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Synthesis and biological evaluation of novel fluoro and iodo quinoline carboxamides as potential ligands of NK-3 receptors for in vivo imaging studies

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Abstract—In order to develop radioligands of human NK-3 receptor (hNK-3r) for imaging studies by positron emission tomography (PET) or single photon emission computed tomography (SPECT), a new series of fluoro- and iodo-quinoline carboxamides were synthesized and evaluated in a target receptor binding assay. Compared to the unsubstituted parent compound SB 223 412 ($K_i = 27 \text{ nM} \pm 9$), affinity was not altered for the analogues 1c and 2c bearing a fluorine in position 8 ($K_i \sim 24-27 \text{ nM}$), and was only slightly reduced for compounds 1b, 2b, 1e and 2e fluorinated or iodinated at the position 7 ($K_i \sim 49-67 \text{ nM}$). A drastic reduction in binding ($K_i > 115 \text{ nM}$) was observed for all other halogenated compounds 1a, 2a, 1d, 2d, 1f and 2f. © 2004 Elsevier Ltd. All rights reserved.

1. Introduction

The three main mammalian tachykinins, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) belong to the family of neuropeptides sharing the common COOH-terminal pentapeptide sequence of Phe-X-Gly-Leu-Met-NH₂. As neurotransmitters, these peptides exert their biological activity via three distinct neurokinin (NK) receptors termed as NK-1, NK-2 and NK-3. SP binds preferentially to the NK-1 receptor, NKA to the NK-2r and NKB to the NK-3r.¹⁻⁴ These receptors have been characterized by pharmacological studies and binding assays, cloned and expressed in stably transfected cells.⁵⁻⁷ Their distribution has been studied by autoradiography, and by in situ hybridization and immunocytochemistry.⁸⁻¹¹ The NK-1 receptor is widely distributed in central (CNS) and peripheral (PNS) nervous system whilst the NK-2 receptor is primarily con-

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fined in PNS. The NK-3 receptor is characterized by a predominant expression in CNS and its involvement in the modulation of central monoaminergic system has been shown. These properties make the NK-3 receptor a potential target for central nervous system disorders such as anxiety, psychosis, depression.^{12–16}

Positron emission tomography (PET) and single photon emission computed tomography (SPECT), are fundamental in vivo imaging techniques for the study of brain receptors. These methods allow their visualization during both physiological and pathophysiological conditions by the interactions of the biological target with a specific radioligand. As of present, no radioligand for the NK-3 receptor has been described. The discovery of SR 142801 (Osanetant)¹⁷ and SB 223412 (Talnetant)^{18–20} as lead compounds given that they are potent and selective non-peptidergic NK-3r antagonists, promises much for future in vivo biological investigations (Scheme 1).

SR 142801, has been tritiated and used in ex vivo imaging studies.^{8,9} However, it displays a lower selectivity than SB 223412. This latter seemed to us particularly attractive as it was found to be metabolically

Keywords: NK-3 receptor; Quinoline carboxamide; Ligand; Fluorine; Iodine.



Scheme 1. h-NK3r antagonists and target molecules.

stable and able to cross the blood-brain barrier. Moreover, SAR studies on the quinoline carboxamide structures showed that chemical modifications on the quinoline ring could be envisaged without loss of affinity at NK-3r.^{19,21} In order to develop ligands for in vivo imaging studies by PET or SPECT, we aimed to design fluoro- and iodoanalogues of SB 223412 that retain the biological activity of the parent compound. Herein, we describe the synthesis of new halogenated methoxy- or hydroxyquinoline carboxamides 1–2 and their in vitro evaluation (Scheme 1).

2. Results and discussion

2.1. Chemistry

Synthesis of the target compounds was based on the Pfitzinger reaction converting isatin into quinolinic acid.^{19,21,22} The previously described isatins **3a–f**, bearing a fluorine or an iodine atom in position 5, 6 or 7 were used as starting materials.^{23,24} The treatment of these latter with 2-methoxyacetophenone under basic conditions led to the corresponding 3-methoxyquinolinic acids **4a–f** in 53–100% yields (Scheme 2). The same reaction from 5-fluoroisatin **3a** and 2-hydroxyacetophenone afforded the corresponding hydroxyacid **5a** in poor yields (34%).

Access to hydroxyacids 5a-f was envisaged by demethylation of methoxyacids 4a-f. In preliminary experiments carried out with HI,^{18,19} yields did not exceed 50%. Then, we turned towards the use of BBr₃; under these conditions, fluorohydroxyacids 5a-c were obtained in high yields (75–93%). In the iodinated series, only the hydroxyacid **5d** substituted in the position 6 was formed in satisfactory yield (70%). Hydroxyacids **5e,f** bearing an iodine in the positions 7 and 8, respectively, could not be isolated.

Methoxy- and hydroxyacids 4-5 were converted into quinoline carboxamides 1-2 by amidation with (S)phenylpropylamine either in the presence of HOBt (1hydroxybenzotriazole) and EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) in CH₂Cl₂, or via an activation with N-hydrosuccinimide in the presence of EDCI in DMF. This step afforded methoxyquinoline carboxamides **1a-f** in yields ranging from 67% to 98%, and the hydroxy analogues 2a-f in only 10–30% yields. Finally, demethylation of methoxycarboxamides 1a-f using BBr₃ was found to be the method of choice to prepare hydroxyacids 2a-f. In both fluoro- and iodo series, yields in **2a**–**f** were in the 64–81% range. Finally, 12 guinoline carboxamides **1a–f** and **2a–f**, bearing either a methoxy or a hydroxy group in the position 3, and either a fluorine or an iodine atom in the positions 6, 7 or 8, were prepared.

2.2. Affinity and selectivity of the synthesized ligands

Affinities of the novel quinoline carboxamides 1-2 at the NK-3 receptor, were evaluated by analyzing the specific displacement of [³H]-senktide binding on CHO cells stably expressing the human recombinant NK-3 receptor (hNK-3r). In each experiment, the results were



Scheme 2. Synthesis of target quinoline carboxamides. Reagents and conditions: (i) KOH, EtOH, 70 °C, 72 h; (ii) EDCI, HCl, Et₃N, HOBt, (*S*)-phenylpropylamine, CH_2Cl_2 , -5 °C for 1 h then rt for 18 h, or (1) EDCI, NHS, DMF, -5 °C for 1 h then rt for 6 h; (2) (*S*)-phenylpropylamine, CH_2Cl_2 , Δ , 4 h; (ii) BBr₃, CH_2Cl_2 , -78 °C for 1.5 h then rt for 3 h; (2) EtOH, -78 °C.

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compared with those obtained on the same in vitro model with endogenous NKB, the senktide itself, the known potent synthetic antagonists SB 223412 and SB 222200²⁵ and finally with endogenous NKA, which exhibits preferential binding to the NK-2 receptors (Table 1).

As described for the SB 223412 reference compound,²⁰ the replacement of the hydroxyl function by a methoxy group in position 3 in the novel halogenated series, did not modify affinities. However, the position and the nature of the halogen atom, was crucial for affinity. The quinolines **1c** and **2c** possessing a fluorine atom in

position 8, displayed similar affinities compared to SB 223412, senktide and NKB, whereas the corresponding iodoanalogues **1f** and **2f** were devoid of significant hNK-3r binding affinity. Compounds **1b**,e and **2b**,e fluorinated or iodinated in position 7, exhibited an approximately 2-fold lower affinity than those of the references. Halogenation, and especially iodination, in position 6 led to a reduction or loss of the binding properties (compounds **1a**,d and **2a**,d).

The haloquinolines **1b**,c,e and **2b**,c,e, which showed the most significant affinities for NK-3r, exhibited no affinity for NK-1r ($K_i > 10\ 000$), determined by specific

Table 1. Binding affinities of the novel quinoline carboxamides compared to NKB, senktide and the parent SB 223412 and SB 222200 compounds at cloned hNK-3 and hNK-1 receptors

Compd	1	Binding affinities (K_i , nM; mean ± SEM) ^a			
		hNK-3 ^b		hNK-1°	
References NKB Senktide SB 223412 SB 222200 NKA SB		$28 (\pm 3) 22 (\pm 1)^d 27 (\pm 9) 31 (\pm 5) >1000 c$		$ \begin{array}{c} 450 (\pm 40) \\ \underline{}^{e} \\ >10,000 \\ >10,000 \\ \underline{}^{e} \\ 4.7 (\pm 1.6) \end{array} $	
Quinolines		1	2	1	2
		(R = Me)	(R = H)	(R = Me)	(R = H)
F OR N Ph	a	162 ± 78	115±15	e	e
F N Ph	b	62±12	49 ± 3	>10,000	>10,000
O NHAIk OR N Ph	с	27±11	24±13	>10,000	>10,000
	d	410±172	>1000	e	c
O NHAIk OR I N Ph	e	57±11	67 ± 26	>10,000	>10,000
OF NHAIk OR N Ph	f	625±35	>1000	c	e

^a Average of two to nine independent determinations (n = 2-9).

^b Inhibition of [³H]-senktide binding on hNK-3r-CHO cell membranes.^{26,27}

^c Inhibition of [¹²⁵I]-Bolton-Hunter SP binding on hNK-3r-CHO cells.

 ${}^{d}K_{d}$ 12 ± 2 nM, determined by three independent saturation experiments.

^eNot determined.

displacement of [¹²⁵I]-BH-SP (Bolton-Hunter Substance P) binding (Table 1). Thus, the selectivity for hNK-3r over hNK-1r was retained after substitution by a fluorine atom in position 7 or 8 or by an iodine in position 7.

3. Conclusion

A new series of halogenated 2-phenylquinoline-4-carboxamides 1–2 was prepared from the corresponding isatins 3. These compounds were evaluated as novel NK-3 ligands. Their binding properties were found strongly dependent on the nature and position of the halogen atom. The fluoro compounds 1b,c and 2b,c and the iodo compounds 1e and 2e displayed a similar or close affinity compared to that of unsubstituted SB 223412 as reference. In view of the in vivo potential use of these quinolines, work is in progress for their labelling with fluorine-18 or iodine-123 for NK-3 receptor imaging studies by PET or SPECT.

4. Experimental

4.1. Chemistry

4.1.1. General considerations. All reagents were used as supplied from commercial sources (Aldrich, Acros or Fluka). Solvents were purchased from Carlo Erba. N,N-Dimethylformamide (DMF) and dichloromethane (CH₂Cl₂) were freshly distilled from calcium hydride under nitrogen prior to use. Pentane, methanol (MeOH), ethanol (EtOH), diethyl ether (Et₂O), tetrahydrofuran (THF) and ethyl acetate (AcOEt) were used as received. ¹H and ¹³C nuclear magnetic resonance spectra were recorded on a Bruker DPX 400 spectrometer at 400 MHz (¹H) or 100.6 MHz (¹³C) or on a Bruker DPX 250 at 250 MHz (¹H) or 62.9 MHz (¹³C), and were calibrated according to the chemical shift of tetramethylsilane. ¹⁹F nuclear magnetic resonance spectra were recorded on a Bruker DPX 400 (376.5 MHz) or Bruker DPX 250 (235 MHz) spectrometer, and were calibrated according to the chemical shift of trifluorotoluene. Chemical shift (δ) are quoted in ppm and coupling constants (J) in Hz. The following abbreviations are used to denote multiplicities: s, singlet; d, doublet; dd, double-doublet; ddd: double-double-doublet; t, triplet; q, quartet; m, multiplet. Infrared spectra were recorded on a Perkin-Elmer 16 PC FT IR spectrophotometer in KBr and peaks are given in cm^{-1} . Low (EI) and high resolution (HRMS, EI) mass spectra were recorded on a JEOL JMS GCMate instrument at 70 eV. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm, concentrations (c) are quoted in g/100 mL. Elemental analyses were performed on a ThermoQuest analyzer CHNS-O, and were within $\pm 0.4\%$ of the calculated values. Melting points (mp) were measured on a Kofler hot-block apparatus. Thin layer chromatography (TLC) was carried out on aluminium or plastic sheets coated with 60 F-254 silica

from Merck (0.1 mm). Plates were revealed with UV detection or by ninhydrine (10% in ethanol) treatment followed by heating. Flash chromatography was carried out using Si 60 (40–63 μ m) silica from Merck.

4.1.2. General procedure for the synthesis of 3-methoxy-4quinolinic acid hydrochlorides 4. A mixture of haloisatin **3** (1 equiv), 2-methoxyacetophenone (1.2 equiv) and KOH pellets (85%, 3 equiv) in ethanol (5–50 mL) was refluxed for 72 h. After concentration under vacuum, the residue was dissolved in H₂O and washed twice with Et₂O. The ice cold aqueous layer was acidified to pH 1 with HCl 37%. The precipitate formed was filtrated, washed with H₂O and dried at 65 °C in an oven to give the expected acid hydrochloride **4**.

4.1.2.1. 6-Fluoro-3-methoxy-2-phenylquinoline-4-carboxylic acid hydrochloride 4a. Acid hydrochloride **4a** was obtained from 5-fluoroisatin **3a** (3.0 g, 18.1 mmol), 2-methoxyacetophenone (3.27 g, 21.8 mmol) and KOH (3.0 g, 53.6 mmol) in EtOH (25 mL): brown solid, mp 214–216 °C; 87% (5.26 g). ¹H NMR (DMSO-*d*₆) δ 3.60 (s, 3H), 7.35–7.62 (m, 4H), 7.67–7.72 (m, 1H), 7.93–7.97 (m, 2H), 8.13–8.18 (m, 1H). ¹³C NMR (DMSO-*d*₆) δ 62.5, 108.4 (d, ²J_{CF} = 23.5 Hz), 120.0 (d, ²J_{CF} = 25.4 Hz), 125.6 (d, ³J_{CF} = 10.4 Hz), 129.3, 129.8, 130.3, 136.7 (d, ⁵J_{CF} = 5.6 Hz), 133.1 (d, ⁴J_{CF} = 9.8 Hz), 137.6, 142.1, 149.1, 154.4, 161.5 (d, ¹J_{CF} = 246.0 Hz), 167.4. ¹⁹F NMR (DMSO-*d*₆) δ –(111.5–111.6) (m). IR (KBr) 1724, 1614, 1504 and 1246. *m*/*z* 297 (M⁺⁻, C₁₇H₁₂FNO₃,100), 296 (99), 222 (43). HRMS (M⁺⁻, C₁₇H₁₂FNO₃) calcd 297.08010, found 297.08123.

4.1.2.2. 7-Fluoro-3-methoxy-2-phenylquinoline-4-carboxylic acid hydrochloride 4b. Acid hydrochloride 4b was obtained from 6-fluoroisatin 3b (5.41 g, 32.9 mmol), 2-methoxyacetophenone (5.93 g, 39.5 mmol) and KOH (5.5 g, 98.2 mmol) in EtOH (40 mL): brown solid, mp 218–222 °C; 90% (9.87 g). ¹H NMR (DMSO-*d*₆) δ 3.58 (s, 3H), 7.52–7.59 (m, 4H), 7.76 (dd, ⁴J_{H5F} = 2.7 Hz, ³J_{H5H6} = 9.9 Hz, 1H), 8.01–8.03 (m, 2H), 8.77 (dd, ⁴J_{H8H6} = 6.2 Hz, ³J_{H8F} = 9.4 Hz, 1H). ¹³C NMR (DMSO-*d*₆) δ 62.1, 113.7 (d, ²J_{CF} = 20.3 Hz), 119.1 (d, ²J_{CF} = 25.4 Hz), 121.8, 127.6 (d, ³J_{CF} = 10.3 Hz), 129.3, 129.4, 129.8, 129.9, 130.4, 133.7, 137.6, 145.7 (d, ³J_{CF} = 12.6 Hz), 147.8 (d, ⁴J_{CF} = 2.8 Hz), 156.0, 158.8 (d, ¹J_{CF} = 248 Hz), 167.5. ¹⁸F NMR (DMSO-*d*₆) δ –(111.1–111.2) (m). IR (KBr) 3422, 1720, 1626, 1232, 1060 and 704. *m*/*z* 297 (M⁺⁺, C₁₇H₁₂FNO₃, 91), 296 (90), 222 (36), 122 (37), 105 (57), 73 (100). HRMS (M⁺⁻, C₁₇H₁₂FNO₃) calcd 297.08010, found 297.08029.

4.1.2.3. 8-Fluoro-3-methoxy-2-phenylquinoline-4-carboxylic acid hydrochloride 4c. Acid hydrochloride 4c was obtained from 7-fluoroisatin 3c (1.35 g, 8.2 mmol), 2-methoxyacetophenone (1.49 g, 9.9 mmol) and KOH (1.50 g, 26.8 mmol) in EtOH (12 mL): brown solid, mp 254 °C (decomposition); 53% (1.45 g). ¹H NMR (DMSO- d_6) δ 3.47 (s, 3H), 7.41–7.54 (m, 6H), 7.83–7.88 (m, 2H). ¹³C NMR (DMSO- d_6) δ 62.0, 113.7 (d,

 ${}^{2}J_{CF} = 19.1 \text{ Hz}$), 120.4 (d, ${}^{3}J_{CF} = 4.3 \text{ Hz}$), 126.1 (d, ${}^{4}J_{CF} = 2.0 \text{ Hz}$), 128.0, 128.8, 129.4, 130.0, 132.8 (d, ${}^{3}J_{CF} = 2.8 \text{ Hz}$), 134.4 (d, ${}^{2}J_{CF} = 12.0 \text{ Hz}$), 137.2, 148.4, 154.6, 156.7 (d, ${}^{1}J_{CF} = 256 \text{ Hz}$), 166.9. ${}^{19}\text{F}$ NMR (DMSO- d_{6}) δ -123.4 (dd, ${}^{4}J_{FH6} = 3.8 \text{ Hz}$, ${}^{3}J_{FH7} = 7.5 \text{ Hz}$). IR (KBr) 2536, 1732, 1468, 1244, 1230, 762 and 702. m/z 297 (M⁺, C₁₇H₁₂FNO₃, 91), 296 (100), 223 (35), 222 (44). HRMS (M⁺, C₁₇H₁₂FNO₃) calcd 297.08010, found 297.08036.

4.1.2.4. 6-Iodo-3-methoxy-2-phenylquinoline-4-carboxylic acid hydrochloride 4d. Acid hydrochloride **4d** was obtained from 5-iodoisatin (2.24 g, 8.22 mmol), 2-meth-oxyacetophenone (1.48 g, 9.9 mmol) and KOH (1.4 g, 25.0 mmol) in EtOH (12 mL): brown solid, mp 202 °C; 100% (3.62 g). ¹H NMR (DMSO-*d*₆) δ 3.57 (s, 3H), 7.43–7.54 (m, 3H), 7.83 (d, ³J_{H7H8} = 8.8 Hz, 1H), 7.93–8.01 (m, 3H), 8.10–8.12 (m, 1H). ¹³C NMR (DMSO-*d*₆) δ 62.5, 95.6, 126.4, 129.3, 129.4, 129.8, 130.1, 130.4, 131.8, 132.0, 133.0, 137.6, 138.3, 143.7, 148.6, 155.5, 167.4. IR (KBr) 3504, 1702, 1448 and 1348. *m/z* 405 (M⁺⁺, C₁₇H₁₂INO₃, 70), 404 (100). HRMS (M⁺⁻, C₁₇H₁₂INO₃) calcd 404.98680, found 404.98511.

4.1.2.5. 7-Iodo-3-methoxy-2-phenylquinoline-4-carboxylic acid hydrochloride 4e. Acid hydrochloride 4e was obtained from 6-iodoisatin 3e (2.24 g, 8.20 mmol), 2-methoxyacetophenone (1.48 g, 9.9 mmol) and KOH (1.4 g, 25.0 mmol) in EtOH (12 mL): light brown solid, mp 230 °C (decomposition); 72% (2.61 g). ¹H NMR (DMSO- d_6) δ 3.59 (s, 3H), 7.55–7.58 (m, 4H), 7.92–7.97 (m, 3H), 8.48 (s, 1H). ¹³C NMR (DMSO- d_6) δ 62.4, 96.3, 124.0, 126.7, 129.3, 129.9, 130.4, 133.9, 136.9, 137.8, 138.3, 145.6, 148.3, 155.6, 167.5. IR (KBr) 3058, 2936, 1716, 1624, 1594, 1350, 1324, 1286, 1234, 1144, 1008, 724 and 698. m/z 405 (M⁺⁺, C₁₇H₁₂INO₃), 100), 404 (69), 97 (21), 84 (23). HRMS (M⁺⁻, C₁₇H₁₂INO₃) calcd 404.98680, found 404.98466.

4.1.2.6. 8-Iodo-3-methoxy-2-phenylquinoline-4-carboxylic acid hydrochloride 4f. Acid hydrochloride 4f was obtained from 7-iodoisatin 3f (1.44 g, 5.29 mmol), 2methoxyacetophenone (0.95 g, 6.35 mmol) and KOH (1.19 g, 21.2 mmol) in EtOH (20 mL), after extraction of the aqueous layer with CH₂Cl₂ (five times), drying over Na₂SO₄, filtration and concentration under vacuum: brown solid, mp 74 °C; 90% (2.09 g). ¹H NMR (DMSO d_6) δ 3.71 (s, 3H), 7.35–7.42 (m, 1H), 7.52–7.61 (m, 3H), 7.96 (dd, ${}^{4}J_{H5H7} = 1.2 \text{ Hz}$, ${}^{3}J_{H5H6} = 9.5 \text{ Hz}$, 1H), 8.25– 8.28 (m, 2H), 8.32 (dd, ${}^{4}J_{H7H5} = 1.2$ Hz, ${}^{3}J_{H6H7} = 7.4$ Hz, 1H). ¹³C NMR (DMSO- d_6) δ 62.1, 104.8, 124.4, 125.3, 128.9, 129.5, 130.1, 133.1, 133.5, 137.0, 139.7, 148.5, 154.4, 166.9, 177.2. IR (KBr) 2936, 1718, 1342, 1196 and 760. m/z 405 (M⁺, C₁₇H₁₂INO₃, 36), 404 (47), 204 (19), 190 (19), 73 (100). HRMS (M⁺, C₁₇H₁₂INO₃) calcd 404.98680, found 404.98622.

4.1.3. General procedure for demethylation of 3-methoxy-4-quinolinic acid hydrochlorides 4. BBr₃ in CH₂Cl₂ (0.7 M, 3.6 equiv) was added dropwise in 1 h to a solution of acid 4 (0.2 M, 1 equiv) in CH₂Cl₂ at -78 °C. After stirring for 1.5 h at -78 °C, then for 3 h at room temperature, the mixture was cooled to -78 °C and ethanol 96% was added. The mixture was concentrated under vacuum and the residue was dissolved in ethanol 96%, then concentrated under vacuum and finally dissolved in a mixture of THF/NaOH aqueous (2 N) 1:1. After stirring for 1 h at 60 °C, HCl 37% was added until pH 1 and a precipitate was formed. The precipitate was filtered off, washed with water and dried in an oven at 65 °C to afford hydroxyacids **5**.

4.1.3.1. 6-Fluoro-3-hydroxy-2-phenylquinoline-4-carboxylic acid hydrochloride 5a. Acid hydrochloride 5a was obtained from methoxyacid 4a (4.0 g, 12.0 mmol) in CH₂Cl₂ (60 mL) and BBr₃ (10.9 g, 43.3 mmol) in CH₂Cl₂ (60 mL): yellow solid, mp 224 °C; 93% (3.55 g). ¹H NMR $(DMSO-d_6)$ δ 7.52–7.60 (m, 4H), 7.77 (dd, ${}^{4}J_{\rm H8F} = 2.7 \,\mathrm{Hz}, \, {}^{3}J_{\rm H8H7} = 9.9 \,\mathrm{Hz}, \, 1\mathrm{H}), \, 8.00-8.02 \,\mathrm{(m, 2H)},$ 8.77 (dd, ${}^{4}J_{H5H7} = 6.2 \text{ Hz}$, ${}^{3}J_{H5F} = 9.5 \text{ Hz}$, 1H). ${}^{13}\text{C}$ NMR (DMSO- d_6) δ 109.1 (d, ${}^{2}J_{CF} = 26$ Hz), 116.0 (d, ${}^{2}J_{CF} = 26$ Hz), 126.0 (d, ${}^{3}J_{CF} = 12$ Hz), 128.3, 129.8, 130.0, 130.1, 131.6 (d, ${}^{3}J_{CF} = 10$ Hz), 136.2, 137.6, 151.5 (d, ${}^{4}J_{CF} = 3$ Hz), 156.4, 161.7 (d, ${}^{1}J_{CF} = 245$ Hz), 171.9. ¹⁹F NMR (DMSO- d_6) δ –111.0 (s). IR (KBr) 1918, 1630, 1600, 1526, 1450, 1406, 1374, 1322, 1292 and 1246. m/z 283 (M⁺, C₁₆H₁₀FNO₃, 29), 265 (32), 238 (100), 237 (72), 208 (22), 183 (18). HRMS $(M^{+}, C_{16}H_{10}FNO_3)$ calcd 283.06445, found 283.06437.

4.1.3.2. 7-Fluoro-3-hydroxy-2-phenylquinoline-4-carboxylic acid hydrochloride 5b. Acid hydrochloride 5b was obtained from methoxyacid 4b (5.1 g, 15.9 mmol) in CH_2Cl_2 (75 mL) and BBr₃ (13.8 g, 55.0 mmol) in CH_2Cl_2 (75 mL): orange solid, mp 228 °C; 92% (4.67 g). ¹H NMR (DMSO- d_6) δ 7.52–7.59 (m, 4H), 7.77 (dd, ${}^{4}J_{\text{H5F}} = 2.7 \,\text{Hz}, \, {}^{3}J_{\text{H5H6}} = 9.9 \,\text{Hz}, \,1\text{H}$), 8.00–8.03 (m, 2H), 8.76 (dd, ${}^{4}J_{H8H6} = 6.2 \text{ Hz}$, ${}^{3}J_{H8F} = 9.4 \text{ Hz}$, 1H). ${}^{13}\text{C}$ NMR (DMSO- d_6) δ 113.4 (d, ${}^2J_{CF} = 20.8 \text{ Hz}$), 116.2, $^{2}J_{\rm CF} = 24.3$ Hz), 123.1, 118.6 128.0 (d, (d, ${}^{3}J_{\rm CF} = 9.2$ Hz), 129.0, 129.4, 129.8, 130.3, 130.5, 130.9, 137.2, 142.5 (d, ${}^{3}J_{CF} = 11.2 \text{ Hz}$), 153.9, 161.0 (d, ${}^{1}J_{CF} = 243 \text{ Hz}$, 172.0. ¹⁹F NMR (DMSO- d_{6}) δ –(115.5– 115.6) (m). IR (KBr) 3448, 1636, 1574, 1522, 1508 and 1320. m/z 283 (M⁺⁻, C₁₆H₁₀FNO₃, 5), 265 (13); 239 (69), 238 (100), 211 (17). HRMS (M⁺, C₁₆H₁₀FNO₃) calcd 283.06445, found 283.05924.

4.1.3.3. 8-Fluoro-3-hydroxy-2-phenylquinoline-4-carboxylic acid hydrochloride 5c. Acid hydrochloride **5c** was obtained from methoxyacid **4c** (0.69 g, 2.1 mmol) in CH₂Cl₂ (12 mL) and BBr₃ (1.86 g, 7.5 mmol) in CH₂Cl₂ (12 mL): yellow solid, mp 150 °C; 75% (0.50 g). ¹H NMR (DMSO-*d*₆) δ 7.35–7.39 (m, 1H), 7.49–7.60 (m, 4H), 8.03–8.06 (m, 2H), 8.49 (d, ³*J*_{H5H6} = 8.7 Hz, 1H). ¹³C NMR (DMSO-*d*₆) δ 111.3 (d, ²*J*_{CF} = 18.2 Hz), 115.7, 121.3 (d, ⁴*J*_{CF} = 4.4 Hz), 128.0, 128.8, 128.9, 130.0, 130.4, 132.3 (d, ³*J*_{CF} = 11.2 Hz), 138.0, 153.0, 155.0, 158.6 (d, ¹*J*_{CF} = 252.1 Hz), 172.0. ¹⁹F NMR (DMSO-*d*₆) δ –(125.1–125.2) (m). IR (KBr) 4374, 4344, 4280, 3060, 1724, 1644, 1448 and 1238. *m*/*z* 283 (M⁺⁺, C₁₆H₁₀FNO₃, 27), 265 (34); 239 (59), 238 (100), 237 (69), 208 (24). HRMS (M⁺⁻, $C_{16}H_{10}FNO_3$) calcd 283.06445, found 283.06523.

4.1.3.4. 3-Hydroxy-6-iodo-2-phenylquinoline-4-carboxylic acid hydrochloride 5d. Acid hydrochloride **5d** was obtained from methoxyacid **4d** (1 g, 2.5 mmol) in CH₂Cl₂ (15 mL) and BBr₃ (1.85 g, 7.4 mmol) in CH₂Cl₂ (15 mL) and BBr₃ (1.85 g, 7.4 mmol) in CH₂Cl₂ (15 mL): yellow solid, mp 236 °C; 70% (0.68 g). ¹H NMR (DMSO-*d*₆) 7.51–7.56 (m, 4H), 7.79 (d, ³J_{H8H7} = 8.7 Hz, 1H), 7.85 (dd, ⁴J_{H7H5} = 1.6 Hz, ³J_{H7H8} = 8.7 Hz, 1H), 8.00–8.04 (m, 2H), 9.27 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ 95.5, 114.0, 128.2, 128.8, 128.9, 130.4, 130.6, 130.7, 131.1, 134.0, 135.3, 153.3, 156.9, 171.9. *m/z* 391 (M⁺, C₁₆H₁₀INO₃, 5), 347 (100), 346 (94), 219 (29), 192 (20), 165 (15), 110 (13), 85 (61). HRMS (M⁺, C₁₆H₁₀INO₃) calcd 390.97055, found 390.96996.

4.1.4. General procedure for amidation of quinolinic acids 4–5. EDCI (2 equiv) was added slowly at $-5 \,^{\circ}$ C to a mixture of Et₃N (2 equiv), (S)-phenylpropylamine (1.1 equiv), HOBt (2 equiv) and acid **4** or **5** (1 equiv) in CH₂Cl₂ (20–40 mL). The mixture was stirred at $-5 \,^{\circ}$ C for 1 h, then at room temperature for 18 h. After washing with aqueous solution of citric acid 5% (three times), then NaHCO₃ 5% (three times) and finally with brine, the organic layer was dried over MgSO₄, filtered off and concentrated under vacuum. Purification by column chromatography (pentane/AcOEt 85:15 containing Et₃N: 1‰) yielded to quinoline carboxamides **1–2**.

4.1.4.1. (S)-N-(1-Phenylpropyl)-6-fluoro-3-methoxy-2phenylquinoline-4-carboxamide 1a. Carboxamide 1a was obtained from methoxyacid 4a (1.35 g, 4.0 mmol): white solid, mp 158 °C; 67% (1.1 g). $[\alpha]_D$ –46.8 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.07 (t, ³J_{HH} = 7.4 Hz, 3H), 1.95– 2.02 (m, 2H), 3.48 (s, 3H), 5.28 (q, ${}^{3}J_{HH} = 8.1$ Hz, 1H), 6.68 (d, ${}^{3}J_{HH} = 8.5$ Hz, 1H), 7.28–7.54 (m, 10H), 7.95– 7.97 (m, 2H), 8.06 (dd, ${}^{4}J_{\rm HF} = 3.1 \,{\rm Hz}, {}^{3}J_{\rm H8H7} = 10.0 \,{\rm Hz},$ 1H). ¹³C NMR (CDCl₃) δ 11.3, 29.5, 56.1, 62.5, 108.7 (d, ${}^{2}J_{\rm CF} = 23.8$ Hz), 119.4 (d, ${}^{2}J_{\rm CF} = 25.9$ Hz), 126.7, 126.8, 127.1, 127.2, 128.1, 129.2, 129.5, 129.7, 132.5 (d, ${}^{3}J_{CF} = 9.4 \text{ Hz}$), 133.7 (d, ${}^{3}J_{CF} = 5.8 \text{ Hz}$), 137.6, 142.7, 149.1, 154.2 (d, ${}^{3}J_{CF} = 2.9 \text{ Hz}$), 161.7 (d, ${}^{14}J_{CF} = 249.2 \text{ Hz}$, 164.7. ${}^{19}\text{F} \text{ NMR} (\text{CDCl}_3) \delta -111.6 (ddd, {}^{4}J_{FH8} = 3 \text{ Hz}, {}^{3}J_{FH5} = 8 \text{ Hz}, {}^{3}J_{FH6} = 10 \text{ Hz}$. IR (KBr) 3262, 2966, 2934, 1636, 1492, 1458, 1446, 1348, 1234, 1186 and 698. m/z 414 (M^{+,}, C₂₆H₂₃FN₂O₂, 16), 385 (23), 281 (19), 280 (100), 237 (17), 149 (13), 86 (49), 84 (77). HRMS (M⁺, C₂₆H₂₃FN₂O₂) calcd 414.17534, found 414.17465. Anal. ($C_{17}H_{12}FNO_3 \cdot \frac{1}{4}H_2O$) C, H, N.

4.1.4.2. (*S*)-*N*-(1-Phenylpropyl)-7-fluoro-3-methoxy-2phenylquinoline-4-carboxamide 1b. Carboxamide 1b was obtained from methoxyacid 4b (2.24 g, 6.7 mmol): white solid, mp 64 °C; 70% (1.93 g). $[\alpha]_D$ –38.0 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 0.93 (t, ³J_{HH} = 7.3 Hz, 3H), 1.78– 1.91 (m, 2H), 3.33 (s, 3H), 5.09–5.13 (q, ³J_{HH} = 8.3 Hz, 1H), 6.65 (d, ³J_{HH} = 8.5 Hz, 1H), 7.22–7.40 (m, 9H), 7.56 (dd, ⁴J_{H8H6} = 2.5 Hz, ³J_{H8F} = 10.0 Hz, 1H), 7.67 (dd, ⁴J_{H5F} = 6.0 Hz, ³J_{H5H6} = 9.2 Hz, 1H), 7.83–7.88 (m, 2H). ¹³C NMR (CDCl₃) 11.3, 29.5, 56.1, 62.5, 113.6 (d, $\label{eq:constraint} \begin{array}{l} ^2J_{\rm CF} = 20.4\,{\rm Hz}), \, 118.2 \, ({\rm d}, \,\,^2J_{\rm CF} = 25.2\,{\rm Hz}), \, 122.6, \, 127.1 \\ ({\rm d}, \,\,^3J_{\rm CF} = 6.1\,{\rm Hz}), \,\, 127.1, \,\, 128.0, \,\, 128.9, \,\, 129.1, \,\, 129.7, \\ 129.9, \,\, 134.7, \,\, 137.6, \,\, 142.1, \,\, 146.3 \,\, ({\rm d}, \,\,^3J_{\rm CF} = 12.6\,{\rm Hz}), \\ 147.9 \, ({\rm d}, \,\,^4J_{\rm CF} = 2.7\,{\rm Hz}), \,\,\, 156.0, \,\,\, 162.9 \,\,\, ({\rm d}, \,\,^1J_{\rm CF} = 149.5\,{\rm Hz}), \,\, 164.8. \,\,^{19}{\rm F}\,\,{\rm NMR}\,\,({\rm CDCl}_3)\,\,-(111.1-111.2)\,\,({\rm m}).\,\,{\rm IR}\,\,({\rm KBr})\,\, 3256, \,2964, \,2934, \,1636, \,1540, \,1504, \\ 1352, \,\, 1216\,\,{\rm and}\,\, 698.\,\,m/z\,\,414\,\,({\rm M}^{+*}, \,\, C_{26}{\rm H_{23}}{\rm FN_2}{\rm O}_2, \,47), \\ 385\,(59), \,281\,\,(54), \,280\,\,(100), \,237\,\,(38), \,210\,\,(33), \,105\,\,(81). \\ {\rm HRMS}\,\,({\rm M}^{+*}, \,\, C_{26}{\rm H_{23}}{\rm FN_2}{\rm O}_2)\,\,{\rm calcd}\,\,\,414.17534, \,\,{\rm found} \\ 414.17345.\,\,{\rm Anal.}\,\,({\rm C}_{26}{\rm H_{23}}{\rm FN_2}{\rm O}_2)\,\,{\rm C},\,{\rm H},\,{\rm N}. \end{array}$

4.1.4.3. (S)-N-(1-Phenylpropyl)-8-fluoro-3-methoxy-2phenylquinoline-4-carboxamide 1c. Carboxamide 1c was obtained from methoxyacid 4c (0.43 g, 1.3 mmol): white solid, mp 68 °C; 73% (0.39 g). $[\alpha]_D$ –44.6 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.03 (t, ³J_{HH} = 7.3 Hz, 3H), 1.90– 1.99 (m, 2H), 3.46 (s, 3H), 5.20 (q, ³J_{HH} = 8.3 Hz, 1H), 6.63 (d, ${}^{3}J_{HH} = 8.5$ Hz, 1H), 7.17–7.54 (m, 11H), 8.00– 8.01 (m, 2H). ${}^{13}C$ NMR (CDCl₃) δ 11.3, 29.6, 56.2, 62.5, 113.3 (d, ${}^{2}J_{CF} = 18.9 \text{ Hz}$), 120.6 (d, ${}^{3}J_{CF} = 4.8 \text{ Hz}$), 127.1, 127.3, 127.5, 127.6, 128.0, 128.9, 129.1, 129.8, ${}^{4}J_{\rm CF} = 2.3$ Hz), 129.9, 134.4 (d, 135.7 (d. ${}^{3}J_{\rm CF} = 12.0 \,{\rm Hz}$, 137.6, 142.0, 149.2, 155.0, 158.5 (d, ${}^{1}J_{CF} = 257.7 \text{ Hz}$, 164.8. ${}^{19}\text{F}$ NMR (CDCl₃) δ -124.3 (ddd, ${}^{5}J_{\text{FH5}} = 3.8 \text{ Hz}, {}^{4}J_{\text{FH6}} = 11.3 \text{ Hz}, {}^{3}J_{\text{FH7}} = 15.1 \text{ Hz}$). IR (KBr) 3422, 3312, 3250, 1636, 1578, 1560, 1540 and 1376. m/z 414 (M⁺, C₂₆H₂₃FN₂O₂, 21), 385 (26), 280 (100), 237 (15), 210 (36), 105 (91). HRMS $(M^{+},$ $C_{26}H_{23}FN_2O_2$) calcd 414.17534, found 414.17553. Anal. $(C_{26}H_{23}FN_2O_2)$ C, H, N.

4.1.4.4. (*S*)-*N*-(1-Phenylpropyl)-6-iodo-3-methoxy-2phenylquinoline-4-carboxamide 1d. Carboxamide 1d was obtained from methoxyacid 4d (0.71 g, 1.60 mmol): white solid, mp 82 °C; 78% (0.65 g). $[\alpha]_{\rm D}$ –62.4 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.09 (t, ³*J*_{HH} = 7.38 Hz, 3H), 2.00–2.06 (m, 2H), 3.53 (s, 3H), 5.28 (q, ³*J*_{HH} = 7.5 Hz, 1H), 6.43 (d, ³*J*_{HH} = 7.6 Hz, 1H), 7.28– 7.55 (m, 8H), 7.84 (d, ³*J*_{HH77} = 8.8 Hz, 1H), 7.91 (dd, ⁴*J*_{H7H5} = 1.9 Hz, ³*J*_{H7H8} = 8.8 Hz), 7.99–8.01 (m, 2H), 8.29 (d, ⁴*J*_{H5H7} = 1.8 Hz, 1H). ¹³C NMR (CDCl₃) δ 9.8, 28.0, 54.8, 61.2, 92.7, 125.7, 125.9, 126.7, 127.5, 127.9, 128.2, 128.4, 130.2, 131.6, 132.3, 136.2, 136.6, 140.5, 143.0, 147.4, 154.1, 163.1. IR (KBr) ν 3250, 2932, 1636, 1534 and 1346. *m*/*z* 523 (MH⁺⁺, C₂₆H₂₃IN₂O₂, 100), 387 (23), 362 (19), 348 (24), 119 (13). HRMS (M⁺⁻, C₂₆H₂₃IN₂O₂) c, H, N.

4.1.4.5. (*S*)-*N*-(1-Phenylpropyl)-8-iodo-3-methoxy-2phenylquinoline-4-carboxamide 1f. Carboxamide 1f was obtained from methoxyacid 4f (0.31 g, 0.70 mmol): white solid, mp 74 °C; 78% (0.65 g). $[\alpha]_D$ –62.4 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.24 (t, ³J_{HH} = 7.2 Hz, 3H), 1.92– 1.99 (m, 2H), 3.52 (s, 3H), 5.21 (q, ³J_{HH} = 8.3 Hz, 1H), 6.50 (d, ³J_{HH} = 8.5 Hz, 1H), 7.14 (dd, ³J_{H6H5} = 8.2 Hz, ³J_{H6H7} = 7.4 Hz, 1H), 7.11–7.39 (m, 5H), 7.48–7.51 (m, 3H), 7.70 (dd, ⁴J_{H5H7} = 1.2 Hz, ³J_{H5H6} = 8.3 Hz, 1H), 8.22–8.26 (m, 3H). ¹³C NMR (CDCl₃) δ 11.3, 29.5, 56.1, 62.6, 104.7, 125.7, 125.8, 127.11, 128.0, 128.8, 128.9, 129.1, 130.0, 130.1, 135.1, 137.4, 139.6, 142.0, 143.8, 149.3, 154.8, 164.7. IR (KBr) 2966, 1636, 1526, 1476, 1446, 1344, 1294, 700 and 692. m/z 522 (M⁺·, C₂₆H₂₃IN₂O₂, 57), 493 (28), 388 (100). HRMS (M⁺·, C₂₆H₂₃IN₂O₂) calcd 522.08044, found 522.0773. Anal. (C₂₆H₂₃IN₂O₂) C, H, N.

4.1.4.6. (S)-N-(1-Phenylpropyl)-7-iodo-3-methoxy-2phenylquinoline-4-carboxamide 1e. EDCI $(0.52 \,\mathrm{g},$ 2.72 mmol) was added by portions at -5 °C to a solution of DMF (15 mL) containing quinolinic acid 4e (1.00 g, and *N*-hydroxysuccinimide $2.27 \,\mathrm{mmol}$ (0.31 g. 2.72 mmol). After stirring at $-5 \,^{\circ}$ C for 1 h, then at room temperature for 6 h, the mixture was concentrated under vacuum. The residue was diluted in ethyl acetate and the organic layer was washed successively with a solution of citric acid 5% (three times), a solution of NaHCO₃ (once) and brine (once), dried over MgSO₄, filtrated off and concentrated under vacuum to afford 1-{[(7-iodo-3methoxy-2-phenylquinolin-4-yl)carbonyl]oxy}pyrrolidine-2,5-dione, which was purified by two consecutive recrystallizations in a mixture of MeOH/Et₂O: white solid, mp 72 °C; 99% (1.13 g). ¹H NMR (CDCl₃) 2.97-2.99 (m, 4H), 3.75 (s, 3H), 7.53–7.55 (m, 3H), 7.92–7.93 (m, 2H), 8.06–8.09 (m, 2H), 8.38 (d, ${}^{4}J_{HH} = 0.8$ Hz, 1H). ¹³C NMR (CDCl₃) δ 26.2, 63.0, 95.4, 123.9, 125.7, 126.0, 129.1, 129.6, 130.3, 137.0, 137.6, 139.1, 145.6, 150.7, 155.5, 161.8, 169.1. IR (KBr) 3242, 2941, 1666, 1534, 1527 and 1346. m/z 502 (M⁺⁻, C₂₁H₁₅IN₂O, 28), 388 (100), 84 (48). HRMS $(M^+, C_{21}H_{15}IN_2O)$ calcd 502.00257, found 502.00169. Anal. (C₂₁H₁₅IN₂O₅) C, H, N. A solution of this pyrrolidine-2,5-dione (0.375 g, and (S)-phenylpropylamine $0.75 \,\mathrm{mmol}$ (0.403 g. 3.0 mmol) in CH₂Cl₂ (5 mL) was refluxed for 4 h. After washing with aqueous solution of citric acid 5% (three times), then NaHCO₃ 5% (three times) and finally with brine, the organic layer was dried over MgSO₄, filtered off and concentrated under vacuum. Purification by column chromatography over silica gel (pentane/AcOEt 85:15) yielded to 1e: white solid, mp $72 \degree C$, 99% $(0.390 \text{ g}). [\alpha]_{D} - 29.4 (c \ 0.5, \text{ MeOH}).$ ¹H NMR (CDCl₃) δ 1.07 (t, ${}^{3}J_{HH} = 7.4$ Hz, 3H), 2.00–2.04 (m, 2H), 3.52 (s, 3H), 5.28 (q, ${}^{3}J_{HH} = 8.2$ Hz, 1H), 6.46 (d, ${}^{3}J_{HH} = 8.5$ Hz, 1H), 7.29-7.41 (m, 1H), 7.42-7.44 (m, 4H), 7.51-7.53 (m, 3H), 7.63 (d, ${}^{3}J_{H5H6} = 8.9$ Hz, 1H), 7.77 (d, ${}^{3}J_{\text{H6H5}} = 8.9 \text{ Hz}, 1\text{H}$, 7.98–8.00 (m, 2H), 8.57 (d, ${}^{4}J_{\text{H8H6}} = 1.6 \text{ Hz}, 1\text{H}$). ${}^{13}\text{C}$ NMR (CDCl₃) δ 9.9, 27.9, 54.6, 60.9, 93.5, 123.3, 124.6, 125.7, 126.6, 127.4, 127.7, 128.3, 128.5, 133.0, 134.9, 136.0, 137.2, 140.6, 144.4, 147.1, 154.1, 163.2. IR (KBr) 3238, 3224, 3144, 3056, 3028, 2962, 2932, 2854, 1632, 1544, 1540 and 696. m/z522 (M⁺·, C₂₆H₂₃IN₂O₂, 22), 493 (26), 388 (100), 84 (58). HRMS $(M^+, C_{26}H_{23}IN_2O_2)$ calcd 522.08044, found 522.07837. Anal. (C₂₆H₂₃IN₂O₂) C, H, N.

4.1.5. Synthesis of 3-hydroxy-4-quinoline carboxamides 2 by demethylation of 3-methoxy-4-quinoline carboxamides 1. Demethylation reactions from 1 were carried out according to the procedure described in the quinolinic acid series.

4.1.5.1. (S)-N-(1-Phenylpropyl)-6-fluoro-3-hydroxy-2phenylquinoline-4-carboxamide 2a. Carboxamide 2a was obtained from methoxycarboxamide 1a (0.200 g, 0.48 mmol) in CH_2Cl_2 (10 mL) and BBr_3 (0.250 g, 1.00 mmol) in CH₂Cl₂ (5 mL): white solid, mp 158 °C; 75% (0.144 g). $[\alpha]_{\rm D}$ -27.0 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.08 (t, ³J_{HH} = 7.4 Hz, 3H), 1.95–2.09 (m, 2H), 5.19 (q, ${}^{3}J_{HH} = 7.7$ Hz, 1H), 6.80 (d, ${}^{3}J_{HH} = 8.1$ Hz, 1H), 7.27–7.54 (m, 9H), 7.56 (dd, ${}^{4}J_{H5H7} = 2.4 \text{ Hz}$, ${}^{3}J_{\text{H5F}} = 10.6 \text{ Hz}, 1 \text{H}$), 7.98–8.01 (m, 3H), 11.2 (s, 1H). ¹³C NMR (CDCl₃) δ 11.4, 29.4, 56.7, 106.6 (d, ${}^{2}J_{\rm CF} = 24.7$ Hz), 116.7 (d, ${}^{2}J_{\rm CF} = 25.1$ Hz), 125.0, 125.1, 127.2, 128.4, 128.6, 129.5, 129.8, 130.0, 133.8 (d, ${}^{3}J_{CF} = 9.9 \text{ Hz}$), 136.9, 140.1, 141.2, 151.9, 161.6 (d, ${}^{1}J_{CF} = 249.4 \text{ Hz}$, 168.1. ¹⁹F NMR (CDCl₃) δ -110.1 (ddd, ${}^{4}J_{\text{FH8}} = 7.1 \text{ Hz}, {}^{3}J_{\text{FH6}} = 14.1 \text{ Hz}, {}^{3}J_{\text{FH5}} = 11.8 \text{ Hz}$). IR (KBr) 3746, 3530, 3448, 3966, 1624, 1586, 1560 and 1544. m/z 400 (M⁺, C₂₅H₂₁FN₂O₂, 20), 282 (35), 265 (41), 237 (78), 135 (53), 119 (55), 91 (100), 84 (81). HRMS $(M^{+}, C_{25}H_{21}FN_2O_2)$ calcd 400.15868, found 400.15845. Anal. (C₂₅H₂₁FN₂O₂) C, H, N.

4.1.5.2. (S)-N-(1-Phenylpropyl)-7-fluoro-3-hydroxy-2phenylquinoline-4-carboxamide 2b. Carboxamide 2b was obtained from methoxycarboxamide **1b** (0.400 g, 0.97 mmol) in CH₂Cl₂ (20 mL) and BBr₃ (0.500 g, 2.00 mmol) in CH₂Cl₂ (10 mL): white solid, mp 112 $^{\circ}$ C; 81% (0.315 g). $[\alpha]_{\rm D}$ =24.0 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.05 (t, ³J_{HH} = 7.3 Hz, 3H), 1.99–2.11 (m, 2H), 5.26 (q, ${}^{3}J_{HH} = 7.7$ Hz, 1H), 6.63 (d, ${}^{3}J_{HH} = 7.8$ Hz, 1H), 7.34-7.46 (m, 4H), 7.50-7.56 (m, 3H), 7.81 (dd, ${}^{4}J_{\rm H5F} = 2.7 \,{\rm Hz}, {}^{3}J_{\rm H5H6} = 9.7 \,{\rm Hz}, {}^{1}{\rm H}, {}^{8}{\rm .50}$ (dd, ${}^{4}J_{\text{H8H6}} = 5.6 \text{ Hz}, {}^{3}J_{\text{H8F}} = 9.3 \text{ Hz}, 1\text{H}), 8.07-8.09 \text{ (m, 2H)},$ 10.9 (sl, 1H). ¹³C NMR (CDCl₃) δ 11.3, 29.5, 56.7, 115.3 (d, ${}^{2}J_{CF} = 20.2 \text{ Hz}$), 118.6 (d, ${}^{2}J_{CF} = 24.7 \text{ Hz}$), 121.1, 123.8 (d, ${}^{3}J_{CF} = 8.9 \text{ Hz}$), 127.1, 128.5, 128.7, 129.5, 130.0, 130.1, 137.0, 141.1, 144.1 (d, ${}^{3}J_{CF} = 12.9 \text{ Hz}$), 153.6, 161.29 (d, ${}^{1}J_{CF} = 248 \text{ Hz}$), 168.0. ${}^{19}\text{F}$ NMR (CDCl₃) δ -(114.6-114.7) (m). IR (KBr) 3318, 3058, 3028, 2964, 2932, 1624, 1578, 1216 and 698. m/z 400 $(M^{+}, C_{25}H_{21}FN_2O_2, 24), 282 (52), 265 (58), 237 (41),$ 119 (63), 106 (39), 91 (100). HRMS $(M^{+}, C_{25}H_{21}FN_2O_2)$ calcd 400.15868, found 400.15811. Anal. $(C_{25}H_{21}FN_2O_2)$ C, H, N.

4.1.6. (S)-N-(1-Phenylpropyl)-8-fluoro-3-hydroxy-2-phenylquinoline-4-carboxamide 2c. Carboxamide 2c was obmethoxycarboxamide tained from 1c $(0.200 \,\mathrm{g})$ 0.48 mmol) in CH_2Cl_2 (10 mL) and BBr_3 (0.250 g, 1.00 mmol) in CH₂Cl₂ (5 mL): white solid, mp 136 °C; 65% (0.126 g). $[\alpha]_D$ –7.6 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.00 (t, ³J_{HH} = 7.4 Hz, 3H), 2.00–2.09 (m, 2H), 5.25 (q, ${}^{3}J_{HH} = 7.6$ Hz), 6.74 (d, ${}^{3}J_{HH} = 8.0$ Hz, 1H), 7.21–7.52 (m, 10H), 7.78 (d, ${}^{3}J_{\text{H5H6}} = 8.6$ Hz, 1H), 8.11–8.13 (m, 2H), 11.4 (sl, 1H). 13 C NMR (CDCl₃) δ 11.3, 29.5, 53.7, 111.5 (d, ${}^{2}J_{CF} = 19.4 \text{ Hz}$), 116.3, 117.6 (d, ${}^{4}J_{CF} = 4.8 \text{ Hz}$), 126.1, 127.1, 128.4, 128.6, 128.8 (d, ${}^{3}J_{\rm CF} = 8.9 \,{\rm Hz}$, 129.5, 130.0, 130.3, 133.3 (d, ${}^{3}J_{\rm CF} = 11.5 \,{\rm Hz}$), 137, 141.1, 152.3, 152.5, 159.4 (d, ${}^{1}J_{CF} = 258 \text{ Hz}$, 168.1. ${}^{19}\text{F}$ NMR (CDCl₃) δ -(124.3-124.4) (m). IR (KBr) 3448, 3442, 3384, 1734, 1718, 1648, 1624, 1578, 1570, 1560, 1540 and 1534. m/z 400 (M⁺⁻, C₂₅H₂₁FN₂O₂, 18), 282 (348), 265 (42), 237 (79), 135 (54), 119 (58), 91 (100), 84 (59). HRMS (M⁺,

 $C_{25}H_{21}FN_2O_2$) calcd 400.15868, found: 400.15886. Anal. ($C_{25}H_{21}FN_2O_2$) C, H, N.

4.1.6.1. (S)-N-(1-Phenylpropyl)-6-iodo-3-hydroxy-2phenylquinoline-4-carboxamide 2d. Carboxamide 2d was obtained from methoxycarboxamide 1d (0.050 g. 0.0958 mmol) in CH_2Cl_2 (6 mL) and BBr_3 (0.100 g, 0.398 mmol) in CH_2Cl_2 (3 mL) after purification by column chromatography (pentane/AcOEt 90:10): white solid, mp 85 °C; 64% (0.031 g). $[\alpha]_{\rm D}$ -26.4 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.07 (t, ³J_{HH} = 7.4 Hz, 3H), 2.01–2.10 (m, 2H), 5.25 (q, ${}^{3}J_{HH} = 7.6$ Hz, 1H), 6.67 (d, ${}^{3}J_{\text{HH}} = 8.2 \text{ Hz}, 1\text{H}$), 7.37–7.53 (m, 8H), 7.82 (dd, ${}^{4}J_{\rm H7H5} = 1.6 \,{\rm Hz}, {}^{3}J_{\rm H7H8} = 8.7 \,{\rm Hz}, {}^{1}{\rm H},$ 7.86 (d, ${}^{3}J_{\text{H8H7}} = 8.7 \text{ Hz}, 1\text{H}$), 8.08 (m, 2H), 8.45 (d, ${}^{4}J_{\text{H5H7}} = 1.1 \text{ Hz}, 1\text{H}$). ${}^{13}\text{C}$ NMR (CDCl₃) δ 11.3, 29.6, 56.6, 94.9, 115.0, 126.0, 127.0, 128.4, 128.7, 129.6, 130.0, 130.1, 131.2, 132.9, 135.7, 136.9, 141.2, 142.0, 151.9, 152.6, 153.0, 167.8. IR (KBr) 2852, 1636, 1576, 1492, 1294, 1182 and 698. m/z 508 (M⁺⁻, C₂₅H₂₁IN₂O₂, 22), 390 (33), 373 (37), 149 (54), 85 (79), 72 (100). HRMS $(M^+, C_{25}H_{21}IN_2O_2)$ calcd 508.06479, found: 508.06158.

4.1.6.2. (S)-N-(1-Phenylpropyl)-7-iodo-3-hydroxy-2phenylquinoline-4-carboxamide 2e. Carboxamide 2e was obtained from methoxycarboxamide 1e (0.193 g, 0.37 mmol) in CH_2Cl_2 (10 mL) and BBr_3 (0.370 g, 1.47 mmol) in CH₂Cl₂ (6 mL): white solid, mp $112 \,^{\circ}$ C; 67% (0.126 g). $[α]_D$ -6.6 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.01 (t, ³J_{HH} = 7.4 Hz, 3H), 1.94–2.10 (m, 2H), 5.20 (q, ³J_{HH} = 7.7 Hz, 1H), 6.67 (d, ³J_{HH} = 8.1 Hz, 1H), 7.33-7.42 (m, 5H), 7.48-7.52 (m, 3H), 7.64 (d, ${}^{4}J_{\rm H6H8} = 1.8$ Hz, ${}^{3}J_{\rm H5H6} = 8.9, 1 \,{\rm H}), 7.73$ (dd, ${}^{3}J_{\text{H6H5}} = 9.0 \text{ Hz}, 1\text{H}, 8.03-8.06 \text{ (m, 2H)}, 8.48 \text{ (d,}$ ${}^{4}J_{\text{H8H6}} = 1.7 \text{ Hz}, 1 \text{H}$, 11.17 (sl, 1H). ${}^{13}\text{C} \text{ NMR} (\text{CDCl}_3)$ δ 11.3, 29.4, 56.6, 91.4, 116.5, 123.3, 123.4, 127.1, 128.5, 128.6, 129.5, 130.0, 130.1, 136.8, 137.0, 140.0, 141.0, 143.8, 151.6, 153.0, 167.9. IR (KBr) 3854, 3448, 3344, 1624, 1560 and 698. m/z 508 (M⁺⁻, C₂₅H₂₁IN₂O₂, 12), 390 (33), 373 (26), 149 (30), 119 (36), 91 (68), 84 (100). HRMS (M⁺, C₂₅H₂₁IN₂O₂) calcd 508.06479, found: 508.06581. Anal. (C₂₅H₂₁IN₂O₂) C, H, N.

4.1.6.3. (*S*)-*N*-(**1**-Phenylpropyl)-8-iodo-3-hydroxy-2phenylquinoline-4-carboxamide **2f**. Carboxamide **2e** was obtained from methoxycarboxamide **1e** (0.034 g, 0.065 mmol) in CH₂Cl₂ (5 mL) and BBr₃ (0.400 g, 1.59 mmol) in CH₂Cl₂ (10 mL): white solid, mp 74 °C; 73% (0.024 g). $[\alpha]_D$ –24.8 (*c* 0.5, MeOH). ¹H NMR (CDCl₃) δ 1.00 (t, ³J_{HH} = 7.4 Hz, 3H), 1.95–2.09 (m, 2H), 5.19 (q, ³J_{HH} = 7.7 Hz, 1H), 6.64 (d, ³J_{HH} = 7.9 Hz, 1H), 7.19 (t, ³J_{H6H5} = ³J_{H6H7} = 8.1 Hz, 1H), 7.25–7.42 (m, 5H), 7.48–7.54 (m, 3H), 7.96 (d, ³J_{H5H6} = 8.2 Hz, 1H), 8.18 (dd, ⁴J_{H7H5} = 0.7 Hz, ³J_{H7H6} = 8.1 Hz, 1H), 8.34 (m, 2H), 11.34 (sl, 1H). ¹³C NMR (CDCl₃) δ 10.9, 29.1, 56.3, 106.5, 116.4, 122.3, 124.1, 126.7, 128.0, 128.2, 129.0, 129.1, 129.8, 130.2, 136.4, 137.2, 140.7, 141.1, 151.8, 151.9, 167.6. IR (KBr) 2958, 2954, 2852, 2358, 1716, 1700, 1684, 1652, 1622 and 698. *m*/*z* 508 (M⁺, C₂₅H₂₁IN₂O₂, 14), 390 (33), 373 (20), 149 (32), 119 (36), 91 (70), 84 (100). HRMS (M^{+} , $C_{25}H_{21}IN_2O_2$) calcd 508.06479, found: 508.06466.

4.2. Biology

Receptor binding assays were performed with crude membranes from Chinese hamster ovary (CHO) cells expressing the human NK-3 receptor (hNK-3r-CHO) purchased from Amersham Biosciences or with CHO cells stably transfected with the human NK-1 receptor (hNK-1r-CHO) cDNA (generous gift from Dr. J. Grassi and Prof. J. Y. Couraud, CEA Saclay) plated in 24-well plates. [³H]-Senktide and [¹²⁵I]-Bolton-Hunter-substance P ([¹²⁵I]-BH-SP) used as radioligands were supplied by Perkin Elmer Life Sciences. The time necessary to reach the steady state was previously determined by kinetic studies; the radioligand dissociation constant (K_d) and the maximum number of binding sites (B_{max}) were obtained by saturation binding experiments.

4.2.1. Radioligand binding assays. Briefly, for hNK3-r competition studies, hNK-3r-CHO membranes (\sim 7 µg of protein) were incubated with 3 nM [³H]-senktide in a total volume of 170 µL of 50 mM Tris HCl (pH7.4), 3 mM MnCl₂, 1 µM phosphoramidon, 40 µg/mL bacitracin and 0.5% bovine serum albumin (BSA), with or without various concentrations of tested compounds, for 60 min at 25 °C. Incubations were stopped by rapid filtration with a Brandell tissue harvester through Whatman GF/C filters that were presoaked for at least 60 min in 0.5% BSA. Membranes were washed three times with ice-cold 50 mM Tris HCl (pH7.4) and bound radioactivity (dpm) was determined by using liquid scintillation counting.

For hNK-1r competition studies, CHO cells expressing hNK-1r (2×10^5 cells/well) were cultured as previously described²⁸ and washed three times with Krebs buffer (120 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO₄, 70 mM NaH₂PO₄, 1.6 mM CaCl₂, 0.04 mM BSA, 30 mg/mL bacitracin and 6 g/L glucose). Cells were then incubated for 100 min at room temperature (until equilibrium) in presence of 15 pM [¹²⁵I]-BH-SP and increasing concentrations of cold competing ligands in a final volume of 200 µL. Finally, cells were washed three times in cold Krebs buffer, lysed with 0.1% Triton X100 containing 1 mg/mL BSA overnight at 4 °C°C and the radioactivity was counted with a Cobra Auto-Gamma counter ard).

Concentration–response curves for each compound, were run using duplicate samples in at least two independent experiments following curve-fitting procedures in SigmaPlot. Specific binding was determined by subtracting total binding from nonspecific binding, which was assessed as the binding in the presence of $10 \,\mu\text{M}$ NKB or SP. Percent inhibition of specific binding was determined for each compound concentration and the IC₅₀ value defined as the concentration required to inhibit 50% of the specific binding, obtained from concentration–response curves. Values presented in Table 1 were the inhibition constants (K_i), which were calculated from the IC_{50} according to Cheng and Prusoff procedure.^29

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