Lithiation of Functionalized Fluoroquinolines: Synthesis of Dihalo-2-phenylquinoline-4-carboxamides and In Vitro Evaluation as NK-3 Receptor

Ligands for Imaging Studies

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CHEMISTRY.

General Considerations. Tetrahydrofuran (THF) was dried by reflux over sodium/benzophenone. Dichloromethane (CH_2Cl_2) was distilled over calcium hydride before use for demethylation reaction. Pentane, methanol, ethanol and ethyl acetate were used as received. ^1H nuclear magnetic resonance spectra were recorded at 400 or 250 MHz and ^{13}C nuclear magnetic resonance spectra were recorded at 100.6 or 62.9 MHz. They were calibrated according to the chemical shift of tetramethylsilane. ^{19}F nuclear magnetic resonance spectra were recorded at 376.5 or 235 MHz, and were calibrated according to the chemical shift of α,α,α -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; br, broad; a, apparent. Infrared spectra were recorded in KBr and peaks are given in cm^{-1} . Optical rotations were measured on a polarimeter with a path length of 1 dm, concentrations (c) are quoted in g/100 mL. Thin layer chromatography (TLC) was carried out on aluminum or plastic silica gel 60 F-254 plates (0.1 mm), with UV detection or ninhydrine (10% in ethanol) treatment followed by heating. Flash chromatography was carried out using Si 60 (40-63 μm) silica.

(*S*)-*N*-(1-Phenylpropyl)-7-fluoro-8-iodo-3-methoxy-2-phenylquinoline-4-carboxamide (*S*)-6b**.** The quinoline (*S*)-**6b** (0.105 g, 78% yield) was obtained from (*S*)-*N*-(1-phenylpropyl)-7-fluoro-3-methoxy-2-phenylquinoline-4-carboxamide (*S*)-**2b** (0.10 g, 0.25 mmol) and isolated, after purification by chromatography on silica gel (eluent CH_2Cl_2) and recrystallization from pentane, as a white solid: mp 152 °C; $[\alpha]_{\text{D}} = -18.4$ ($c = 0.5$ MeOH); ^1H NMR (CDCl_3) δ 8.28-8.25 (m, 2H), 7.79 (dd, $J = 5.9, 9.1$ Hz, 1H), 7.57-7.51 (m, 3H), 7.43-7.38 (m, 4H), 7.35-7.32 (m, 1H), 7.21 (dd, $J = 7.3, 9.1$ Hz, 1H), 6.60 (d, $J = 8.2$ Hz, 1H), 5.23 (q, $J = 8.2$ Hz, 1H), 3.53 (s, 3H),

2.05-1.94 (m, 2H), 1.05 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 164.6, 163.2 (d, $J = 248$ Hz), 155.6, 148.8 (d, $J = 3.1$ Hz), 144.8 (d, $J = 6.6$ Hz), 142.0, 137.1, 135.3 (d, $J = 1.2$ Hz), 130.3, 130.2, 129.1, 128.8, 128.0, 127.2, 127.1, 122.6, 117.5 (d, $J = 27.9$ Hz), 88.9 (d, $J = 23.2$ Hz), 62.6, 56.1, 29.5, 11.4; ^{19}F NMR δ -86.9 (dd, $J = 5.9, 7.0$ Hz); IR (KBr) ν 3270, 2960, 2355, 1643, 1556, 1346, 1319, 1211, 1129, 1028, 732, 690 cm^{-1} . Anal. calcd for $\text{C}_{26}\text{H}_{22}\text{FIN}_2\text{O}_2$: C, 57.79; H, 4.10; N, 5.18; found: C, 57.61; H, 4.29; N, 5.15.

(S)-N-(1-Phenylpropyl)-8-fluoro-7-iodo-3-methoxy-2-phenylquinoline-4-carboxamide (S)-

6c. The quinoline (S)-**6c** (76 mg, 28% yield) was obtained from (S)-N-(1-phenylpropyl)-8-fluoro-3-methoxy-2-phenylquinoline-4-carboxamide (S)-**2c** (0.20 g, 0.50 mmol) and isolated, after purification by chromatography on silica gel (eluent CH_2Cl_2 containing 0.1% of Et_3N) and recrystallization from pentane, as a white solid: mp 82 °C; $[\alpha]_{\text{D}} = -28.6$ ($c = 0.5$ MeOH); ^1H NMR (CDCl_3) δ 8.01-7.98 (m, 2H), 7.64 (dd, $J = 5.7, 8.9$ Hz, 1H), 7.49-7.47 (m, 3H), 7.36-7.24 (m, 6H), 6.73 (d, $J = 8.4$ Hz, 1H), 5.14 (q, $J = 8.0$ Hz, 1H), 3.41 (s, 3H), 1.94-1.89 (m, 2H), 1.00 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 164.3, 158.2 (d, $J = 256$ Hz), 155.4, 149.4, 142.0, 137.1, 136.5, 135.0 (d, $J = 13.4$ Hz), 134.4 (d, $J = 2.2$ Hz), 130.2, 130.0, 129.1, 128.9, 128.0, 127.4, 127.1, 121.6 (d, $J = 5.3$ Hz), 80.3 (d, $J = 24.4$ Hz), 62.4, 56.3, 29.5, 11.4; ^{19}F NMR δ -101.9 (dd, $J = 1.0, 5.7$ Hz); IR (KBr) ν 3271, 2966, 2934, 1636, 1550, 1457, 1350, 1304, 1224, 1112, 1054, 887, 756, 698 cm^{-1} . Anal. calcd for $\text{C}_{26}\text{H}_{22}\text{FIN}_2\text{O}_2$: C, 57.79; H, 4.10; N, 5.18; found: C, 57.92; H, 4.23; N, 5.10.

(S)-N-(1-phenylpropyl)-7-fluoro-3-hydroxy-8-iodo-2-phenylquinoline-4-carboxamide (S)-

7b. The quinoline (S)-**7b** (42 mg, 94% yield) was obtained from (S)-N-(1-phenylpropyl)-7-fluoro-

8-iodo-3-methoxy-2-phenylquinoline-4-carboxamide (*S*)-**6b** (46 mg, 0.085 mmol), and isolated, after recrystallization from MeOH/pentane, as a light brown solid: mp 166-168 °C; $[\alpha]_D = -12.0$ ($c = 0.2$ MeOH); ^1H NMR (CDCl_3) δ 10.99 (s, 1H), 8.35-8.33 (m, 2H), 7.93 (dd, $J = 9.2, 5.6$ Hz, 1H), 7.54-7.24 (m, 9H), 6.55 (d, $J = 7.8$ Hz, 1H), 5.21 (q, $J = 7.5$ Hz, 1H), 2.08-1.96 (m, 2H), 1.01 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 167.7, 161.9 (d, $J = 246$ Hz), 153.1, 151.4, 142.6 (d, $J = 6.2$ Hz), 141.0, 136.6, 130.6, 130.4, 129.5, 128.7, 128.5, 127.1, 124.0 (d, $J = 9.3$ Hz), 121.2, 117.7 (d, $J = 27.7$ Hz), 117.5, 91.1 (d, $J = 23.0$ Hz), 56.8, 29.4, 11.3; ^{19}F NMR (CDCl_3) δ -90.2 (t, $J = 6.0$ Hz); IR (KBr) ν 3556, 1684, 1654, 1624, 1610, 1560, 1544 cm^{-1} ; EI-MS (220 °C, 70 eV, 300 mA) m/z 526 (M^+ , 69), 408 (58), 391 (45), 363 (22), 208 (23), 119 (87), 106 (27), 91 (100); HRMS calcd for $\text{C}_{25}\text{H}_{20}\text{FIN}_2\text{O}_2$: 526.0553, found: 526.0558.

(*S*)-*N*-(1-phenylpropyl)-8-fluoro-3-hydroxy-7-iodo-2-phenylquinoline-4-carboxamide (*S*)-7c.

The quinoline (*S*)-**7c** (36 mg, 62% yield) was obtained from ((*S*)-*N*-(1-phenylpropyl)-7-fluoro-8-iodo-3-methoxy-2-phenylquinoline-4-carboxamide (*S*)-**6c** (60 mg, 0.11 mmol), and isolated, after purification by chromatography on silica gel (eluent CH_2Cl_2), as a white solid: mp 160-162 °C; $[\alpha]_D = -5.2$ ($c = 0.5$ MeOH); ^1H NMR (CDCl_3) δ 11.32 (s, 1H), 8.11-8.09 (m, 2H), 7.75 (dd, $J = 5.7, 9.0$ Hz, 1H), 7.52-7.47 (m, 4H), 7.43-7.32 (m, 5H), 6.65 (d, $J = 8.0$ Hz, 1H), 5.18 (q, $J = 7.6$ Hz, 1H), 2.09-1.96 (m, 2H), 1.01 (t, $J = 7.4$ Hz, 3H); ^1H NMR ($\text{MeOH-}d_4$) δ 8.00-7.95 (m, 2H), 7.72 (dd, $J = 5.8, 8.9$ Hz, 1H), 7.55-7.52 (m, 3H), 7.46-7.44 (m, 2H), 7.41-7.37 (m, 2H), 7.32-7.29 (m, 1H), 7.19 (d, $J = 8.9$ Hz, 1H), 5.51 (t, $J = 7.4$ Hz, 1H), 1.92 (dq, $J = 7.4, 7.3$ Hz, 2H), 1.04 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($\text{MeOH-}d_4$) δ 166.2, 158.3 (d, $J = 254$ Hz), 153.8, 146.8, 142.9, 137.3, 136.0, 132.7 (d, $J = 13.9$ Hz), 129.8, 129.6, 128.6, 128.3, 127.9, 127.3, 126.9, 120.6 (d, $J = 5.2$ Hz), 76.8 (d, $J = 24.3$ Hz), 56.3, 29.5, 10.5; ^{19}F NMR (CDCl_3) δ -100.5 (d, $J = 5.7$

Hz);•IR (KBr) ν 3368, 1636, 1624, 1618, 1560, 1544, 1534, 1522, 1458, 696 cm^{-1} ; EI-MS (220 °C, 70 eV, 300 mA) m/z 526 (M^+ , 13), 408 (33), 390 (29), 363 (15), 119 (63), 91 (100); HRMS calcd for $C_{25}H_{20}FIN_2O_2$: 526.05536, found: 526.05693.

BIOLOGY.

General Considerations. 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) (generous gift from Dr J. Grassi and Pr J.Y. Couraud, CEA Saclay) plated in 24-well plates. [^3H]-Senktide and [^{125}I]-Bolton-Hunter-substance P ([^{125}I]-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.

Radioligand Binding Assays. Briefly, for hNK3-r competition studies, hNK-3r-CHO membranes ($\sim 7 \mu\text{g}$ of protein) were incubated with 3 nM [^3H]-senktide in a total volume of 170 μL of 50 mM Tris HCl (pH 7.4), 3 mM MnCl_2 , 1 μM phosphoramidon, 40 $\mu\text{g/mL}$ bacitracin and 0.5 % bovine serum albumin (BSA), with or without various concentrations of test 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 (pH 7.4) 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 described¹ 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 μ L. 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 and the radioactivity was counted with a Cobra Auto-Gamma counter (Packard).

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 non-specific binding, which was assessed as the binding in the presence of 10 μ 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 IC₅₀ according to Cheng and Prusoff procedure².

References and Notes.

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