Letters

A Non-Peptide NK_1 Receptor Agonist Showing Subpicomolar Affinity

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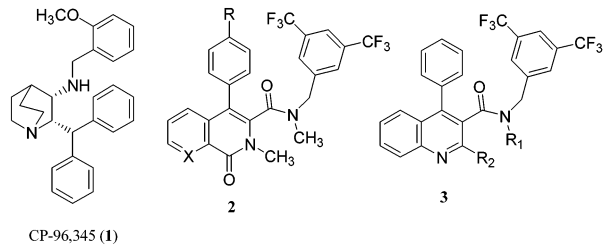
Abstract: 3-Quinolinecarboxamides have been synthesized and evaluated for their binding to the human NK_1 receptor. Several secondary amide derivatives show NK_1 receptor affinity in the picomolar range. The most active compound, hydroxymethylcarboxamide **3h** showing an IC_{50} value in the subpicomolar range, behaved as an agonist of NK_1 receptor in endothelial cell proliferation, inositol phosphate turnover, and NO-mediated cyclic GMP accumulation, thus proving it to be the first non-peptide NK_1 receptor agonist showing very high potency.

Introduction. Substance P (SP) is an undecapeptide member of the tachykinin family. The comparative analysis of the pharmacological properties of various tachykinins has provided the evidence for the existence of three receptor subtypes (termed NK_1, NK_2, and NK_3) showing different preferences in the interaction with their endogenous ligands. All the tachykinin receptors identified up to now belong to the family of the G-protein-coupled receptors (GPCRs) and are linked to the inositol phosphate signal transduction pathway.[1] By interacting with the NK_1 receptor, SP elicits a variety of biological responses including the transmission of pain and stress signals, smooth muscle contraction, the induction of neurogenic inflammation, endothelium-dependent vasodilation, and angiogenesis. Since 1991, the discovery[2] of the first selective non-peptide NK_1 antagonist, CP-96,345 (**1**, Chart 1), has catalyzed much research effort in this area within the framework of the pharmaceutical industry, and a large number of potent NK_1 antagonists have been developed in the past decade.[3] In 1995, researchers from Takeda discovered the potent antagonist properties of 4-phenylisoquinolinone and naphthyridinone derivatives **2**,[4] which are apparently unrelated to the most known NK_1 receptor antagonists. Despite the huge amount of work performed in this field and the large number of non-peptide NK_1 receptor antagonists described so far, the agonism for this receptors still appears to be confined to peptide compounds (since potent non-peptide agonists are, as far as we know, still lacking).

Chart 1



On the other hand, similar research efforts on the development of non-peptide angiotensin II (AT_1) or cholecystokinin-2 (CCK_2) receptor antagonists (AT_1 and CCK_2 receptors are GPCRs as NK_1) showed that slight structural variations of certain antagonists caused the intrinsic efficacy to shift toward agonism.[5] Our experience in the study of the interaction of arylpiperazine, tropane, and quinuclidine ligands with the 5-HT_3 receptor (a ligand-gated ion channel) suggests that the transformation of an antagonist into an agonist by means of slight structural modifications is confined to certain compounds only.[6] Thus, we started a program of structural manipulation of the original Takeda antagonists **2** leading to the synthesis (see Supporting Information) and the pharmacological characterization of 3-quinolinecarboxamides **3** related to our quinolinecarboxamide peripheral benzodiazepine receptor ligands.[7] These studies led to the discovery of the first non-peptide NK_1 receptor agonist endowed with subpicomolar affinity.

Results and Discussion. Compounds **3a**−**r** were tested for their activity in inhibiting the specific binding of [^{125}I]BH-SP (100 pM) to native human NK_1 receptor expressed in astrocytoma UC11MG cells compared with unlabeled SP and reference non-peptide antagonist L-703,606 [8]. The binding inhibition could be described by a single site for most of the tested compounds, while a second lower affinity component was necessary to fit the data of compounds **3c**,**n** more satisfactorily. The binding of [^{125}I] SP was confirmed to be due to NK_1 receptors because it was completely displaced by the non-peptide antagonist to the human NK_1 receptors L-703,606, with an IC_{50} of 1.6 nM. The results of the binding studies (Table 1) reveal that some secondary

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Table 1. Binding Affinities of **3a**−**r** to Human Endogenous NK_1 Receptor in UC11MG Cells

compd	R_1	R_2	binding IC_{50}, nM[a] (SEM, %)	binding %[b]
3a	H	H	>1000	
3b	CH_3	H	0.030(22)	83[c]
3c	H	CH_3	0.075(12)	78[d]
3d	CH_3	CH_3	3.1(6)	73[c]
3e	H	CH_2Cl	26(5)	57[c]
3f	H	CH_2Br	0.010(17)	71[c]
3g	CH_3	CH_2Br	52(17)	100
3h	H	CH_2OH	0.00010(13)	100
3i	CH_3	CH_2OH	16(8)	75[c]
3j	CH_3	CH_2OCH_3	11(21)	100
3k	H	CH_2NH_2	0.0017(25)	100
3l	CH_3	CH_2NH_2	9.0(13)	100
3m	H	$CH_2N(H)CH_3$	0.011(20)	82[c]
3n	H	$CH_2N(CH_3)_2$	0.22(7)	40[e]
3o	H	$CH_2N(CH_3)CH_2C_6H_5$	180(36)	71[c]
3p	H	$CH_2N(CH_2CH_2)_2NCH_3$	>1000	
3q	H	CH_2-phthalimido	>1000	
3r	H	$CH_2OSi(CH_3)_2C(CH_3)_3$	17(13)	100
SP			0.12(8)	100
L-703,606			1.6(9)	100

[a] Each value was calculated from at least three independent experiments performed in duplicate and represents the concentration giving half the maximum inhibition of [^{125}I]SP specific binding to human endogenous NK_1 receptor in UC 11 MG cells. [b] Percent of labeled SP total binding that is inhibited by the compound under scrutiny with the reported IC_{50} value. [c] These compounds failed to completely inhibit the specific [^{125}I]SP binding even at the maximal concentration tested. [d] An IC_{50} value of 3900 nM was calculated for the second binding component (22% of the [^{125}I]SP specific binding) of **3d**. [e] An IC_{50} value of 11 nM was calculated for the second binding component (60%) of **3n**.

amide derivatives (**3c**,**f**,**h**,**k**,**m**) show NK_1 receptor affinity in the picomolar range. In particular, hydroxymethyl derivative **3h** shows a subpicomolar IC_{50} value and is, to our knowledge, the most potent NK_1 receptor ligand so far described (Figure 1).

On the other hand, tertiary amides **3d**,**g**,**i**,**l** were much less potent than their secondary counterparts **3c**,**f**,**h**,**k**. One exception is represented by the simplest *N*-methyl-3-quinolinecarboxamide derivative **3b**, which is more than 30 000 times more potent than the corresponding secondary derivative **3a** (Chart 2). The apparent discrepancy can be easily explained in light of the different conformational preferences shown by **3a** with respect to those of the remaining compounds. The presence of the hydrogen substituents in position 2 of the quinoline nucleus and on the amide nitrogen could allow **3a** to populate conformations in which the amide hydrogen interacts with the π electron cloud of the pendent phenyl (N−H···π hydrogen bond) and the C═O group interacts with the hydrogen atom in position 2 (C−H···O hydrogen bond).[9] Conversely, such conformations are not populated in **3b** because of the steric repulsion between N−CH_3 and the pendent phenyl group. This hypothesis is strongly supported by the chemical shift of the proton in position 2 (9.32 ppm in **3a** and 8.87 ppm in **3b** in $CDCl_3$). Therefore, the bioactive conformation of these 3-quinolinecarboxamide derivatives **3** appears to be characterized by the out-of-plane orientation of the carbonyl dipole guaranteed by the presence of substituents in position 2 of the quinoline nucleus.

The structure−affinity relationship (SAFIR) analysis of these ligands reveals a dualism in the SAFIR trends

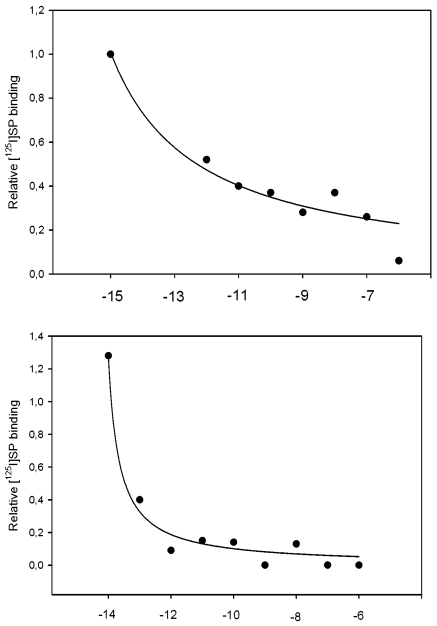
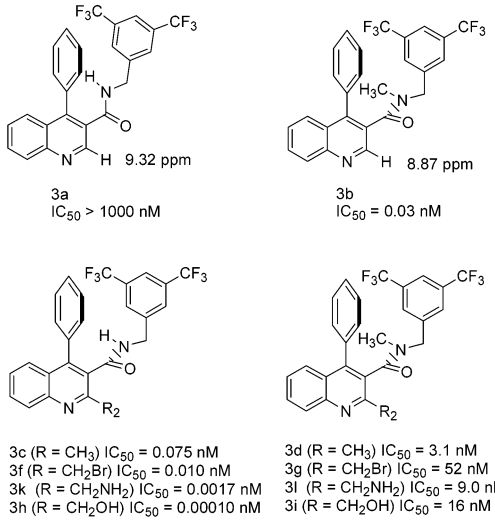


Figure 1. Curves of the inhibition of [^{125}I]SP (100 pM) binding to NK_1 receptors in UC11MG cells by various concentrations of **3h** (top) and **3k** (bottom). The values on the ordinates are relative bindings (referenced to the [^{125}I]SP binding in the absence of the compound, taken as 1.0), and those on the abscissas are the logarithms of the compound concentrations (log *C*). The single inhibition points are the averages of at least three different experiments, each run in duplicate. The continuous curves represent the best fits to the data.

Chart 2



between secondary and tertiary amides. In the secondary amide series, the replacement of a hydrogen atom of the methyl group in position 2 of **3c** with a bromine atom (**3f**), an amino group (**3k**), or a hydroxy group (**3h**) enhances the NK_1 receptor affinity from 1 to almost 3 orders of magnitude. The most notable enhancing effects involve compounds **3h**,**k** showing H-bond donor substituents, which could play a major role in the stabilization of the ligand−receptor complex.[10] Additional substituents on the amine nitrogen of **3k** progressively decrease the affinity as a consequence of the steric bulk increase (compare **3k** with **3m**−**p**), suggesting a limited dimension of the pocket accommodating the substituents in position 2. In the tertiary amide series, the 2-unsubstituted derivative **3b** (R_2 = H) is the most

potent compound and even the introduction of small groups [CH_3 (**3d**), CH_2OH (**3i**), CH_2OCH_3 (**3j**), CH_2NH_2 (**3l**), and CH_2Br (**3g**)] produces dramatic affinity decreases. Taken together, the SAFIR data suggest that secondary amide derivatives **3c**,**e**,**f**,**h**,**k**,**m**−**o**,**r** bind NK_1 receptor with different modalities with respect to tertiary amides **3b**,**d**,**g**,**i**,**j**,**l**. Probably, the N−H group of the secondary amides plays a key role in the interaction with the NK_1 receptor and affects the orientation of these amides in the binding crevice, while the binding of tertiary amides is governed by the strong directional interaction of the C═O group. Secondary amides could perhaps also stabilize a different conformation of the NK_1 receptor.

Pharmacological Characterization of 3h. Because it showed the highest affinity for NK_1 receptor, **3h** was selected for pharmacological characterization in cultured endothelial cells previously demonstrated to possess the NK_1 receptor.[11] Postcapillary endothelial cells isolated from bovine heart (CVEC) proliferate and migrate when stimulated with NK_1 selective agonists, thus leading to angiogenesis. NK_1 receptor mediated angiogenesis has been demonstrated to be linked to the nitric oxide synthase/cyclic GMP pathway.[11,12] We selected the microvascular endothelium as a target tissue to evaluate the properties of the compound for the potential therapeutic implications NK_1 ligands show on angiogenesis-dependent diseases. Compound **3h** was tested on CVEC proliferation, inositol phosphate turnover, and cyclic GMP accumulation. Thus, sparse and synchronized CVEC were exposed to increasing concentrations (0.01−100 nM) of **3h**.

Cell proliferation, measured after 48 h of incubation, was induced by the compound at all the concentrations tested. A bell-shaped dose−response curve was obtained, as previously reported for SP peptides,[13] without any cytotoxic effect at higher concentrations. The maximal effect was reached at 0.1 nM of **3h** and 1 nM SP (40% increase over basal response) (Figure 2A), while Sar9-SP-sulfone induced the maximal effect at 10 nM (162 ± 3 counted cells per well vs 120 ± 4 in basal condition, $P < 0.01$). To verify whether **3h** behaves as an NK_1 agonist, cell proliferation was evaluated in CVEC treated with the NK_1 receptor antagonist L-703,606[14] (1 nM). The presence of L-703,606 completely impaired the proliferative effect of **3h**, thus supporting the fact that **3h** is a NK_1 agonist (Figure 2B).

Tritiated inositol monophosphate (IP1) accumulation was measured by ion exchange chromatography in CVEC exposed for 15−60 min to increasing concentrations of substance P or **3h** (1−1000 nM).

Compound **3h** produced a concentration-dependent increase in IP1 levels, producing maximal stimulation at 1000 nM (Figure 3). IP1 accumulation was detected with SP only at 10 μM and after 1 h of incubation (38 ± 2% increase over basal response).

Compound **3h** and SP were then tested on cGMP production. As shown in Figure 4A, both compounds induced a concentration-dependent activation of guanylate cyclase. SP produced a characteristic bell-shaped response curve, while **3h** continued to increase cGMP accumulation at doses higher than 100 nM. Cyclic GMP levels increased 4 times in the presence of 100 nM **3h**,

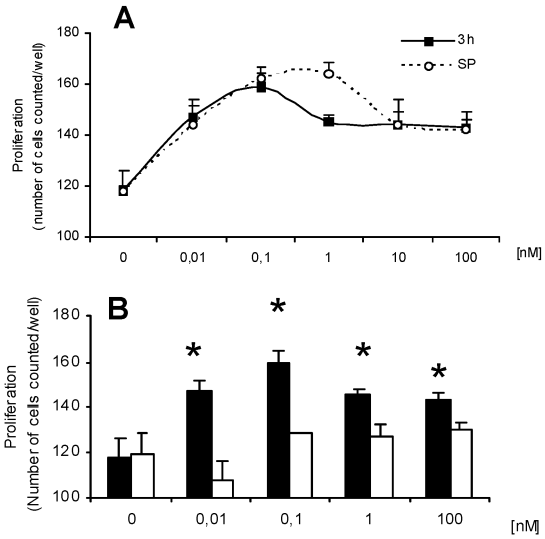


Figure 2. Effect of **3h** on CVEC proliferation: (A) comparison with SP, with cells that were incubated with increasing concentrations (0.01−100 nM) of **3h** and SP; (B) effect of NK_1 antagonist, with cells that were incubated with **3h** (0.01−100 nM) either in the absence (black bars) or in the presence of L-703,606 (1 nM) (white bars). Cellular proliferation was evaluated after 48 h of exposure to the test substances. Fixed and stained cells were microscopically counted, and the data were expressed as total number of cells counted per well. The values are the mean ± SEM of five experiments run in triplicate; $P < 0.05$ for **3h** alone vs basal response (Student's *t* test).

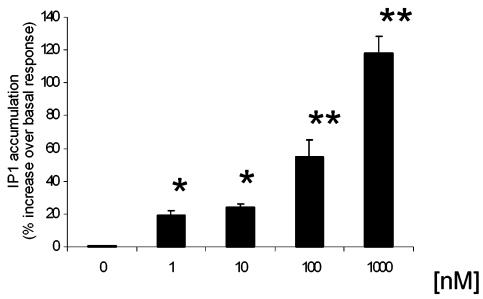


Figure 3. Effect of **3h** on IP1 levels in postcapillary endothelial cells. IP1 accumulation was measured by ion exchange chromatography in [^{3}H]myoinositol labeled CVEC treated with **3h** for 15 min. Data are expressed as % increase over basal activity. The values are the mean ± SEM of at least three determinations; $P < 0.05$ for **3h** alone vs basal control conditions (Student's *t* test).

while no increase was observed when CVEC was pretreated with L-703,606 or L-NMMA (L^W-nitromonomethyl-L-arginine, a NOS inhibitor)[15] (Figure 4B), supporting the involvement of NK_1 and NOS pathway activation. These data thus demonstrate that **3h** behaves like an NK_1 receptor agonist in endothelial cells, stimulating their proliferation and producing an intracellular accumulation of IP1 and NO-mediated cyclic GMP.

Conclusions. 3-Quinolinecarboxamides bearing the 3,5-bis(trifluoromethyl)benzyl group as the main substituent of the amide nitrogen and different substituents in position 2 of the quinoline have been synthesized and evaluated for their potential ability to inhibit the specific binding of [^{125}I]BH-SP to native human NK_1 receptor. This structure−affinity relationship study has led to the discovery of several potent NK_1 receptor ligands en-

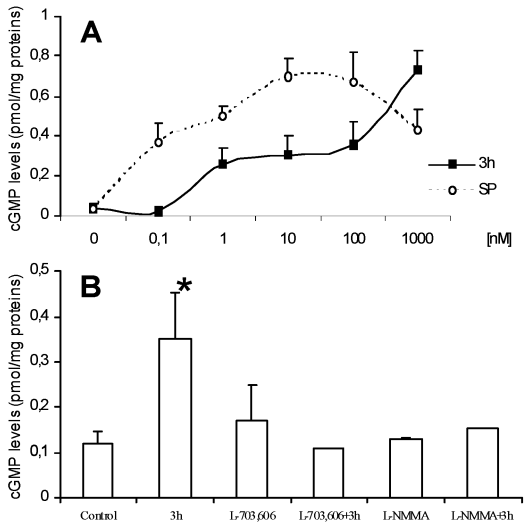


Figure 4. Effect of **3h** on cyclic GMP accumulation in CVEC: (A) effect of increasing concentrations of SP and **3h**, where cyclic GMP levels were measured by enzyme immunoassay in CVEC monolayers treated with stimuli for 10 min; (B) involvement of NK_1 and NOS activation in which L-703,-606 (1 nM) or L-NMMA (200 μM) was given 45 min before the addition of **3h** (100 nM). The data are expressed as pmol of cyclic GMP/mg of protein. The values are the mean ± SEM of at least three determinations; $P < 0.05$ for **3h** alone vs basal control conditions (Student's *t* test).

dowed with picomolar affinity. In particular, the hydroxymethylcarboxamide **3h** showed an IC_{50} value in the subpicomolar range and behaved as an agonist of NK_1 receptor in the endothelial cell proliferation, inositol phosphate turnover, and NO-mediated cyclic GMP accumulation. The compound displayed the highest potency (subnanomolar) in the functional assay on microvascular endothelium (cell proliferation), while it was considerably weaker in the intracellular signal assays. This finding, also observed in the case of SP, may reflect the poor association between these signals and cell growth. In conclusion, **3h** is the first nonpeptide NK_1 receptor agonist showing very high potency and theoretically useful as a tool in the study of NK_1 receptor function. Moreover, the discovery of nonpeptide tachykinin agonists seems to be of therapeutical relevance in angiogenesis-dependent diseases where endothelial cell proliferation is required to promote vascularization and healing of ischemic or damaged tissues.

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Supporting Information Available: Details of the synthesis and characterization of **3** (chemistry, NMR, MS, and pharmacology). This material is available free of charge via the Internet at http://pubs.acs.org.

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