme 2.[22] The 3,5-diisothiocyanate **5** was obtained by reaction of the 3,5-diamine with di-(2-pyridyl) thionocarbonate. Initial efforts to synthesize 3,5-diisothiocyanate derivative **5** resulted in the formation of 3,5-dithiocarbamate compound **6**. The 3,5-diacetamide **7** was synthesized from the amine by addition of acetic anhydride at room temperature. The 3,5-dibromoacetamide **8** was synthesized from the 3,5-diamine **4** by the controlled addition of bromoacetyl bromide in THF at 0 °C.

Results of the evaluation of the NK_1/NK_2 receptor antagonism and nonreversibility of the target compounds by functional assay using guinea pig trachea are presented in Table 1. The smooth muscle assays were conducted on the guinea pig trachea, which was activated by the NK_1-selective agonist ASM-SP (Ac-[Arg^6, Sar^9, Met $(O_2)^{11}$]-SP(6–11)) for NK_1 receptors and by the NK_2-selective agonist [β-Ala[8]]NKA(4–10) for NK_2 receptors.[23] The antagonist potencies of the test compounds are expressed as mean pK_b values.

The lead compound, DNK 333 **1b**, was found to exhibit potent and reversible NK_1 and NK_2 receptor antagonist activities in this assay, with pK_b values of 9.2 and 7.43, respectively. The 3,5-dinitro substituted derivative **3** exhibited a lower NK_1 receptor antagonist potency with a pK_b value of 8.4, while the NK_2 receptor activity was increased to a pK_b value of 7.87, thus providing a more balanced NK_1/NK_2 receptor antagonist activity. Since the 3,5-dinitro derivative **3** lacks the electrophilic moieties, it exhibited reversible antagonism at both NK_1 and NK_2 receptors (Table 1). Overall, 3,5-dinitro substituted derivative which exhibits similarity in size and shape to DNK333 (Fig. 2)[24] showed a potent and balanced dual NK_1/NK_2 receptor antagonist activity. Remaining compounds in the series did not exhibit antagonist activity at either NK_1 or NK_2 receptors, when studied at concentrations up to 1 μM. The calculated log *P* and molecular volumes of the target compounds were compared to analyze the biological results obtained. The lack of antagonist activity in 3,5-dithiocarbamate derivative **6**



Scheme 1. Reagents: (i) 3,5-bis(trifluoromethyl)benzoyl chloride, Et_3N, CH_2Cl_2; (ii) 3,5-dinitrobenzoyl chloride, toluene/satd $NaHCO_3$ solution.

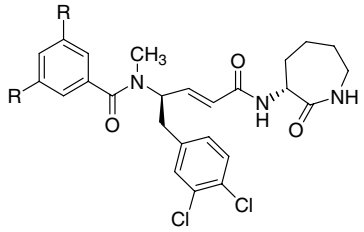


Scheme 2. Reagents and conditions: (i) Zn/NH_2–NH_2·HCOOH, MeOH, rt; (ii) di-(2-pyridyl)-thionocarbonate, CH_2Cl_2, rt; (iii) methanol in work up; (iv) acetic anhydride, $CHCl_3$, rt; (v) bromoacetyl bromide, THF, 0 °C.

and in 3,5-dibromoacetamide **8** might be attributed to the steric factors (Table 2, Fig. 2)[24], whereas relatively higher hydrophilicity of the 3,5-diamino substituted benzamide **4** and increased steric hindrance coupled with reduced hydrophobicity might explain low potency of 3,5-diacetamide **7** (Table 2). The reason for inactivity of 3,5-diisothiocyanate substituted benzamide **5** in the smooth muscle experiments is less clear. Subtle differences in the shape and electronic character of the isothiocyanate group compared to the trifluoromethyl and nitro groups together with its high lipophilicity might be playing a role.

In conclusion, a series of analogues **3**–**8** derived from the modification of 3,5 bis(trifluoromethyl)benzamide moiety of DNK333 **1b** has been successfully synthesized starting from commercially available optically active Boc-D-3,4-dichlorophenylalanine. For comparison and validation purposes, the lead compound, **1b** (DNK333), was also synthesized. Synthesis of the target

Table 1. NK_1 and NK_2 receptor antagonist potencies of substituted DNK333 analogues in guinea pig trachea



Compound	R	NK_1 receptor		NK_2 receptor	
		Maximum response in presence of antagonists (Control = 97.0 ± 1.2%)	pK_b[a,b]	Maximum response in presence of antagonists (Control = 98.3 ± 0.6%)	pK_b[b,c]
DNK333 (**1b**)	CF_3	89.4 ± 2.1	9.2 ± 0.4	97.0 ± 0.1	7.43 ± 0.4
3	NO_2	96.1 ± 2.9	8.4 ± 0.3	96.1 ± 1.3	7.87 ± 0.4

[a] pK_b are determined against ASM-SP (Ac-[Arg^6, Sar^9, Met $(O_2)^{11}$]-SP(6–11)) in isolated guinea pig trachea; values are means ± SEM from four experiments.
[b] pK_b is the negative log of K_b where K_b = [antagonist]/(dose ratio − 1).
[c] pK_b are determined against [β-Ala^8]NKA(4–10) in isolated guinea pig trachea.

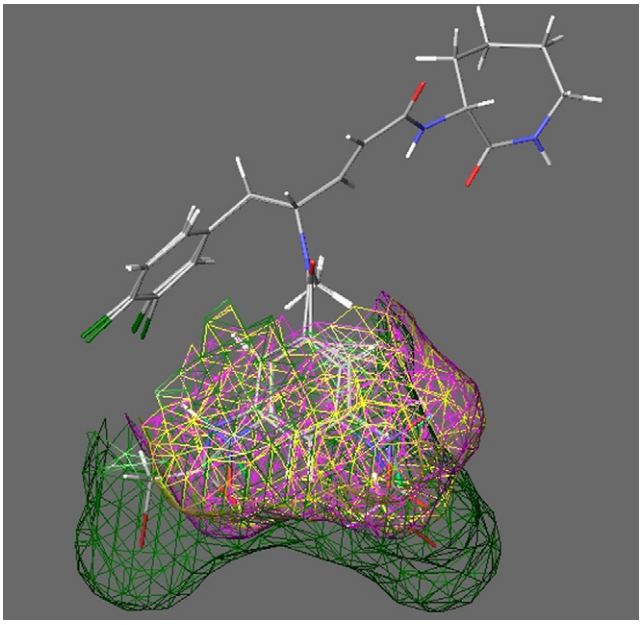


Figure 2. Superimposition of 3,5-dinitro analogue **3** (yellow) and 3,5-dibromoacetamide analogue **8** (green) using DNK333 (magenta) as a template in Maestro 7.5 environment.

Table 2. Partition coefficients and molecular volumes of target compounds

Compound	R	miLog P[a]	Molecular volume (Å^3)[a]
1b	CF_3	5.33	490.45
3	NO_2	3.45	474.53
4	NH_2	1.69	450.44
5	NCS	6.27	497.42
6	$NHC(S)OCH_3$	4.03	559.48
7	$NHCOCH_3$	1.97	523.76
8	$NHCOCH_2Br$	3.36	560.01

[a] Calculated octanol/water partition coefficient log P and molecular volumes were obtained by using Molinspiration software (http://www.molinspiration.com).

compounds consisted of synthesis of the chiral intermediate **2** followed by acylation to obtain 3,5-dinitro substituted benzamide **3**. The 3,5-diamino substituted derivative **4** was prepared by highly efficient reduction of the 3,5-dinitro derivative **3** using hydrazinium monoformate and zinc. The diamine derivative **4** was reacted with various reagents to provide the target compounds **5**–**8**. The smooth muscle data indicated that 3,5-dinitrobenzamide **3** exhibited a potent and balanced dual NK_1/NK_2 receptor antagonist activity with pK_b of 8.4 and 7.87 for the NK_1 and NK_2 receptors, respectively. The NK_2 antagonist potency of compound **3** was greater than that of the lead compound, while the NK_1 receptor antagonist potency was reduced. As expected, compound **3** exhibited reversible antagonism of both NK_1 and NK_2 receptors. Other compounds in the series were inactive in the smooth muscle assay using guinea pig trachea, suggesting strict steric, electronic, and lipophilic requirements for substituents in the benzamide region of DNK333.

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24. The compounds were constructed using fragment dictionary of Maestro 7.5 and geometry optimized using the Optimized Potentials for Liquid Simulations-all atom (OPLS-AA) force field with the steepest descent followed by truncated Newton conjugate gradient protocol. Partial charges were computed using the OPLS-AA force field. Molecular surfaces were computed using molecular surface option in the Maestro with a probe radius of 1.4 Å.