



Non-peptide NK₁ receptor ligands based on the 4-phenylpyridine moiety

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ABSTRACT

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The quinoline nucleus of the previously described 4-phenylquinoline-3-carboxamides NK₁ receptor ligands **7** has been transformed into either substituted or azole—(i.e., triazole or tetrazole) fused pyridine moieties of compounds **9** and **10**, respectively, in order to obtain NK₁ receptor ligands showing lower molecular weight or higher hydrophilicity. The program of molecular manipulations produced NK₁ receptor ligands showing affinity in the nanomolar range. In particular, 4-methyl-1-piperazinyl derivative **9j** showed an IC₅₀ value of 4.8 nM and was proved to behave as a NK₁ antagonist blocking Sar⁹-SP-sulfone induced proliferation and migration of microvascular endothelial cells. Therefore, compound **9j** has been labeled with [¹¹C]CH₃I (*t*_{1/2} = 20.4 min, *β*⁺ = 99.8%) starting from the corresponding des-methyl precursor **9i** using with a radiochemical yield of about 10% (not decay corrected) and a specific radioactivity >1 Ci/μmol in order to be used as a radiotracer in next PET studies.

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1. Introduction

The neurokinin (NK) receptors belong to the family of seven-transmembrane G-protein-coupled receptors (GPCRs) and are classified into three subtypes: NK₁, NK₂ and NK₃. The undecapeptide member of the tachykinin family Substance P (SP) is the endogenous ligand for NK₁.¹ SP interacts with the NK₁ receptor eliciting a wide variety of biological effects. These include smooth muscle contraction, the transmission of pain and stress signals, the

nausea and vomiting (CINV). In 1995, the potent antagonist properties of 4-phenylisoquinolinone and naphthyridinone derivatives **3** were discovered by researchers from Takeda.⁶ The studies performed at Takeda produced TAK-637 (**4**),⁷ which was reported to be an orally bioavailable antagonist of NK₁ receptor in the intestinal smooth muscles and potentially useful in the treatment of functional bowel diseases such as irritable bowel syndrome (IBS).⁸ It is noteworthy that TAK-637 is apparently unrelated to the best known NK₁ receptor antagonists.

induction of neurogenic inflammation, endothelium-dependent vasodilation, and angiogenesis. The first selective non-peptide NK₁ receptor antagonist CP-96,345² (**1**, Fig. 1) was discovered by Pfizer medicinal chemists and stimulated a great interest in this area by the pharmaceutical industry. This research effort produced a large number of potent NK₁ receptor antagonists in the past decade.³ The control of both chemotherapy⁴ and post-surgery nausea and vomiting⁵ represents the major therapeutic applications of non-peptide NK₁ receptor antagonists. In fact, aprepitant (**2**) was approved by the FDA in 2003 under the brand name Emend[®] for the prevention of acute and delayed chemotherapy-induced

The structure of compound **4** was then simplified to obtain axially chiral 2,3,4,5-tetrahydro-6*H*-pyrido[2,3-*b*][1,5]oxazocin-6-one derivative **5**, which confirmed the importance of both *aR* axial chirality and the stacked conformation of the two phenyl moieties for receptor recognition.⁹

In 2006, researchers from Hoffmann-La Roche disclosed the results obtained within a large medicinal chemistry program devoted to the synthesis of novel NK₁ receptor antagonists and culminated in the discovery of netupitant (**6a**) and befetupitant (**6b**).¹⁰

Early work performed in our laboratory led to the development of a series of 4-phenylquinoline-3-carboxamide derivatives **7** (Fig. 2). Among them, secondary amide **7h** (R₁ = H, R₂ = CH₂OH) showed affinity in the picomolar range for native human NK₁ receptor expressed in astrocytoma UC11MG cells and behaved as an agonist of NK₁ receptor in endothelial cell proliferation, inositol

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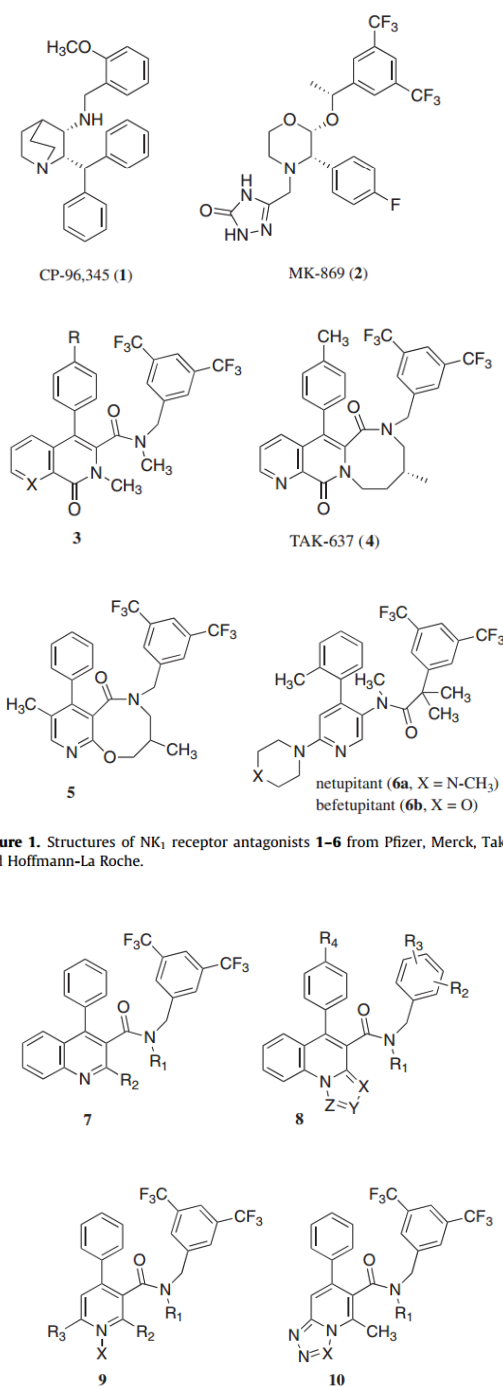
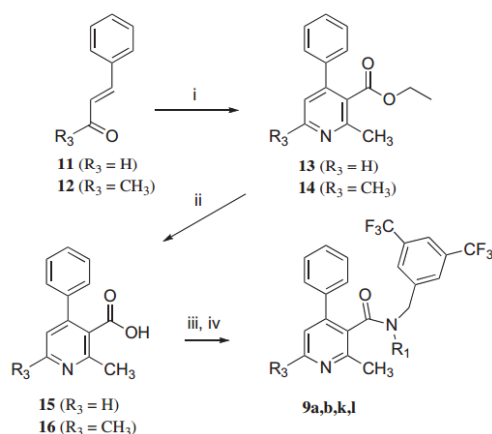
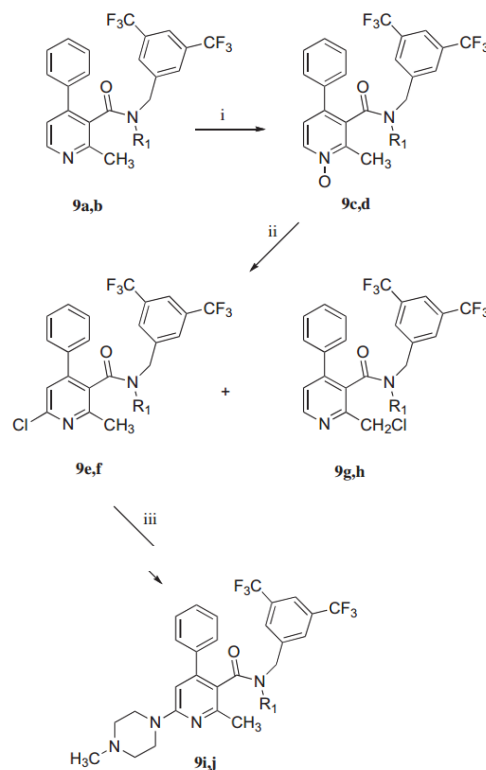


Figure 1. Structures of NK₁ receptor antagonists **1–6** from Pfizer, Merck, Takeda, and Hoffmann-La Roche.

phosphate turnover, and NO-mediated cyclic GMP accumulation, thus proving to be the first non-peptide NK₁ receptor agonist showing very high potency.¹¹ On the other hand, the corresponding tertiary amide derivative **7i** (R₁ = CH₃, R₂ = CH₂OH) showed nanomolar affinity for native human NK₁ receptor expressed in



Scheme 1. Reagents: (i) CH₃-C(NH₂)=CH-COOC₂H₅, piperidine, DMF; (ii) NaOH, H₂O, C₂H₅OH; (iii) SOCl₂; (iv) 3,5-(CF₃)₂C₆H₃CH₂NH₂, CH₂Cl₂, TEA, or 3,5-(CF₃)₂C₆H₃CH₂N(H)CH₃·HCl, CH₂Cl₂, TEA.



Scheme 2. Reagents: (i) *m*-CPBA, CHCl₃; (ii) POCl₃; (iii) *N*-methylpiperazine.

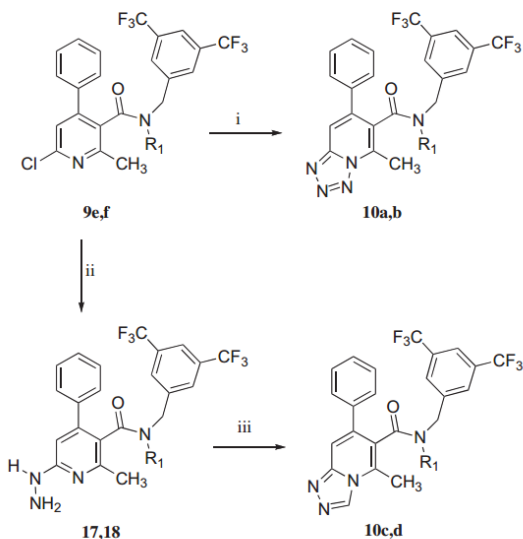
astrocytoma UC11MG cells.¹¹ Subsequently, the results obtained with compounds **7** led to the design of tricyclic carboxamide derivatives **8**, in which the substituent in position 2 of the quinoline nucleus of compounds **7** takes part in a five-membered heterocycle (azole moiety). Within the series of compounds **8**, the tertiary amide derivatives were found to show affinity in the nanomolar range for recombinant human NK₁ receptor, whereas the

corresponding secondary amides were about one order of magnitude less active.¹² Furthermore, the systematic structure variation suggested that electronic features of the tricyclic moiety play a role in modulating the interaction of these amide derivatives with their receptor.¹² As a further step of our large program of structural manipulations, quinoline nucleus of **7** has been transformed into substituted or azole—(i.e., triazole or tetrazole) fused pyridine moieties of compounds **9** and **10**, respectively, in order to obtain NK₁ receptor ligands showing lower molecular weight or higher hydrophilicity.

2. Results and discussion

2.1. Chemistry

The synthesis of 4-phenylpyridine-3-carboxamide derivatives **9** and **10** was carried out by means of the multistep procedure reported in Schemes 1–3. Condensation between ethyl 3-amino-crotonate and the appropriate unsaturated carbonyl derivative (**11** or **12**, Scheme 1) in DMF in the presence of piperidine gave 4-phenylnicotinate derivatives **13**¹³ and **14**,¹⁴ which were



Scheme 3. Reagents: (i) NaN₃, DMF; (ii) NH₂NH₂·H₂O, C₂H₅OH; (iii) HCOOH.

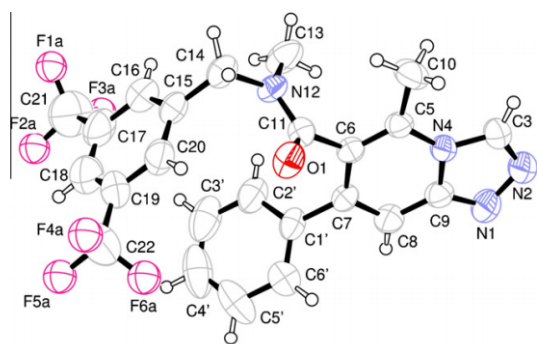
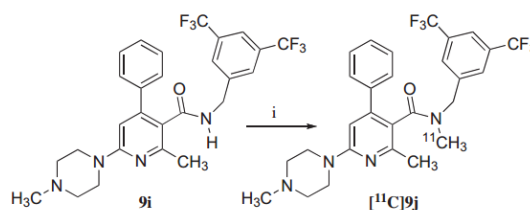


Figure 3. X-ray crystal structure of compound **10d**. Fluorine atoms bound to C21 and to C22 have site occupation factors of 0.61(3) and 0.53(3), respectively.



Scheme 4. Reagents: (i) [¹¹C]CH₃I, TBAOH, DMF.

hydrolyzed with 2 M NaOH to give acid derivatives **15** and **16**. Amidation of **15** and **16** under standard conditions provided target amides **9a,b,k,l**.

Compounds **9a** and **9b** were subjected to oxidation with *m*-CPBA (Scheme 2) to obtain the corresponding N-oxides **9c,d**, which were in turn reacted with phosphorus oxychloride to give chloroderivates **9e,f** and **9g,h**. Reaction of imidoyl chlorides **9e,f** with an excess of *N*-methylpiperazine at 130–140 °C gave the target *N*-methylpiperazino derivatives **9i,j** in good yield.

Moreover, the reaction of imidoyl chlorides **9e,f** with sodium azide gave target tetrazolo[1,5-*a*]pyridine derivatives **10a,b** (Scheme 3). On the other hand, the preparation of [1,2,4]triazolo[4,3-*a*]pyridine derivatives **10c,d** required the two-step procedure shown in Scheme 3, consisting in the reaction of **9e,f** with hydrazine hydrate to obtain hydrazino derivatives **17** and **18**, respectively, which were cyclized with formic acid to obtain the target compounds **10c,d**.

The structure of [1,2,4]triazolo[4,3-*a*]pyridine derivative **10d** was confirmed by X-ray crystallography (Fig. 3). Crystallographic conformation of **10d** shows the two phenyl rings arranged in a parallel, stacked conformation similar to that shown by (*aR*)-**4** in its crystallographic structure,⁷ but different from the corresponding one of secondary amide derivative **8a** (R₁ = R₄ = H, R₂ = 3-CF₃, R₃ = 5-CF₃, X = Y = Z = CH).¹²

2.2. Radiosynthesis

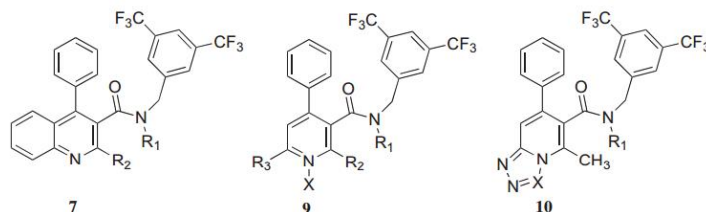
Compound [¹¹C]**9j** was synthesized by *N*-[¹¹C]methylation of the corresponding *des*-methyl precursor **9i** with [¹¹C]methyl iodide in the presence of tetrabutylammonium hydroxide (TBAOH) at 80 °C in dimethylformamide (DMF) for 4 min (Scheme 4), with a radioactivity incorporation yield of 65%.

The overall radiosynthesis, including ¹¹C-methylation, HPLC purification and radiopharmaceutical formulation for intravenous administration, was completed in an average time of 35 min from

the end of bombardment (EOB), with a radiochemical yield of about 10% (not decay corrected) and a specific radioactivity >1 Ci/μmol at the end of synthesis (EOS). In a typical experiment, starting from ca. 1 Ci of [¹¹C]CO₂, 80 mCi of [¹¹C]**9j** were obtained with a chemical and radiochemical purity of 95% and 100%, respectively. The identity of the final ¹¹C-radioligand was confirmed by coinjection of the final radiopharmaceutical with its cold standard **9j** on reverse-phase high-performance liquid chromatography (HPLC) (see Section 4).

2.3. Binding studies

Compounds **9a–l** and **10a–d** were tested for their activity in inhibiting the specific binding of [¹²⁵I]BH-SP to human recombinant NK₁ receptor stably expressed in CHO cells.¹⁵ Owing to the discrepancies observed in the structure–affinity relationships between compounds **8** and **7**,^{11,12} some of the previously-published 3-quinolinecarboxamide derivatives **7** were tested in the above test system. The results of the binding studies obtained with 'old

Table 1Binding affinities of compounds **7**, **9** and **10** for human recombinant NK₁ receptor stably expressed in CHO cells

Compd	R ₁	R ₂	R ₃	X	MW	IC ₅₀ (nM) ^a
7a	H	H			474.4	208 ± 13
7b	CH ₃	H			488.4	92 ± 5.0
7c	H	CH ₃			488.4	257 ± 45
7d	CH ₃	CH ₃			502.4	19 ± 1.2
7h	H	CH ₂ OH			504.4	227 ± 27
7i	CH ₃	CH ₂ OH			518.5	42 ± 5.2
9a	H	CH ₃	H		438.4	653 ± 202
9b	CH ₃	CH ₃	H		452.6	98 ± 0.20
9c	H	CH ₃	H	O	454.4	342 ± 47
9d	CH ₃	CH ₃	H	O	468.4	32 ± 2.4
9e	H	CH ₃	Cl		472.8	270 ± 32
9f	CH ₃	CH ₃	Cl		486.8	91 ± 8.0
9g	H	CH ₂ Cl	H		472.8	235 ± 47
9h	CH ₃	CH ₂ Cl	H		486.8	52 ± 8.3
9i	H	CH ₃	N-Methylpiperazino		536.5	53 ± 11
9j	CH ₃	CH ₃	N-Methylpiperazino		550.5	4.8 ± 1.1
9k	H	CH ₃	CH ₃		452.4	434 ± 51
9l	CH ₃	CH ₃	CH ₃		466.4	41 ± 6.7
10a	H			N	479.4	279 ± 26
10b	CH ₃			N	493.4	57 ± 18
10c	H			CH	478.4	698 ± 130
10d	CH ₃			CH	492.4	53 ± 15
SP						1.5 ± 0.10

^a Each value was calculated from at least three independent experiments performed in duplicate and represents the concentration giving half the maximum inhibition of [¹²⁵I]SP specific binding to human recombinant NK₁ receptor stably expressed in CHO cells.

compounds **7a–c,h** (Table 1) confirm the discrepancy between the results obtained with the two test systems. This result suggests the existence of differences between recombinant NK₁ receptors expressed in CHO and native human NK₁ receptors expressed in astrocytoma UC11MG cells. A survey of the literature showed the existence of differences between native and recombinant proteins in a number of different receptors. For instance, human recombinant 5-HT_{1A} receptors expressed in HEK293 cells exist in high and low agonist affinity states, whereas rat cortex 5-HT_{1A} receptors are primarily in the high agonist affinity state.¹⁶ Moreover, researchers from GlaxoSmithKline reported that NK₁ receptor affinity they observed for NKP-608 was intermediate between the corresponding value obtained by Vassout (pIC₅₀ = 7.9) in gerbil and that obtained by Rupniak (pIC₅₀ = 10.1) with recombinant human NK₁ receptors.¹⁷ It is noteworthy that secondary amide **7h** showing the highest difference behaves as an agonist. On the contrary, the corresponding tertiary amide **7i** shows similar affinities in the two different test systems. These observations suggest that the integration between binding studies and suitable functional studies is of primary importance in order to correctly characterize the interaction of these amide derivatives with NK₁ receptors.

Because of its determinant role in the interaction with NK₁ receptor binding site,^{6,7,12,18} 3,5-bis(trifluoromethyl)benzyl was kept unmodified in the whole series of compounds **9** and **10**, while the second amide substituent was alternatively a methyl group or a hydrogen atom in order to evaluate the effect of the transformation of the secondary amides in the corresponding tertiary ones. In agreement with the results obtained with compounds **8**, the amide methyl group of tertiary amides **9b,d,f,h,j,l** and **10b,d** enhances the

affinity of the secondary amides **9a,c,e,g,i,k** and **10a,c** to a variable extent (from 3 to 19 times). The structure–affinity relationships concerning the variation of the heterocyclic moiety, suggest that the human recombinant NK₁ receptor expressed in CHO cells is moderately sensitive to the small structure modulations shown by compounds **9a–h,k,l** and **10a–d**. In particular, the presence of the methyl group in position 2 of the quinoline moiety is beneficial for the NK₁ receptor affinity of tertiary amide **7d** (compare **7d** vs **7b**), while the deletion of its fused benzene ring produces a significant decrease in the receptor affinity (compare **7d** with **9b**). The introduction of a chlorine atom in place of the fused benzene ring as in compound **9f** does not succeed in restoring the original NK₁ receptor affinity (i.e., the one shown by **7d**). Similarly, the N-oxidation (as in compound **9d**), the introduction of a methyl group (**9l**) or the fusion of differentazole (e.g., triazole or tetrazole) moieties at *a*-edge of pyridine ring of compound **9d** leads only to small increases in the receptor affinity (compare **9d,l** and **10b,d** vs **9b**), whereas the introduction of a bulkier, basic *N*-methylpiperazine moiety, as in compound **9j**, produces a more significant increase in NK₁ receptor affinity so that the latter compound is the most interesting in the present small series. This result fully agrees with those described by Hoffmann and coworkers¹⁰ and

stimulated a deeper biological characterization of **9j**.

It should be noted that the above structure–affinity relationships are roughly (but not perfectly) paralleled in the secondary amide subseries **9a,c,e,g,i,k** and **10a,c**. A more evident dualism in the SAFIR trends between secondary and tertiary amides was observed with compounds **7** using native human NK₁ receptor expressed in astrocytomas UC11MG cells as test system (see Ref. 11).

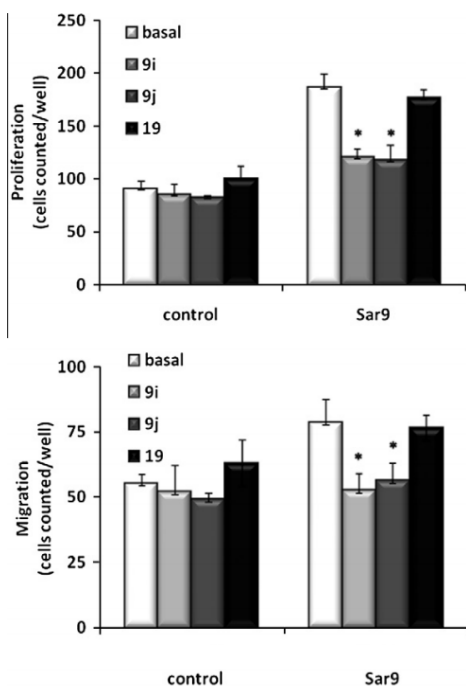


Figure 4. Proliferation (top) and migration (bottom) of CVEC in response to 10 nM Sar⁹-SP-sulfone (Sar9); effect of **9i**, **9j**, and **19**. Top panel: Sparse and synchronized endothelial cells were exposed to test substances for 48 h and the number of fixed and stained cells was microscopically counted. Bottom panel: Cell migration toward the NK₁ agonist in the absence/presence of test compounds was evaluated after 4 h of incubation. Data are means $\pm$ SEM of three experiments. * $P < 0.05$ versus agonist alone (Student's *t* test).

Table 2
Inhibition of Sar⁹-SP-sulfone induced proliferation and migration. Data are expressed as % inhibition of Sar⁹-SP-sulfone induced activity

Compound-dose	Proliferation (% inhibition)	Migration (% inhibition)
9i 1 μ M	76.3 $\pm$ 6	100 $\pm$ 1.4
100 nM	33.5 $\pm$ 4.5	100 $\pm$ 8
9j 1 μ M	79.7 $\pm$ 7	99 $\pm$ 3
100 nM	7.6 $\pm$ 3	100 $\pm$ 2
19 1 μ M	11 $\pm$ 6	9 $\pm$ 7
100 nM	0	0

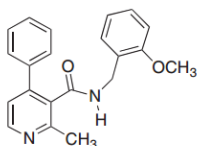


Figure 5. Structure of control substance **19** (see Section 4).

It is remarkable that crystallographic conformation of **10d** shows the two phenyl rings arranged in a parallel, stacked conformation, which appears to be quite similar to that shown by (*aR*)-**4** in its crystallographic structure⁷ and is believed to be an important prerequisite for the interaction with NK₁ receptor binding site. The crystallographic result suggests that tertiary amides **9b**, **d**, **f**, **h**, **j**, **l** and

10b, **d** are able to adopt the postulated pharmacophorically relevant arrangement.⁹ Thus, the comparison between the affinities shown by **10d** and **9j** suggests for *N*-methylpiperazine moiety an important role in the interaction with NK₁ receptor.

2.4. Pharmacological characterization

Since *N*-methylpiperazino derivatives **9i**, **j** exhibited the highest affinity for the NK₁ receptor, these compounds were selected for a preliminary pharmacological characterization in the same test system used in the study of parent quinoline agonist **7h**.¹¹ Post-capillary endothelial cells isolated from bovine heart (CVEC) proliferate and migrate when stimulated with NK₁ selective agonists, thus leading to angiogenesis.¹¹ Compounds **9i** and **9j** were tested on CVEC proliferation and migration and found to be lacking of any agonist activity (Fig. 4).

On the other hand, they completely inhibited the effects induced by NK₁ receptor agonist Sar⁹-SP-sulfone (Fig. 4 and Table 2), suggesting that the modification of **7h** leading to **9i**, **j** abolishes the agonist activity. In the same assays, the negative control substance **19** (Fig. 5, IC₅₀ > 1000 nM) was devoid of any activity.

While endothelial migration was completely impaired by 100 nM and 1 μ M drug concentrations of **9i** and **9j**, the compounds were able to reduce cell proliferation only at 1 μ M concentration (Table 2), suggesting that short term biological assay (4 h migration) is more powerful. In conclusion, our results document the activity of compounds **9i** and **9j** as NK₁ antagonists.

3. Conclusions

On the basis of the intriguing results obtained with compounds **7** and **8**, a small series of NK₁ receptor ligands based on the 4-phenylpyridine scaffold was designed, synthesized, and evaluated for its potential ability to inhibit the specific binding of [¹²⁵I]BH-SP to recombinant human NK₁ receptor in order to obtain compounds possessing lower molecular weight or higher hydrophilicity. The structure–affinity relationship study confirms the higher potency of tertiary amides with respect to the corresponding secondary ones and suggests that the human recombinant NK₁ receptor expressed in CHO cells is moderately sensitive to small structure modulations in the heterocyclic moiety of compounds **9a–h**, **k**, **l** and **10a–d**. However, the introduction of a *N*-methylpiperazine moiety in position 2 of pyridine ring produced compound **9j** showing a NK₁ receptor affinity in the low nanomolar range. This result stimulated a deeper biological characterization of **9j**, which was proved to behave as a NK₁ receptor antagonist blocking Sar⁹-SP-sulfone induced proliferation and migration of microvascular endothelial cells. Thus, compound **9j** was labeled with ¹¹C

(*t*_{1/2} = 20.4 min, β^+ = 99.8%) starting from the corresponding des-methyl precursor **9i** by using [¹¹C]CH₃I in the presence of tetrabutylammonium hydroxide in DMF with a radiochemical yield of about 10% (not decay corrected) and a specific radioactivity > 1 Ci/ μ mol in order to be used as a radiotracer in next PET studies.

4. Experimental section

4.1. Chemistry

All chemicals used were of reagent grade. Yields refer to purified products and are not optimized. Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Merck silica gel 60 (230–400 mesh) was used for column chromatography. Merck TLC plates, silica gel 60 F254 were used for TLC. ¹H NMR spectra were recorded at 200 MHz (Bruker