Bioorganic & Medicinal Chemistry 16 (2008) 6850–6859

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis, and structure–affinity relationship studies in NK_1 receptor ligands based on azole-fused quinolinecarboxamide moieties

Andrea Cappelli [a,*], Germano Giuliani [a], Maurizio Anzini [a], Daniela Riitano [b], Gianluca Giorgi [c], Salvatore Vomero [a]

[a] Dipartimento Farmaco Chimico Tecnologico and European Research Centre for Drug Discovery and Development, Università degli Studi di Siena, Via A. Moro, 53100 Siena, Italy
[b] Dipartimento di Farmacologia, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy
[c] Dipartimento di Chimica, Università degli Studi di Siena, Via A. Moro, 53100 Siena, Italy

ARTICLE INFO

Article history:
Received 28 December 2007
Revised 22 May 2008
Accepted 28 May 2008
Available online 12 June 2008

Keywords:
Neurokinin
NK1 receptor
Substance P
Synthesis
Amide derivatives

ABSTRACT

The substituent in position 2 of the quinoline nucleus of NK_1 receptor ligands **5** has been constrained into different five-membered heterocyclic moieties in order to obtain information on the binding site pocket interacting with this apparently critical portion of ligands **5**. This structure–affinity relationship study led to the discovery of novel tricyclic NK_1 receptor ligands **6** showing affinity in the nanomolar range to the sub-micromolar one. The systematic structure variation suggests that electronic features of the tricyclic moiety play a role in modulating the interaction of these amide derivatives with their receptor.

1. Introduction

The neurokinin (NK) receptors are members of the seven-transmembrane G-protein-coupled receptor family (GPCRs) and are divided into three subtypes: NK_1, NK_2, and NK_3. The endogenous ligand for NK_1 is the undecapeptide member of the tachykinin family, Substance P (SP).[1]

By interacting with the NK_1 receptor, SP elicits a wide 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 receptor antagonist CP-96,345 (**1**, Fig. 1) has catalyzed a great amount of research in this area on the part of the pharmaceutical industry, and a large number of potent NK_1 receptor antagonists have been developed in the past decade.[3] Non-peptide NK_1 receptor antagonists have proved to be highly effective in the control of both chemotherapy[4] and post-surgery nausea and vomiting.[5] By way of example, aprepitant (**2**, Emend) has been approved as an adjunctive therapy for chemotherapy-induced emesis. In 1995, researchers from Takeda discovered the potent antagonist properties of 4-phenylisoquinolinone and naphthyridinone derivatives **3**,[6] which are apparently unrelated to the best known NK_1 receptor antagonists. The studies performed at Takeda led to the development of TAK-637 (**4**),[7] which was reported to be an orally bioavailable antagonist of NK_1 receptor in the intestinal smooth muscles and potentially useful in the treatment of functional bowel diseases such as irritable bowel syndrome (IBS).[8]

The program of structural manipulation of original Takeda antagonists **3** led to the synthesis of 3-quinolinecarboxamide derivatives **5**[9] related to our quinolinecarboxamide peripheral benzodiazepine receptor ligands.[10] Several derivatives **5** showed NK_1 receptor affinity in the picomolar range, and the most active compound (**5h**, $R_1 = H$, $R_2 = CH_2OH$) behaved as an agonist of NK_1 receptor in endothelial cell proliferation, inositol phosphate turnover, and NO-mediated cyclic GMP accumulation, thus proving to be the first non-peptide NK_1 receptor agonist showing very high potency. Moreover, the structure–affinity relationship analysis of the series suggested a limited dimension for the pocket accommodating the substituents in position 2.[9] The outstanding results obtained with these 3-quinolinecarboxamides stimulated the design of tricyclic carboxamide derivatives **6**, in which the substituent in position 2 of the quinoline nucleus of compounds **5** takes part in a five-membered heterocycle (azole moiety), in order to obtain further information on the structural prerequisites for the interaction with NK_1 binding site.

* Corresponding author. Tel.: +39 0577 234320; fax: +39 0577 234333.
E-mail address: cappelli@unisi.it (A. Cappelli).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.05.067

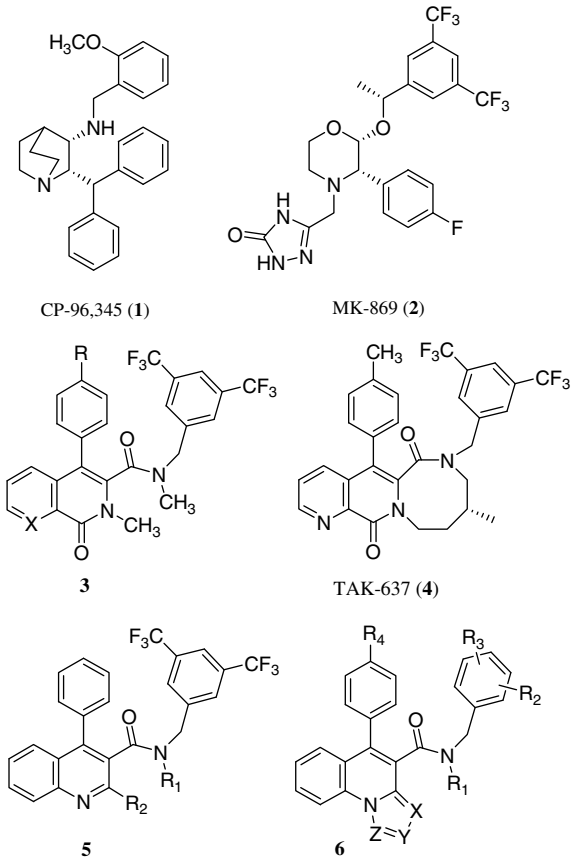
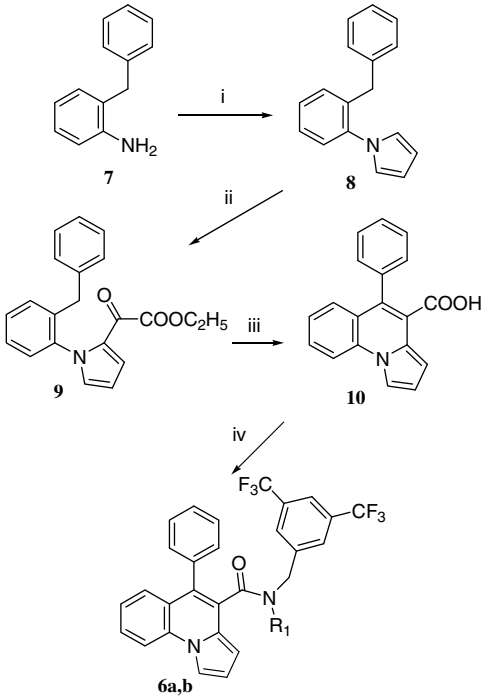


Figure 1. Structures of compounds **1–6**.



Scheme 1. Reagents: (i) 2,5-Dimethoxytetrahydrofuran, CH_3COOH; (ii) $ClCOCOOC_2H_5$, C_6H_6; (iii) NaH, DMF; (iv) 3,5-$(CF_3)_2C_6H_3CH_2NH_2$, HOBt, DCC, CH_2Cl_2 or 3,5-$(CF_3)_2C_6H_3CH_2N(H)CH_3{\cdot}HCl$, TEA, HOBt, DCC, CH_2Cl_2.

2. Results and discussion

2.1. Chemistry

The synthesis of pyrrolo[1,2-*a*]quinoline derivatives **6a**,**b** was accomplished starting from commercially available 2-benzylaniline **7** as reported in Scheme 1.

Compound **7** was subjected to Clauson–Kaas reaction, with 2,5-dimethoxytetrahydrofuran in glacial acetic acid to give intermediate **8** which reacted with ethyl oxalylchloride to give **9**. This intermediate was then cyclized in the presence of sodium hydride as the base to obtain the carboxylic acid derivate **10** instead of the expected ester. Reaction between **10** and the appropriate amines in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) afforded the target compounds **6a**,**b**. The structure of pyrrolo[1,2-*a*]quinoline derivative **6a** was confirmed by X-ray crystallography (Fig. 2).

The procedure for the preparation of imidazo[1,2-*a*]quinoline amides **6c**,**d** is shown in Scheme 2. Chloroquinoline derivate **11**[11] was reacted with 2-aminoethanol to obtain **12**, which was cyclized in phosphorus oxychloride to give the dihydroderivative **13**. The heating of **13** in DMSO at 120 °C gave ester **14**, which was hydrolyzed with 2 M NaOH to give acid **15**. Target amides **6c**,**d** were prepared from acid **15** by means of the same procedure described for the corresponding pyrrolo[1,2-*a*]quinoline amides **6a**,**b** (Fig. 3).

A different approach to the synthesis of imidazo[1,5-*a*]quinoline amides **6e**,**f** is reported in Scheme 3. Chloroquinoline derivate **16**[11] was reacted with *tert*-butyl isocyanoacetate in the presence of potassium *tert*-butoxide to obtain diester **17**, which was cleaved with formic acid to **18**. Acid **18** was decarboxylated under nitrogen at 200 °C to give monoester **19**, which was in turn hydrolyzed with

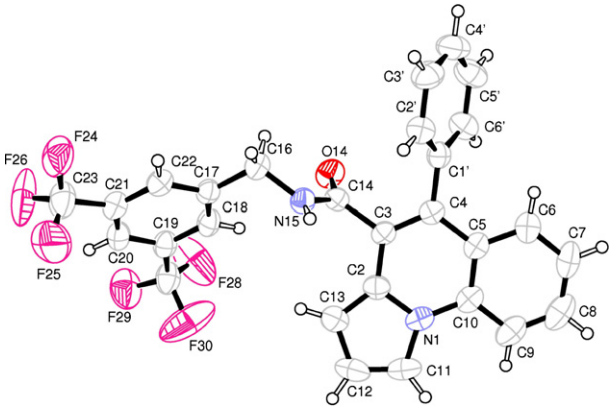
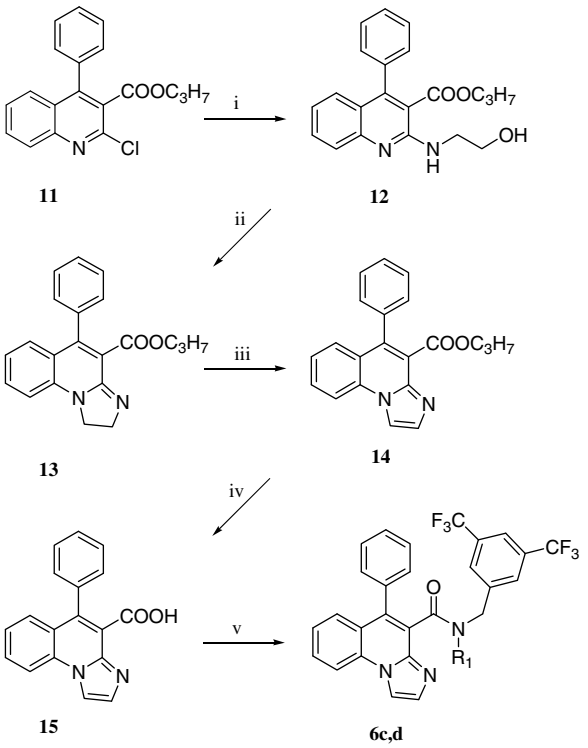


Figure 2. Crystal structure of compound **6a**. Ellipsoids enclose 50% probability.

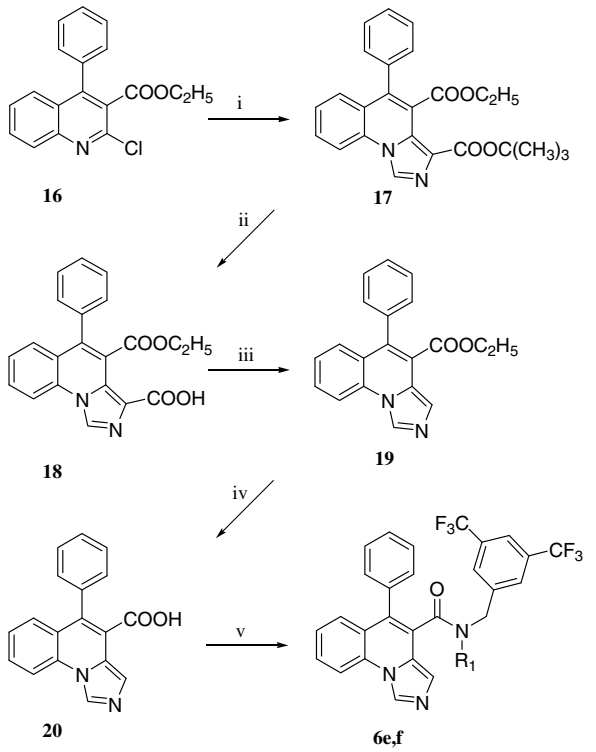
2 M NaOH to give acid **20**. The target amides **6e**,**f** were prepared from acid **20** by means of the same procedure described for the corresponding pyrrolo[1,2-*a*]quinoline amides **6a**,**b**.

The synthesis of [1,2,4]triazolo[4,3-*a*]quinoline derivates **6g**,**h** was carried out starting from acid **21**[11] as shown in Scheme 4. Acid **21** was converted into the corresponding amides **22** and **23**,[12] which reacted with phosphorus oxychloride to give chloroderivates **24** and **25**.[12] The reaction of **24** and **25** with hydrazine hydrate gave hydrazinoderivatives **26** and **27**, respectively, which were cyclized with formic acid to obtain the target compounds **6g**,**h**.

The synthesis of tetrazolo[1,5-*a*]quinoline-4-carboxamides **6i–p** is described in Scheme 5. The preparations of 2-chloroquinoline derivative **16**,[11] 4-(4-methylphenyl)quinoline derivative **28**,[13] and 4-(4-fluorophenyl)quinoline derivative **29**[14] were performed by means of procedures described in the literature. The reaction of 2-chloroquinoline derivates **16**, **28**, **29** with sodium



Scheme 2. Reagents: (i) $HOCH_2CH_2NH_2$, C_2H_5OH; (ii) $POCl_3$; (iii) Δ, DMSO; (iv) NaOH, C_2H_5OH; (v) 3,5-$(CF_3)_2C_6H_3CH_2NH_2$, HOBt, DCC, CH_2Cl_2 or 3,5-$(CF_3)_2C_6H_3CH_2N(H)CH_3$·HCl, HOBt, DCC, TEA, CH_2Cl_2.



Scheme 3. Reagents: (i) *tert*-Butyl isocyanoacetate, *t*-BuOK, DMF; (ii) HCOOH; (iii) Δ, no-solvent (neat), N_2; (iv) NaOH, C_2H_5OH; (v) 3,5-$(CF_3)_2C_6H_3CH_2NH_2$, HOBt, DCC, CH_2Cl_2 or 3,5-$(CF_3)_2C_6H_3CH_2N(H)CH_3$·HCl, HOBt, DCC, TEA, CH_2Cl_2.

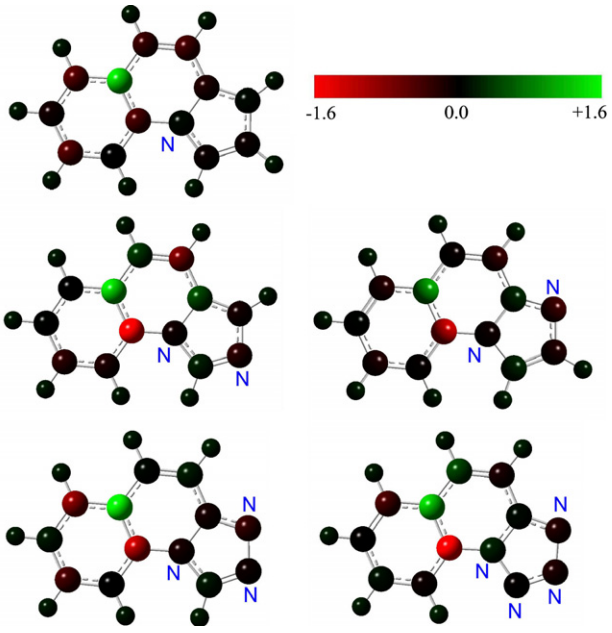
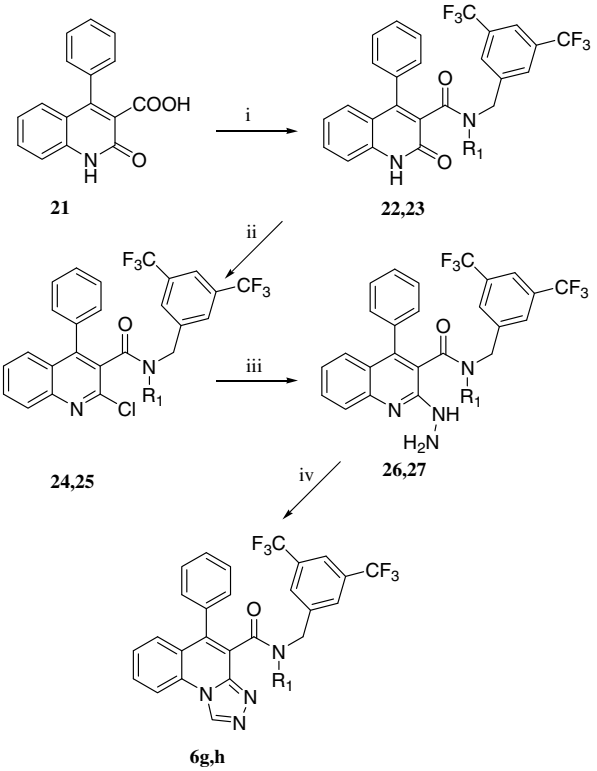
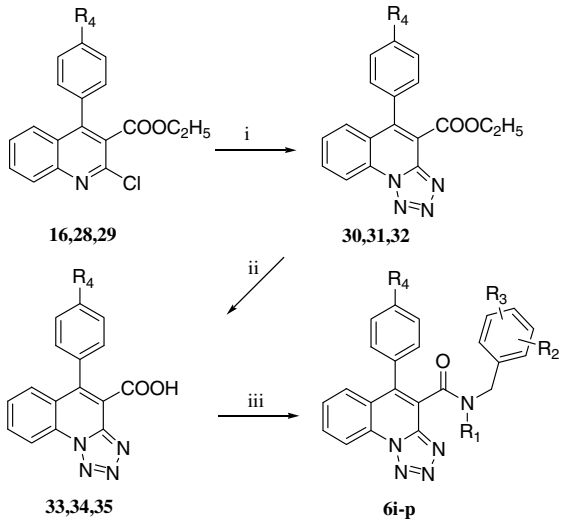


Figure 3. Mulliken charge distribution in the isolated tricyclic systems calculated at B3LYP/6-31+G** level of theory using Gaussian 03 package. The code-color scheme is also reported.

azide gave the key intermediates **30**, **31**, **32** (Scheme 5) which were hydrolyzed to obtain the required carboxylic acids **33**, **34**, **35**. The activation of these acids with thionyl chloride followed by the reaction with the suitable amines gave tetrazolo[1,5-*a*]quinoline-4-carboxamides **6i–p**.



Scheme 4. Reagents: (i) a—$SOCl_2$, CH_2Cl_2; b—3,5-$(CF_3)_2C_6H_3CH_2NH_2$, TEA, CH_2Cl_2 or 3,5-$(CF_3)_2C_6H_3CH_2N(H)CH_3$·HCl, TEA, CH_2Cl_2 (ii) $POCl_3$, CH_2Cl_2; (iii) $NH_2NH_2·H_2O$, C_2H_5OH; (iv) HCOOH.



Scheme 5. Reagents: (i) NaN_3, DMF; (ii) NaOH, C_2H_5OH; (iii) a—$SOCl_2$, CH_2Cl_2; b—$R_2R_3C_6H_3CH_2N(H)R_1$, TEA, CH_2Cl_2.

2.2. Binding studies

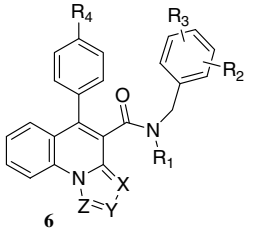
Compounds **6** were assayed for their activity in inhibiting the specific binding of [^{125}I]BH-SP to human recombinant NK_1 receptor stably expressed in CHO cells.[15] The results of the binding studies, expressed as IC_{50} values in Table 1, reveal that compounds **6a–p** show NK_1 receptor affinities in the nanomolar range to the micromolar one and the affinity modulation is affected by both the amide substituents and the azole moiety fused at *a*-edge of the quinoline nucleus.

In agreement with the structure–affinity relationships described in the literature,[6,7,12] the amide substituents of compounds **6** appear to play a fundamental role in the interaction of these tricyclic amide derivatives with NK_1 receptor. Indeed, the 3,5-bistrifluoromethylbenzyl group is confirmed to be an optimal substituent from the point of view of the binding potency (compare **6i** with **6o**,**p**) and the amide methyl group of tertiary amides **6b**,**d**,**f**,**h**,**j**,**l**,**n** enhances the affinity of the secondary amides **6a**,**c**,**e**,**g**,**i**,**k**,**m** to a variable extent (from 7 to 60 times).

The most remarkable result of the present structure–affinity relationship study is that the effects of the variation of the fused azole moiety are significant (in the range of one order of magnitude) in both the sub-micromolar affinity secondary amides **6a**,**c**,**e**,**g**,**i**,**k**,**m**, and in the series of the nanomolar affinity tertiary amide ligands **6b**,**d**,**f**,**h**,**j**,**l**,**n**. Owing to the evident similarity in the steric features of the different tricyclic systems, this result suggests a role of the electron distribution in the observed affinity modulation. In order to evaluate this hypothesis, Mulliken charges were calculated at B3LYP/6-31+G** level of theory in the models of the tricyclic systems. The results obtained show a different charge distribution as an effect related to both the different position and the number of nitrogen atoms present in the fused azole moiety. In particular, the presence of a single (quinoline) nitrogen atom in the simplified model of **6a,b** tricyclic system leads to a uniform electron distribution in the azole portion. The introduction of additional nitrogen atoms produces the formation of more pronounced localized dipoles within the tricyclic system, which may find suitable counterparts for interaction in the binding site. In particular, the comparison of partial charge distribution in the azole moieties of tertiary amides **6b**,**d**,**f**,**h**,**j** supports the important presence of a nitrogen atom in position 2 of the tricyclic system (compound **6f**). In fact, when the additional nitrogen is present in position 3 as in compound **6d**, the affinity is about one order of magnitude lower with respect to **6f** and similar to that shown by **6b**. Moreover, when two additional nitrogen atoms are present in positions 2 and 3 of **6h** tricyclic system, the NK_1 receptor affinity is just slightly lower with respect to **6f**.

Finally, the presence of a nitrogen atom in position 3 of the tricyclic nucleus is assumed to affect the conformational preferences

Table 1
Binding affinities of azole-fused quinolinecarboxamide derivatives **6** for human recombinant NK_1 receptor stably expressed in CHO cells and chemical shift values of NH signal in the ^{1}H NMR spectra of secondary amides **6a**,**c**,**e**,**g**,**i**



6

Compound	X–Y–Z	R_1	R_2	R_3	R_4	IC_{50} (nM)	NH chemical shift (ppm)
6a	=CH–CH=CH–	H	3-CF_3	5-CF_3	H	390 ± 47	5.79
6b	=CH–CH=CH–	CH_3	3-CF_3	5-CF_3	H	36 ± 7.5	
6c	=N–CH=CH–	H	3-CF_3	5-CF_3	H	1233 ± 335	10.12
6d	=N–CH=CH–	CH_3	3-CF_3	5-CF_3	H	38 ± 12	
6e	=CH–N=CH–	H	3-CF_3	5-CF_3	H	146 ± 48	5.90
6f	=CH–N=CH–	CH_3	3-CF_3	5-CF_3	H	4.3 ± 1.6	
6g	=N–N=CH–	H	3-CF_3	5-CF_3	H	542 ± 37	9.82
6h	=N–N=CH–	CH_3	3-CF_3	5-CF_3	H	9.0 ± 4.0	
6i	=N–N=N–	H	3-CF_3	5-CF_3	H	311 ± 52	9.09
6j	=N–N=N–	CH_3	3-CF_3	5-CF_3	H	26 ± 3.0	
6k	=N–N=N–	H	3-CF_3	5-CF_3	CH_3	401 ± 31	
6l	=N–N=N–	CH_3	3-CF_3	5-CF_3	CH_3	55 ± 8.0	
6m	=N–N=N–	H	3-CF_3	5-CF_3	F	235 ± 9.0	
6n	=N–N=N–	CH_3	3-CF_3	5-CF_3	F	21 ± 8.2	
6o	=N–N=N–	H	2-OCH_3	H	H	2749 ± 329	
6p	=N–N=N–	H	3-OCH_3	5-OCH_3	H	780 ± 145	
SP						1.5 ± 0.1	

of secondary amides **6c**,**g**,**i**. This assumption appears to be supported by the chemical shift value ($CDCl_3$ as the solvent, Table 1) of the ^1H NMR signal attributed to NH of secondary amides bearing a nitrogen atom in position 3 of the tricyclic nucleus. Therefore, the presence and the intensity of this intramolecular interaction may play a role in the affinity modulation of secondary amides **6c**,**g**,**i**.

On the other hand, the replacement of the hydrogen atom in para-position of the pendent phenyl group with a methyl group or a fluorine atom has negligible effects on the receptor affinity (compare **6i** with **6k**,**m** and **6j** with **6l**,**n**).

3. Conclusions

A small series of 3-quinolinecarboxamides bearing different five-membered heterocycles condensed at the *a*-edge of the quinoline nucleus were designed, synthesized, and evaluated for their potential ability to inhibit the specific binding of [^{125}I]BH-SP to recombinant human NK_1 receptor in order to obtain further information on the structural prerequisites for the interaction of NK_1 binding site with this apparently central portion of ligands **5**. The structure–affinity relationship study confirms the importance of the amide substituents in the interaction with the receptor and, remarkably, reveals a clear modulating effect of the fused azole moiety in both secondary and tertiary amide ligands. The results of Mulliken charges calculations performed at B3LYP/6-31+G** level of theory with models of the different tricyclic systems support the role of charge distribution in the interaction of tertiary amides **6b**,**d**,**f**,**h**,**j** with the receptor. Moreover, the presence and the intensity of an intramolecular H-bond interaction in the secondary amides bearing a nitrogen atom in position 3 of the tricyclic system may play an additional role in the affinity modulation within the secondary amide derivatives.

Finally, the discrepancies observed in the structure–affinity relationships between compounds **6** and **5**[9] warrant further investigations (i.e., the evaluation of previously published 3-quinolinecarboxamide derivatives **5** in the human recombinant NK_1 receptor expressed in CHO cells). Therefore, further studies are now in progress in order to obtain information about the interaction of these heterocyclic amide derivatives with NK_1 receptor.

4. Experimental

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. Microanalyses were carried out by means of a Perkin-Elmer 240C or a Perkin-Elmer Series II CHNS/O Analyzer 2400. 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 with a Bruker AC 200 spectrometer in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in ppm and the coupling constants (J) in Hz. Mass spectra were recorded on either a Varian Saturn 3 spectrometer or a ThermoFinnigan LCQ-Deca.

4.1.1. 1-(2-Benzylphenyl)-1*H*-pyrrole (8)

A mixture of 2-aminodiphenyl (**7**, 0.30 g, 1.64 mmol) in glacial acetic acid (15 mL) with 2,5-dimethoxytetrahydrofuran (0.26 mL, 1.96 mmol) was stirred at 80 °C for 2 h. The solvent was then removed under reduced pressure and the residue was diluted with ethyl acetate. The mixture was washed with brine and water, the organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography with *n*-hexane/ethyl acetate (8:2) as the eluent gave compound **8** (0.32 g) in 84% yield. ^1H NMR ($CDCl_3$): 3.93 (s, 2H), 6.35 (t, J = 1.8, 2H), 6.79 (t, J = 1.9, 2H), 7.07 (m, 2H), 7.22–7.35 (m, 7H).

4.1.2. Ethyl 2-[1-(2-Benzylphenyl)-1*H*-pyrrol-2-yl]-2-oxoacetate (9)

To a solution of **8** (0.15 g, 0.65 mmol) in benzene (15 mL) was added ethyl oxalylchloride (0.22 mL, 1.97 mmol), and the resulting mixture was refluxed for 4 h. After concentration under reduced pressure, the reaction mixture was diluted with ethyl acetate, washed with saturated $NaHCO_3$ solution, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by flash chromatography with *n*-hexane/ethyl acetate (8:2) as the eluent gave the pure product **9** (0.15 g, yield 69%). ^1H NMR ($CDCl_3$): 1.38 (t, J = 7.0, 3H), 3.63 (d, J = 15.6, 1H), 3.80 (d, J = 15.6, 1H), 4.36 (q, J = 7.0, 2H), 6.34 (m, 1H), 6.83 (m, 1H), 6.98 (m, 2H), 7.11–7.41 (m, 7H), 7.47 (m, 1H). MS(ESI): m/z 334 ($M+H^+$).

4.1.3. 5-Phenylpyrrolo[1,2-*a*]quinoline-4-carboxylic acid (10)

To a solution of **9** (0.80 g, 2.4 mmol) in anhydrous DMF (8 mL) was added NaH (0.115 g, 4.8 mmol), and the resulting mixture was heated for 3 h at 120 °C under argon. The solvent was then removed under reduced pressure, the resulting mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate as the eluent gave pure acid **10** as a yellow oil which crystallized on standing (0.50 g, yield 73%, mp 168–169 °C). ^1H NMR ($CDCl_3$): 6.85 (m, 2H), 7.18–7.61 (m, 8H), 7.95 (m, 2H). MS(E-SI, negative ions): m/z 286 ($M-H^+$).

4.1.4. *N*-[3,5-Bis(trifluoromethyl)benzyl]-5-phenylpyrrolo[1,2-*a*]quinoline-4-carboxamide (6a)

To an ice-cooled mixture of **10** (0.115 g, 0.40 mmol) and 3,5-bis(trifluoromethyl)benzylamine (0.105 g, 0.43 mmol) in dry dichloromethane (20 mL) was added HOBt (0.058 g, 0.43 mmol), and the resulting mixture was stirred at 0–5 °C for 10 min. Afterwards, a solution of DCC (0.112 g, 0.54 mmol) in the same solvent (10 mL) was added dropwise. The reaction mixture was stirred overnight at room temperature, the precipitate was filtered-off, and the filtrate was washed in sequence with water and brine, dried, and concentrated under reduced pressure. The residue was subjected to flash chromatography on silica gel with dichloromethane as the eluent to give **6a** (0.14 g, 68%). An analytical sample crystallized from diethyl ether melted at 202–203 °C. ^1H NMR ($CDCl_3$): 4.40 (d, J = 6.2, 2H), 5.79 (t, J = 6.0, 1H), 6.70 (m, 1H), 6.85 (m, 1H), 7.17–7.58 (m, 10H), 7.76 (s, 1H), 7.92 (m, 2H). MS(E-SI): m/z 513 ($M+H^+$). Anal. ($C_{28}H_{18}F_6N_2O$) C,H,N.

4.1.5. *N*-[3,5-Bis(trifluoromethyl)benzyl]-*N*-methyl-5-phenylpyrrolo[1,2-*a*]quinoline-4-carboxamide (6b)

This compound was prepared from **10** (0.50 g, 1.74 mmol), 15 mL of dry dichloromethane, HOBt (0.27 g, 2.0 mmol), DCC (0.54 g, 2.6 mmol), 0.50 mL of TEA, and *N*-[3,5-bis(trifluoromethyl)benzyl]methylamine hydrochloride (0.59 g, 2.0 mmol) by the same procedure described for the synthesis of **6a**. The mixture was purified by flash chromatography with CH_2Cl_2 as the eluent to obtain **6b** (0.58 g, yield 63%, melting point 161 °C). The ^1H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^1H NMR ($CDCl_3$): 2.71 (s), 2.74 (s), 3.93 (d, J = 15.7), 4.46 (d, J = 14.8), 4.65 (d, J = 15.7), 4.79 (d, J = 14.8), 6.46 (m), 6.59 (m), 6.85 (m), 7.19–7.70 (m), 7.79 (s), 7.94 (m). MS(ESI): m/z 527 ($M+H^+$). Anal. ($C_{29}H_{20}F_6N_2O$) C,H,N.

4.1.6. Propyl 2-(2-hydroxyethylamino)-4-phenylquinoline-3-carboxylate (12)

A mixture of **11** (0.40 g, 1.23 mmol) in absolute ethanol (15 mL) with 2-aminoethanol (0.30 mL, 4.97 mmol) was refluxed for 15 h. The solvent was then removed under reduced pressure and the residue was diluted with ethyl acetate. The mixture was washed with water, the organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography with petroleum ether/ethyl acetate (6:4) as the eluent gave compound **12** as an orange oil, which crystallized on standing (0.32 g, yield 74%, mp 74–75 °C). ^{1}H NMR ($CDCl_3$): 0.59 (t, J = 7.5, 3H), 0.96–1.14 (m, 2H), 3.71–3.91 (m, 6H), 6.10 (br s, 1H), 7.02–7.29 (m, 4H), 7.39–7.54 (m, 4H), 7.65 (d, J = 8.4, 1H). MS(ESI): m/z 351 ($M+H^+$).

4.1.7. Propyl 1,2-dihydro-5-phenylimidazo[1,2-*a*]quinoline-4-carboxylate (13)

A mixture of **12** (0.18 g, 0.51 mmol) in phosphorous oxychloride (10 mL) was refluxed for 6 h. The excess of $POCl_3$ was then decomposed with ice-water, and the precipitate was extracted with dichloromethane. The organic phase was dried over sodium sulfate and concentrated under reduced pressure, to give compound **13** as a yellow solid (0.12 g, yield 71%, melting point 158 °C). ^{1}H NMR ($CDCl_3$): 0.66 (t, J = 7.1, 3H), 1.28 (m, 2H), 3.88–4.16 (m, 6H), 6.72–6.87 (m, 2H), 7.01 (d, J = 7.3, 1H), 7.26–7.40 (m, 6H). MS(ESI): m/z 333 ($M+H^+$).

4.1.8. Propyl 5-phenylimidazo[1,2-*a*]quinoline-4-carboxylate (14)

Compound **13** (0.30 g, 0.90 mmol) was dissolved in DMSO (10 mL) and the resulting mixture was heated at 120 °C for 48 h. The mixture was diluted with water and extracted with dichloromethane, the organic phase was dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by flash chromatography with CH_2Cl_2/ethyl acetate (7:3) as the eluent gave **14** as an off-white solid (0.19 g, 64%, melting point 117 °C). ^{1}H NMR ($CDCl_3$): 0.71 (t, J = 7.4, 3H), 1.36 (m, 2H), 4.06 (t, J = 6.8, 2H), 7.31–7.69 (m, 8H), 7.71 (s, 1H), 7.94 (d, J = 8.4, 1H), 8.08 (s, 1H). MS(ESI): m/z 331 ($M+H^+$).

4.1.9. 5-Phenylimidazo[1,2-*a*]quinoline-4-carboxylic acid (15)

A mixture of **14** (0.14 g, 0.42 mmol) in ethanol (10 mL) with 2 N NaOH (4.0 mL) was refluxed for 2 h. The solvent was then removed under reduced pressure, and the residue was diluted with water and acidified with 2 N HCl (pH 6). The precipitate was collected by filtration, washed with diethyl ether, and dried under reduced pressure to give 0.10 g of **15** as a white solid melting at 223 °C (yield 83%). ^{1}H NMR (DMSO-d_6): 7.35–7.62 (m, 7H), 7.86–7.93 (m, 2H), 8.57 (d, J = 8.4, 1H), 8.96 (s, 1H). MS(ESI): m/z 289 ($M+H^+$).

4.1.10. *N*-[3,5-Bis(trifluoromethyl)benzyl]-5-phenylimidazo[1,2-*a*]quinoline-4-carboxamide (6c)

This compound was prepared from **15** (0.10 g, 0.35 mmol), 10 mL of dry dichloromethane, HOBt (0.057 g, 0.42 mmol), DCC (0.11 g, 0.53 mmol), and 3,5-bis(trifluoromethyl)benzylamine (0.13 g, 0.53 mmol) by the same procedure described for the synthesis of **6a**. The mixture was purified by flash chromatography with $CHCl_3$/ethyl acetate (7:3) as the eluent to give **6c** as a white solid (0.11 g, 61%, melting point 208 °C). ^{1}H NMR ($CDCl_3$): 4.74 (d, J = 5.9, 2H), 7.28–7.51 (m, 7H), 7.68–7.74 (m, 3H), 7.82 (s, 2H), 7.98 (d, J = 8.3, 1H), 8.15 (s, 1H), 10.12 (t, J = 6.0, 1H). MS(ESI): m/z 514 ($M+H^+$). Anal. ($C_{27}H_{17}F_6N_3O$) C,H,N.

4.1.11. *N*-[3,5-Bis(trifluoromethyl)benzyl]-*N*-methyl-5-phenylimidazo[1,2-*a*]quinoline-4-carboxamide (6d)

This compound was prepared from **15** (0.29 g, 1.0 mmol), 20 mL of dry dichloromethane, HOBt (0.20 g, 1.5 mmol), DCC (0.31 g, 1.5 mmol), 0.40 mL of TEA and *N*-[3,5-bis(trifluoromethyl)benzyl]methylamine hydrochloride (0.44 g, 1.5 mmol) by the same procedure described for the synthesis of **6a**. The crude product was purified by flash chromatography with $CHCl_3$/ethyl acetate (7:3) as the eluent to obtain **6d** as a white solid (0.41 g, yield 78%). An analytical sample melted at 206 °C. The ^{1}H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^{1}H NMR ($CDCl_3$): 2.78 (s), 2.80 (s), 3.93 (d, J = 15.6), 4.49 (d, J = 15.2), 4.70 (d, J = 15.7), 5.08 (d, J = 15.1), 7.21–7.75 (m), 7.98 (m), 8.11 (m). MS(ESI): m/z 528 ($M+H^+$). Anal. ($C_{28}H_{19}F_6N_3O$) C,H,N.

4.1.12. 3-(*tert*-Butyl) 4-ethyl 5-phenylimidazo[1,5-*a*]quinoline-3,4-dicarboxylate (17)

To an ice-cooled solution of **16** (0.60 g, 1.9 mmol) in anhydrous DMF (15 mL) were added *tert*-butyl isocyanoacetate (0.76 g, 5.38 mmol) and potassium *tert*-butoxide (0.65 g, 5.76 mmol), and the resulting mixture was stirred at 0–5 °C for 30 min. Afterwards, the solution was stirred at room temperature for 30 min and then heated at 80 °C for 10 h. The final mixture was neutralized with glacial acetic acid, poured into crushed ice and extracted with ethyl acetate. The combined extract was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by washing with diethyl ether to give 0.65 g of **17** (yield 81%). An analytical sample melted at 159–161 °C. ^{1}H NMR ($CDCl_3$): 1.02 (t, J = 7.0, 3H), 1.63 (s, 9H), 4.12 (q, J = 7.0, 2H), 7.33–7.48 (m, 7H), 7.66 (m, 1H), 8.07 (d, J = 8.3, 1H), 8.68 (s, 1H). MS(ESI): m/z 439 ($M+Na^+$).

4.1.13. 4-(Ethoxycarbonyl)-5-phenylimidazo[1,5-*a*]quinoline-3-carboxylic acid (18)

A mixture of compound **17** (0.60 g, 1.44 mmol) in formic acid (15 mL) was heated at 50 °C for 3 h. The reaction mixture was then concentrated under reduced pressure while the remaining formic acid was azeotropically removed with toluene. The residue was washed with ethanol to give 0.50 g of **18** as an off-white solid (yield 96%). An analytical sample melted at 245–247 °C. ^{1}H NMR (DMSO-d_6): 0.87 (t, J = 6.8, 3H), 3.91 (q, J = 6.9, 2H), 7.04–7.83 (m, 8H), 8.59 (d, J = 8.3, 1H), 9.36 (s, 1H), 12.38 (s, 1H). MS(ESI, negative ions): m/z 359 ($M-H^+$).

4.1.14. Ethyl 5-phenylimidazo[1,5-*a*]quinoline-4-carboxylate (19)

Compound **18** (0.20 g, 0.55 mmol) was heated without solvent at 190–200 °C until the end of CO_2 bubbling. The cooled residue was dissolved in ethyl acetate (30 mL), washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate as the eluent gave **19** as a yellow solid (0.075 g, 43%, melting point 113–114 °C). ^{1}H NMR ($CDCl_3$): 0.95 (t, J = 6.9, 3H), 4.09 (q, J = 6.9, 2H), 7.27–7.48 (m, 7H), 7.59 (m, 1H), 7.72 (s, 1H), 8.01 (d, J = 8.1, 1H), 8.69 (s, 1H). MS(ESI): m/z 317 ($M+H^+$).

4.1.15. 5-Phenylimidazo[1,5-*a*]quinoline-4-carboxylic acid (20)

A mixture of **19** (0.20 g, 0.63 mmol) in ethanol (8.0 mL) with 2 N NaOH (4.0 mL) was refluxed for 2 h. The reaction mixture was acidified with 2 N HCl (pH 6), and the solvent was removed under reduced pressure. The residue was washed with ethanol, and the organic solution was evaporated under reduced pressure to give 0.12 g of **20** as a brown solid (yield 66%, mp > 300 °C). ^{1}H NMR

(DMSO-d_6): 7.18–7.54 (m, 9H), 8.34 (d, J = 8.0, 1H), 9.02 (s, 1H). MS(ESI): m/z 289 ($M+H^+$).

4.1.16. *N*-[3,5-Bis(trifluoromethyl)benzyl]-5-phenylimidazo[1,5-*a*]quinoline-4-carboxamide (6e)

A solution of HOBt (0.058 g, 0.43 mmol) in dry dichloromethane (10 mL) was added to an ice-cooled solution of **20** (0.105 g, 0.36 mmol) and 3,5-bis(trifluoromethyl)benzylamine (0.105 g, 0.43 mmol) in the same solvent (10 mL). After stirring at 0–5 °C for 10 min, a solution of DCC (0.112 g, 0.54 mmol) in dichloromethane (10 mL) was added dropwise, and the resulting mixture was stirred at room temperature for 20 h. The precipitate was filtered-off and the filtrate was washed in sequence with water and brine, then dried, and evaporated under reduced pressure. The residue was purified by column chromatography with *n*-hexane/ethyl acetate (2:8) as the eluent to give **6e** as a white solid (0.12 g, yield 65%). An analytical sample melted at 191 °C. ^{1}H NMR ($CDCl_3$): 4.43 (d, J = 6.0, 2H), 5.90 (t, J = 5.9, 1H), 7.33 (m, 7H), 7.50 (s, 2H), 7.61 (m, 1H), 7.68 (s, 1H), 7.77 (s, 1H), 8.02 (d, J = 8.1, 1H), 8.78 (s, 1H). MS(ESI): m/z 514 ($M+H^+$). Anal. ($C_{27}H_{17}F_6N_3O$) C,H,N.

4.1.17. *N*-[3,5-Bis(trifluoromethyl)benzyl]-*N*-methyl-5-phenylimidazo[1,5-*a*]quinoline-4-carboxamide (6f)

This compound was prepared from **20** (0.20 g, 0.69 mmol), 15 mL of dry dichloromethane, HOBt (0.113 g, 0.83 mmol), DCC (0.215 g, 1.04 mmol), 0.5 mL of TEA and *N*-[3,5-bis(trifluoromethyl)benzyl]methylamine hydrochloride (0.305 g, 1.04 mmol) by means of the same procedure described for the synthesis of **6e**. The crude product was purified by flash chromatography with *n*-hexane/ethyl acetate (3:7) as the eluent to obtain **6f** (0.22 g, yield 60%, melting point 175 °C). The ^{1}H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^{1}H NMR ($CDCl_3$): 2.73 (s), 2.77 (s), 4.09 (d, J = 16.0), 4.32 (d, J = 14.7), 4.57 (d, J = 16.0), 4.95 (d, J = 14.7), 7.20–7.71 (m), 7.79 (s), 8.03 (d, J = 8.3), 8.72 (s). MS(ESI): m/z 528 ($M+H^+$). Anal. ($C_{28}H_{19}F_6N_3O$) C,H,N.

4.1.18. *N*-[3,5-Bis(trifluoromethyl)benzyl]-1,2-dihydro-2-oxo-4-phenylquinoline-3-carboxamide (22)

A mixture of acid **21** (0.10 g, 0.38 mmol) in thionyl chloride (5 mL) was refluxed for 3 h. The thionyl chloride excess was azeotropically removed with toluene and the residue was immediately dissolved into dichloromethane (15 mL). To the resulting solution 3,5-bis(trifluoromethyl)benzylamine (0.14 g, 0.57 mmol) and 0.30 mL of TEA were added, and the reaction mixture was refluxed for 4 h. The final mixture was diluted with CH_2Cl_2 (15 mL), washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by washing with diethyl ether to give 0.125 g of pure **22** as a white solid (yield 67%). Melting point 251 °C (literature[12] mp 251–252 °C). ^{1}H NMR ($CDCl_3$): 4.59 (d, J = 6.0, 2H), 7.09–7.54 (m, 10H), 7.71 (m, 2H), 8.29 (t, J = 5.9, 1H), 11.30 (br s, 1H). MS(ESI): m/z 513 ($M+Na^+$).

4.1.19. *N*-[3,5-Bis(trifluoromethyl)benzyl]-1,2-dihydro-*N*-methyl-2-oxo-4-phenylquinoline-3-carboxamide (23)

A mixture of acid **21** (0.50 g, 1.88 mmol) in thionyl chloride (8 mL) was refluxed for 3 h. The thionyl chloride excess was azeotropically removed with toluene and the residue was immediately dissolved into dichloromethane (20 mL). To the resulting solution 3,5-bis(trifluoromethyl)benzylamine hydrochloride (0.60 g, 2.04 mmol) and 0.5 mL of TEA were added, and the reaction mixture was refluxed for 4 h. The final mixture was diluted with CH_2Cl_2 (20 mL), washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Crude compound **23** was purified by washing with diethyl ether to obtain a white solid (0.60 g, yield 63%). Melting point 263–264 °C (literature[12] mp 262–264 °C). The ^{1}H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^{1}H NMR ($CDCl_3$): 2.73 (s), 2.87 (s), 4.08 (d, J = 16.2), 4.60 (d, J = 15.2), 4.70–4.82 (m), 7.09–7.77 (m), 12.43 (br s). MS(ESI): m/z 527 ($M+Na^+$).

4.1.20. *N*-[3,5-Bis(trifluoromethyl)benzyl]-2-chloro-4-phenylquinoline-3-carboxamide (24)

A mixture of **22** (0.10 g, 0.20 mmol) in phosphorus oxychloride (10 mL) was refluxed for 4 h. The excess of $POCl_3$ was decomposed with ice-water, and the precipitate was extracted with dichloromethane. The combined extracts were dried over sodium sulfate and concentrated under reduced pressure to give a crude product, which after purification by flash chromatography CH_2Cl_2/ethyl acetate (9:1) afforded **24** as a white solid (0.085 g, yield 84%, mp 172 °C). ^{1}H NMR ($CDCl_3$): 4.41 (d, J = 6.0, 2H), 6.10 (t, J = 5.9, 1H), 7.26–7.53 (m, 9H), 7.73 (m, 2H), 8.01 (d, J = 8.4, 1H). MS(ESI): m/z 509 ($M+H^+$).

4.1.21. *N*-[3,5-Bis(trifluoromethyl)benzyl]-2-chloro-*N*-methyl-4-phenylquinoline-3-carboxamide (25)

This compound was prepared from **23** (0.56 g, 1.1 mmol) and phosphorus oxychloride (10 mL) by the same procedure described for the synthesis of **20**. It was purified by flash chromatography with *n*-hexane/ethyl acetate (8:2) as the eluent to obtain **25** (0.51 g, yield 89%); melting point 148 °C (literature[12] mp 147–148 °C). The ^{1}H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^{1}H NMR ($CDCl_3$): 2.70 (s), 2.75 (s), 4.04 (d, J = 15.9), 4.48 (d, J = 15.6), 4.53 (d, J = 14.9), 4.69 (d, J = 14.9), 7.21 (m), 7.34–7.65 (m), 7.77 (m), 8.09 (d, J = 8.4). MS(ESI): m/z 523 ($M+H^+$).

4.1.22. *N*-[3,5-Bis(trifluoromethyl)benzyl]-2-hydrazino-4-phenylquinoline-3-carboxamide (26)

To a solution of **24** (0.48 g, 0.94 mmol) in ethanol (15 mL) was added an excess of hydrazine hydrate (200 μL, 4.1 mmol), and the resulting mixture was refluxed for 3 h. The solvent was then removed under reduced pressure and the residue was diluted with CH_2Cl_2 (20 mL); the organic phase was washed with water, dried over sodium sulfate, and concentrated under reduced pressure to obtain **26** as a brown solid (0.40 g, 84%, mp 163 °C). ^{1}H NMR ($CDCl_3$): 4.30 (d, J = 6.0, 2H), 5.67 (t, J = 6.0, 1H), 7.11–7.61 (m, 10H), 7.77 (m, 2H). MS(ESI): m/z 505 ($M+H^+$).

4.1.23. *N*-[3,5-Bis(trifluoromethyl)benzyl]-2-hydrazino-*N*-methyl-4-phenylquinoline-3-carboxamide (27)

The title compound was prepared from **25** (0.48 g, 0.92 mmol), 15 mL of ethanol, and hydrazine hydrate (180 μL, 3.7 mmol) by the same procedure described for the synthesis of **26** to obtain **27** as an orange oil (0.40 g, yield 84%). The oil obtained was used in the next step of the synthesis without purification. MS(ESI): m/z 519 ($M+H^+$).

4.1.24. *N*-[3,5-Bis(trifluoromethyl)benzyl]-5-phenyl[1,2,4]triazolo[4,3-*a*]quinoline-4-carboxamide (6g)

A mixture of **26** (0.36 g, 0.71 mmol) in formic acid (20 mL) was heated at 80 °C for 4 h. The formic acid excess was removed under reduced pressure, and the residue was dissolved into $CHCl_3$ and washed with water. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate/TEA (9:1) as the eluent gave pure **6g** as a white solid (0.35 g, yield 96%). An analytical sample melted at 226 °C. ^{1}H NMR ($CDCl_3$): 4.73 (d, J = 6.0, 2H), 7.29 (m, 2H), 7.49 (m, 6H), 7.76 (m, 3H), 8.06 (d, J = 8.3, 1H),

9.36 (s, 1H), 9.82 (t, J = 5.8, 1H). MS(ESI): m/z 515 ($M+H^+$). Anal. ($C_{26}H_{16}F_6N_4O$) C,H,N.

4.1.25. *N*-[3,5-Bis(trifluoromethyl)benzyl]-*N*-methyl-5-phenyl[1,2,4]triazolo[4,3-*a*]quinoline-4-carboxamide (6h)

This compound was prepared from **27** (0.36 g, 0.69 mmol), 15 mL of formic acid by the same procedure described for the synthesis of **6g**. It was purified by flash chromatography with ethyl acetate/TEA (9:1) as the eluent to obtain **6h** as a yellow oil which crystallized from diethyl ether (0.51 g, yield 88%). An analytical sample melted at 224 °C. The ^{1}H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^{1}H NMR ($CDCl_3$): 2.85 (s), 4.29 (d, J = 15.2), 4.63 (d, J = 16.0), 5.27 (d, J = 15.0), 7.16–7.79 (m), 8.07 (m), 9.36 (s), 9.39 (s). MS(ESI): m/z 529 ($M+H^+$). Anal. ($C_{27}H_{18}F_6N_4O$) C,H,N.

4.1.26. Ethyl 5-phenyltetrazolo[1,5-*a*]quinoline-4-carboxylate (30)

A mixture of **16** (0.80 g, 2.57 mmol) in anhydrous DMF (15 mL) with sodium azide (0.33 g, 5.1 mmol) was heated at 80 °C for 36 h. The solvent was then removed under reduced pressure and the residue was diluted with ethyl acetate (20 mL). The organic phase was washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by recrystallization from diethyl ether to give 0.52 g of **30** as white solid (yield 64%, mp 154–156 °C). ^{1}H NMR ($CDCl_3$): 1.03 (t, J = 7.0, 3H), 4.21 (q, J = 7.0, 2H), 7.36–7.69 (m, 7H), 7.91 (m, 1H), 8.77 (d, J = 8.3, 1H). MS(ESI): m/z 319 ($M+H^+$).

4.1.27. Ethyl 5-(4-methylphenyl)tetrazolo[1,5-*a*]quinoline-4-carboxylate (31)

The title compound was prepared from **28** (0.70 g, 2.15 mmol) by the same procedure described for the synthesis of **30** to obtain **31** (0.43 g, yield 60%) as a white solid melting at 177 °C. ^{1}H NMR ($CDCl_3$): 1.08 (t, J = 6.9, 3H), 2.48 (s, 3H), 4.25 (q, J = 7.0, 2H), 7.32 (m, 4H), 7.59–7.74 (m, 2H), 7.92 (m, 1H), 8.78 (d, J = 8.4, 1H). MS(ESI): m/z 333 ($M+H^+$).

4.1.28. Ethyl 5-(4-fluorophenyl)tetrazolo[1,5-*a*]quinoline-4-carboxylate (32)

The title compound was prepared from **29** (0.80 g, 2.43 mmol) by the same procedure described for the synthesis of **30** to obtain **32** (0.70 g, yield 86%) as a white solid. An analytical sample melted at 173 °C. ^{1}H NMR ($CDCl_3$): 1.09 (t, J = 7.0, 3H), 4.24 (q, J = 7.0, 2H), 7.19–7.42 (m, 4H), 7.64 (m, 2H), 7.92 (m, 1H), 8.76 (d, J = 8.2, 1H). MS(ESI): m/z 337 ($M+H^+$).

4.1.29. 5-Phenyltetrazolo[1,5-*a*]quinoline-4-carboxylic acid (33)

A mixture of **30** (0.20 g, 0.63 mmol) in ethanol (10 mL) with 2 M NaOH (4.0 mL) was refluxed for 3 h. The solvent was then removed under reduced pressure, and the residue was diluted in water and neutralized with 2 N HCl. The precipitate was collected by filtration, washed with *n*-hexane, and dried under reduced pressure to give 0.17 g of **33** as a white solid (yield 93%). An analytical sample melted at 256–258 °C. ^{1}H NMR (DMSO-d_6): 7.42–7.53 (m, 6H), 7.71 (m, 1H), 7.97 (m, 1H), 8.68 (d, J = 8.3, 1H), 13.81 (s, 1H). MS(ESI): m/z 313 ($M+Na^+$).

4.1.30. 5-(4-Methylphenyl)tetrazolo[1,5-*a*]quinoline-4-carboxylic acid (34)

The title compound was prepared from **31** (0.13 g, 0.40 mmol) by the same procedure described for the synthesis of **33** to obtain **34** as a white solid (0.10 g, yield 83%). An analytical sample melted at 265 °C. ^{1}H NMR (DMSO-d_6): 2.40 (s, 3H), 7.34 (m, 4H), 7.57 (m, 1H), 7.74 (m, 1H), 8.00 (m, 1H), 8.69 (d, J = 8.4, 1H), 13.80 (br s, 1H). MS(ESI): m/z 327 ($M+Na^+$).

4.1.31. 5-(4-Fluorophenyl)tetrazolo[1,5-*a*]quinoline-4-carboxylic acid (35)

The title compound was prepared from **32** (0.40 g, 1.19 mmol) by the same procedure described for the synthesis of **33** to obtain **35** as a white solid (0.35 g, yield 95%). An analytical sample melted at 267 °C. ^{1}H NMR (DMSO-d_6): 7.36–7.56 (m, 5H), 7.75 (m, 1H), 8.02 (m, 1H), 8.70 (d, J = 8.2, 1H). MS(ESI): m/z 331 ($M+Na^+$).

4.1.32. *N*-[3,5-Bis(trifluoromethyl)benzyl]-5-phenyltetrazolo[1,5-*a*]quinoline-4-carboxamide (6i)

The title compound was prepared from acid **33** (0.030 g, 0.10 mmol) and 3,5-bis(trifluoromethyl)benzylamine (0.050 g, 0.20 mmol) by the same procedure described for the synthesis of compound **22**, and **6i** was obtained as a white solid (0.030 g, yield 58%, mp 193–195 °C). ^{1}H NMR ($CDCl_3$): 4.75 (d, J = 5.9, 2H), 7.31 (m, 2H), 7.51–7.69 (m, 6H), 7.75 (s, 2H), 7.96 (m, 1H), 8.79 (d, J = 8.3, 1H), 9.09 (t, J = 6.0, 1H). MS(ESI): m/z 516 ($M+H^+$). Anal. ($C_{25}H_{15}F_6N_5O$) C,H,N.

4.1.33. *N*-[3,5-Bis(trifluoromethyl)benzyl]-*N*-methyl-5-phenyltetrazolo[1,5-*a*]quinoline-4-carboxamide (6j)

The title compound was prepared from acid **33** (0.050 g, 0.17 mmol) and *N*-[3,5-bis(trifluoromethyl)benzyl]methylamine hydrochloride (0.10 g, 0.34 mmol) by the same procedure described for the synthesis of compound **23**, and **6j** was obtained as a white solid (0.071 g, yield 79%, mp 222–224 °C). The ^{1}H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^{1}H NMR ($CDCl_3$): 2.80 (s), 2.86 (s), 4.19 (d, J = 15.9), 4.35 (d, J = 15.0), 4.57 (d, J = 16.0), 5.19 (d, J = 15.0), 7.18–7.95 (m), 8.78 (m). MS(ESI): m/z 530 ($M+H^+$). Anal. ($C_{26}H_{17}F_6N_5O$) C,H,N.

4.1.34. *N*-[3,5-Bis(trifluoromethyl)benzyl]-5-(4-methylphenyl)tetrazolo[1,5-*a*]quinoline-4-carboxamide (6k)

The title compound was prepared from acid **34** (0.090 g, 0.30 mmol) and 3,5-bis(trifluoromethyl)benzylamine (0.14 g, 0.58 mmol) by the same procedure described for the synthesis of compound **22**, and **6k** was obtained as a white solid (0.12 g, yield 76%). An analytical sample of **6k** melted at 227 °C. ^{1}H NMR ($CDCl_3$): 2.46 (s, 3H), 4.76 (d, J = 6.0, 2H), 7.18–7.35 (m, 4H), 7.62–7.77 (m, 5H), 7.94 (m, 1H), 8.75 (d, J = 8.3, 1H), 8.99 (t, J = 5.9, 1H). MS(ESI): m/z 530 ($M+H^+$). Anal. ($C_{26}H_{17}F_6N_5O$) C,H,N.

4.1.35. *N*-[3,5-Bis(trifluoromethyl)benzyl]-*N*-methyl-5-(4-methylphenyl)tetrazolo[1,5-*a*]quinoline-4-carboxamide (6l)

The title compound was prepared from acid **34** (0.090 g, 0.30 mmol) and *N*-[3,5-bis(trifluoromethyl)benzyl]methylamine hydrochloride (0.18 g, 0.61 mmol) by the same procedure described for the synthesis of compound **23**, and was obtained as a white solid (0.13 g, yield 80%). An analytical sample of **6l** melted at 183 °C. The ^{1}H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^{1}H NMR ($CDCl_3$): 2.41 (s), 2.49 (s), 2.82 (s), 2.89 (s), 4.21 (d, J = 16.0), 4.40 (d, J = 15.1), 4.57 (d, J = 15.9), 5.21 (d, J = 15.1), 7.10 (m), 7.26 (m), 7.40–7.73 (m), 7.80 (s), 7.90 (m), 8.77 (m). MS(ESI): m/z 544 ($M+H^+$). Anal. ($C_{27}H_{19}F_6N_5O$) C,H,N.

4.1.36. *N*-[3,5-Bis(trifluoromethyl)benzyl]-5-(4-fluorophenyl)tetrazolo[1,5-*a*]quinoline-4-carboxamide (6m)

The title compound was prepared from acid **35** (0.15 g, 0.49 mmol) and 3,5-bis(trifluoromethyl)benzylamine (0.24 g,

0.99 mmol) by the same procedure described for the synthesis of compound **22**, and was obtained as a white solid (0.20 g, yield 77%). An analytical sample of **6m** was purified by flash chromatography with dichloromethane/ethyl acetate (9:1) as the eluent (mp 232–233 °C). ^{1}H NMR ($CDCl_3$): 4.76 (d, J = 6.0, 2H), 7.26 (m, 4H), 7.55–7.78 (m, 5H), 7.96 (t, J = 7.4, 1H), 8.77 (d, J = 8.3, 1H), 9.21 (t, J = 6.0, 1H). MS(ESI): m/z 534 ($M+H^+$). Anal. ($C_{25}H_{14}F_7N_5O$) C,H,N.

4.1.37. *N*-[3,5-Bis(trifluoromethyl)benzyl]-5-(4-fluorophenyl)-*N*-methyltetrazolo[1,5-*a*]quinoline-4-carboxamide (6n)

The title compound was prepared from acid **35** (0.15 g, 0.49 mmol) and *N*-[3,5-bis(trifluoromethyl)benzyl]methylamine hydrochloride (0.29 g, 0.99 mmol) by the same procedure described for the synthesis of compound **23**, and was obtained as a white solid (0.19 g, yield 71%). An analytical sample of **6n** was purified by flash chromatography with dichloromethane/ethyl acetate (9:1) as the eluent (mp 252 °C). The ^{1}H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^{1}H NMR ($CDCl_3$): 2.84 (s), 2.91 (s), 4.22 (d, J = 15.9), 4.42 (d, J = 15.0), 4.58 (d, J = 15.9), 5.16 (d, J = 15.0), 7.07–7.36 (m), 7.53–7.76 (m), 7.82 (s), 7.93 (m), 8.80 (m). MS(ESI): m/z 570 ($M+Na^+$). Anal. ($C_{26}H_{16}F_7N_5O$) C,H,N.

4.1.38. *N*-(2-Methoxybenzyl)-5-phenyltetrazolo[1,5-*a*]quinoline-4-carboxamide (6o)

The title compound was prepared from acid **33** (0.20 g, 0.69 mmol) and 2-methoxybenzylamine (0.18 mL, 1.38 mmol) by the same procedure described for the synthesis of compound **22**, and was obtained as a white solid (0.22 g, yield 78%). An analytical sample of **6o** melted at 222 °C. ^{1}H NMR ($CDCl_3$): 3.88 (s, 3H), 4.58 (d, J = 5.8, 2H), 6.86 (m, 2H), 7.18–7.32 (m, 4H), 7.45–7.59 (m, 5H), 7.89 (m, 1H), 8.47 (t, J = 5.9, 1H), 8.74 (d, J = 8.2, 1H). MS(ESI): m/z 410 ($M+H^+$). Anal. ($C_{24}H_{19}N_5O_2$) C,H,N.

4.1.39. *N*-(3,5-Dimethoxybenzyl)-5-phenyltetrazolo[1,5-*a*]quinoline-4-carboxamide (6p)

The title compound was prepared from acid **33** (0.20 g, 0.69 mmol) and 3,5-dimethoxybenzylamine (0.23 g, 1.38 mmol) by the same procedure described for the synthesis of compound **22**, and was obtained as a white solid (0.28 g, yield 92%). An analytical sample of **6p** melted at 215 °C. ^{1}H NMR ($CDCl_3$): 3.78 (s, 6H), 4.54 (d, J = 5.6, 2H), 6.35 (t, J = 1.9, 1H), 6.48 (d, J = 1.8, 2H), 7.34 (m, 2H), 7.52–7.66 (m, 5H), 7.92 (m, 1H), 8.44 (t, J = 5.6, 1H), 8.76 (d, J = 8.2, 1H). MS(ESI): m/z 440 ($M+H^+$). Anal. ($C_{25}H_{21}N_5O_3$) C,H,N.

4.2. X-ray crystallography

A single crystal of **6a** was submitted to X-ray data collection on a Siemens P4 four-circle diffractometer with a graphite monochromated Mo-Kα radiation (l = 0.71069 Å) at 293 K. The structure was solved by direct methods implemented in SHELXS-97 program.[16] The refinements were carried out by full-matrix anisotropic least-squares on F2 for all reflections for non-H atoms by means of SHELXL-97 program.[17]

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 670069. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk).

4.3. In vitro binding assays

4.3.1. Ligands and reagents

Monoiodinated [^{125}I]-Bolton-Hunter reagent labeled substance P (2200 Ci/mmol) was purchased from Perkin-Elmer Life Science. Unlabeled Substance P was purchased from Bachem. Cell culture media, G418, and fetal calf serum were from Invitrogen.

4.3.2. Cell culture

Permanently transfected CHO cells were grown in a mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (1:1) supplemented with 10% fetal calf serum, 100 U/mL penicillin G, 100 mg/mL streptomycin sulfate, and 100 mg/mL G418 (Life Technologies, Inc.) at 37 °C in a humidified atmosphere of 5% CO_2.

4.3.3. Transfection of cells

Wild-type h-NK_1 receptor were transfected in CHO cells using Lipofectin (Life Technologies, Inc.), and stably expressing clones were isolated following selection with 400 mg/mL G418 (Geneticin) after 3–6 weeks.

4.3.4. Radioreceptor binding assays

Enriched plasma membranes from transfected stable cells were prepared as described[15] and stored (2 mg/mL) at −80 °C. The compounds were dissolved into 1 mM DMSO and stored at −20 °C. Radioreceptor binding assays were made in 1 mL reaction mixture containing 50 mM Hepes–Tris, pH 7.4, 5 mM $MgCl_2$, 10 mM leupeptin, 0.1 mg/mL bacitracin, 0.1% (w/v) bovine serum albumin, and 2–3 μg of membrane proteins from transfected CHO cells. The concentration of the radiotracer was kept constant at 10 pM in the presence of increasing concentrations of the compounds to be tested. Reactions lasted for 90 min at room temperature and were terminated by rapid filtration into GF/B glass fiber filtering microplates (Filtermate 196; Packard Instruments, Meriden, CT). Filters were washed three times with 1 mL of ice-cold 50 mM Tris–HCl, pH 7.4, and allowed to dry for a few hours. The plates were counted in a Top Count (Packard Instruments) after the addition (50 μL) of Microscint 20 (Packard) to each well. The data of the dose–response experiments were analyzed by means of Allfit[18] program to compute the IC_{50} values.

4.4. Theoretical calculations

Density functional theory calculations were performed on different tricyclic structures by means of Gaussian 03[19] implemented on a IBM SP RS/6000 Power 5 supercomputer at Cineca in Bologna (Italy). All geometries were fully optimized without any constraints at the Becke 3LYP (B3LYP)[20] method with the 6-31G+(d,p) level of theory. The final lowest energy geometries were confirmed as a minimum on the potential energy surface by normal-mode vibrational frequency calculations that produced all real frequencies.

Acknowledgments

The authors thank Prof. Stefania D'Agata D'Ottavi for the careful reading of the manuscript. This work was financially supported by MUR (Ministero dell'Università e della Ricerca)—PRIN (Programmi di ricerca di Rilevante Interesse Nazionale).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.05.067.

References and notes

1. Hokfelt, T.; Pernow, B.; Wahren, J. *J. Int. Med.* **2001**, *249*, 27.
2. Snider, R. M.; Constantine, J. W.; Lowe, J. A., III; Longo, K. P.; Lebel, W. S.; Woody, H. A.; Drozda, S. E.; Desai, M. C.; Vinick, F. J.; Spencer, R. W.; Hess, H.-J. *Science* **1991**, *251*, 437.
3. Sorbera, L. A.; Silvestre, J.; Castaner, J. *Drugs Future* **1999**, *24*, 254.
4. Bleiberg, H. *Curr. Opin. Oncol.* **2000**, *12*, 284.
5. Diemunsch, P.; Grelot, L. *Drugs* **2000**, *60*, 533.
6. Natsugari, H.; Ikeura, Y.; Kiyota, Y.; Ishichi, Y.; Ishimaru, T.; Saga, O.; Shirafuji, H.; Tanaka, T.; Kamo, I.; Doi, T.; Otsuka, M. *J. Med. Chem.* **1995**, *38*, 3106.
7. Natsugari, H.; Ikeura, Y.; Kamo, I.; Ishimaru, T.; Ishichi, Y.; Fujishima, A.; Tanaka, T.; Kasahara, F.; Kawada, M.; Doi, T. *J. Med. Chem.* **1999**, *42*, 3982.
8. (a) Venkova, K.; Sutkowski-Markmann, D. M.; Greenwood-Van Meerveld, B. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 1046; (b) Okano, S.; Nagaya, H.; Ikeura, Y.; Natsugari, H.; Inatomi, N. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 559.
9. Cappelli, A.; Giuliani, G.; Pericot Mohr, G.; Gallelli, A.; Anzini, M.; Vomero, S.; Cupello, A.; Scarrone, S.; Matarrese, M.; Moresco, R. M.; Fazio, F.; Finetti, F.; Morbidelli, L.; Ziche, M. *J. Med. Chem.* **2004**, *47*, 1315.
10. Anzini, M.; Cappelli, A.; Vomero, S.; Seeber, M.; Menziani, M. C.; Langer, T.; Hagen, B.; Manzoni, C.; Bourguignon, J.-J. *J. Med. Chem.* **2001**, *44*, 1134.
11. Anzini, M.; Cappelli, A.; Vomero, S.; Campiani, G.; Cagnotto, A.; Skorupska, M. *Il Farmaco* **1991**, *46*, 1435.
12. Natsugari, H.; Ikeda, H.; Ishimaru, T.; Doi, T. (Takeda Chemical Industries, Ltd.) Condensed Heterocyclic Compounds, their Production and Use. Eur. Pat. Appl. EP 585,913, 9 March 1994, JP Appl. 92/ 237,481, 4 September 1992; *Chem. Abstr*. **1995**, *122*, 56051x.
13. Cappelli, A.; Pericot Mohr, G.; Gallelli, A.; Rizzo, M.; Anzini, M.; Vomero, S.; Mennuni, L.; Ferrari, F.; Makovec, F.; Menziani, M. C.; De Benedetti, P. G.; Giorgi, G. *J. Med. Chem.* **2004**, *47*, 2574.
14. Suzuki, M.; Iwasaki, H.; Fujikawa, Y.; Kitahara, M.; Sakashita, M.; Sakoda, R. *Bioorg. Med. Chem.* **2001**, *9*, 2727.
15. Riitano, D.; Werge, T. M.; Costa, T. *J. Biol. Chem.* **1997**, *272*, 7646.
16. Sheldrick, G. M. SHELXS-97, Rel. 97-2, A Program for Automatic Solution of Crystal Structures, Gottingen University, 1997.
17. Sheldrick, G. M. SHELXL-97, Rel. 97-2, A Program for Crystal Structure Refinement, Gottingen University, 1997.
18. De Lean, K. W.; Munson, P. J.; Rodbard, D. *Am. J. Physiol.* **1978**, *235*, E97.
19. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, Revision D.02, Gaussian, Inc., Wallingford, CT, 2004.
20. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.