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Lithiation of Functionalized Fluoroquinolines: Synthesis of Dihalo-2-phenylquinoline-4-carboxamides and in Vitro Evaluation as NK-3 Receptor Ligands for Medical Imaging Studies

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Abstract: Fluoroiodo-2-phenylquinoline-4-carboxamides, analogues of NK-3 antagonist SB 223412, were synthesized and evaluated as NK-3 ligands with the aim of developing radioligands suitable for both Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) studies. The key step utilizes metalation directed by the fluorine atom for iodination of the quinoline ring.

The NK-3 receptor belongs to the seven-transmembrane G-protein coupled receptor family and mediates the action of neurokinin B (NKB).[1] This neurotransmitter produces an array of biological responses including regulation of pain transmission and neurogenic inflammation, and it has been proposed to play a physiopathological role in a range of central (CNS) and peripheral (PNS) nervous system disorders such as anxiety, epilepsy, schizophrenia, Parkinson's disease, emesis, and asthma.[2] To study the biodistribution and the quantification of this receptor in living brain using medical imaging, we recently prepared a series of fluoro- and iodo-2-phenylquinoline-4-carboxamides as NK-3 receptor ligands (Figure 1).[3] These new molecules are analogues of SB 223412 (*S*)-**1** (Talnetant),[4] a highly potent and selective human NK-3 antagonist (CHO h-NK-3; $K_i = 1.0$ nM). The fluoro compounds (*S*)-**2** and (*S*)-**3** were synthesized with the aim of developing radioligands for Positron Emission Tomography (PET), a very sensitive and quantitative imaging technique. The iodo analogues (*S*)-**4** and (*S*)-**5** were prepared for further developments by Single Photon Emission Computed Tomography (SPECT), which is routinely employed in nuclear medicine centers. SPECT differs from PET by a coarser spatial and temporal resolution, reduced sensitivity, and less quantification capability. However, SPECT uses easily available gamma emitter (in our case iodine-123, $t_{1/2} = 13.2$ h), whereas PET requires high cost facilities due to the use of tracers labeled with positron emitters (in our case, fluorine-18, $t_{1/2} = 109.7$ min) produced by a cyclotron. Here, we report the synthesis of the 2-phenylquinoline-4-carboxamides (*S*)-**6** and (*S*)-**7** bearing a fluorine and an iodine atom and their in vitro evaluation as NK-3 ligands. Such a ligand, suitable for a labeling either with a positron or with a gamma emitter, would allow imaging studies by PET that could validate further developments in SPECT.

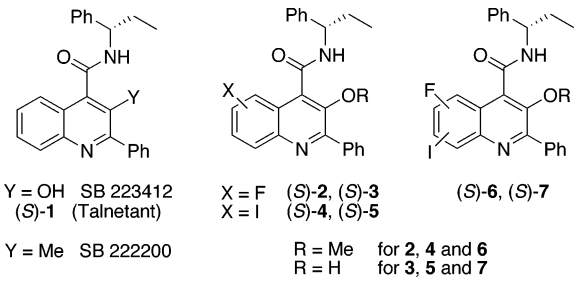

FIGURE 1. NK-3 antagonist SB 223412, its monohalogenated analogues for PET or SPECT, and the dihalo target molecules for PET and SPECT.

Lithiation is a useful method for functionalizing various aromatic or heteroaromatic cycles since lithiated derivatives display a high reactivity toward many electrophilic reagents.[5] Fluorine being a good directing group, and having in hand the fluoro-2-phenylquinoline-4-carboxamides (*S*)-**2** and (*S*)-**3**,[3] we undertook the study of their conversion into the dihalo analogues (*S*)-**6** and (*S*)-**7** following a lithiation−iodination sequence. To our knowledge, the directed ortho-metalation (DoM) strategy in the fluoroquinoline series mainly involves the deprotonation onto the pyridine ring.[6] A few deprotonations on the benzene ring were reported.[6e,f,7] In these cases, the

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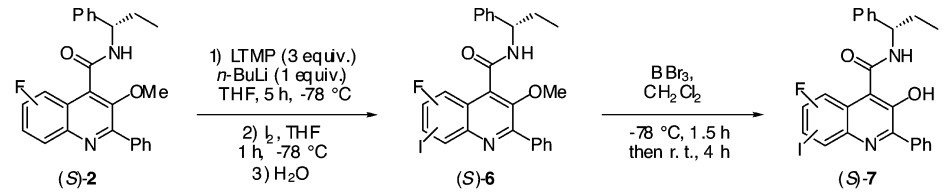
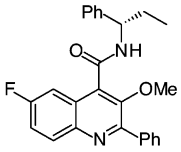
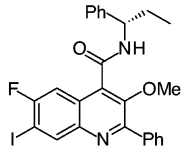
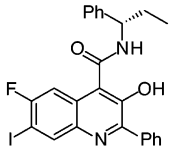
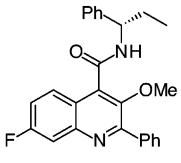
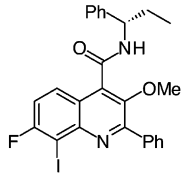
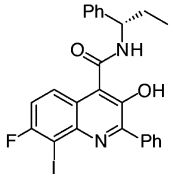
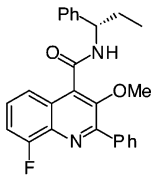
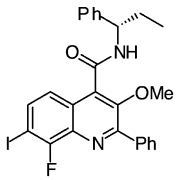
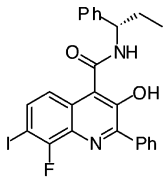
TABLE 1. Synthesis of the Dihaloquinolinecarboxamides 6−7 via Metalation of Fluoro-3-methoxy-2-phenylquinoline-4-carboxamides 2



entry	starting compd **2**	dihalomethoxyproduct **6**	yield (%)	dihalohydroxyproduct **7**	yield (%)
1	 (*S*)-**2a**	 (*S*)-**6a**	61	 (*S*)-**7a**	71
2	 (*S*)-**2b**	 (*S*)-**6b**	78	 (*S*)-**7b**	94
3	 (*S*)-**2c**	 (*S*)-**6c**	28	 (*S*)-**7c**	62

starting fluoroquinolines were free of substitution,[7a] or contain either an alkoxy group at C6[7b] or a bromine atom and a trifluoromethyl group at C2 and C4.[6e,f] In our case, all the pyridine ring positions are substituted (a phenyl moiety at C2, a methoxy or a hydroxy group at C3, and a secondary amide function at C4). Moreover, our substrates possess a benzylic proton on a stereogenic center.

Although quinoline is prone to nucleophilic addition due to its low LUMO level, the use of an alkyllithium as a base was first envisaged. Indeed, the substituents on the pyridine ring and the presence of the fluorine atom would direct deprotonation onto the benzene ring.[8] However, the treatment of 3-methoxy derivatives (*S*)-**2** with *n*-BuLi and then iodine under several conditions of temperature and amounts of base led to a complex mixture of products including demethoxylated quinolines.

We then turned to the hindered and less nucleophilic lithium 2,2,6,6-tetramethylpiperidide (LTMP).[9] Optimization studies of the metalation−iodination sequence starting from quinolines (*S*)-**2a**, (*S*)-**2b**, and (*S*)-**2c** showed that 3 equiv of LTMP and 1 equiv of *n*-BuLi were necessary to obtain the fluoroiodoquinolines (*S*)-**6a**, (*S*)-**6b**, and (*S*)-**6c** in reasonable yields (Table 1). Yields were strongly dependent on the position of the fluorine atom on the phenyl ring, the best result being found for the 7-fluoroquinoline (*S*)-**2b**. In all cases, the deprotonation−iodination sequence occurred with an excellent regiocontrol. Surprisingly, iodine was introduced regiospecifically in position 7 starting from 6-fluoroquinoline (*S*)-**2a**, leading to the corresponding fluoroiodoquinoline (*S*)-**6a** in 61% yield (entry 1). Indeed, the previously described metalation of the free 6-fluoroquinoline using LDA was

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directed by fluorine as well but preferentially to the position 5 (86:13 ratio between 5- and 7-lithio derivatives, 75% total yield).[7a] The steric hindrance of LTMP and of the quinoline (*S*)-**2a** due to the carboxamide function at C4 could be responsible for obtaining (*S*)-**6a**. Regioselective metalation of 7-fluoroquinoline (*S*)-**2b** occurred at C8, as described for unsubstituted 7-fluoroquinoline[7a] and for 7-fluoroquinoline substituted at C2 and C4 by a trifluoromethyl group and a bromine atom.[6e,f] 7-Fluoro-8-iodoquinoline (*S*)-**6b** was obtained in 78% yield. Metalation−iodination of 8-fluoroquinoline (*S*)-**2c** took place at the sole position adjacent to the fluorine atom to afford the fluoro iodo compound (*S*)-**6c** in 28% yield. No starting fluoroquinoline (*S*)-**2c** was recovered, and this reaction led mainly to unidentified degradation byproducts. However, this is the first example of metalation of a 8-fluoroquinoline.

To check the absence of racemization, the starting fluoroquinolines (*S*)-**2a**, (*S*)-**2b**, and (*S*)-**2c** were metalated with 3 equiv of LTMP and 1 equiv of *n*-BuLi as stated previously, then reprotonated with H_2O. In all cases, the optical rotations of **2a**, **2b**, and **2c** were found unchanged after this treatment.

The previous lithiation−iodination procedure applied to the hydroxylated compounds (*S*)-**3** did not lead to the desired iodo compounds. In all cases, the reactants were quantitatively recovered. All attempts using different conditions of temperature or amounts of LTMP were unsuccessful. Finally, the fluoro iodo hydroxy compounds (*S*)-**7** were obtained in 62−94% yields by demethylation of the corresponding methoxy analogues (*S*)-**6** using boron tribromide (Table 1).

The affinities of the dihaloquinolines **6a**−**c** and **7a**−**c** were determined by specific displacement of NK-3 agonist tritiated senktide binding. In each experiment, the results were compared with those obtained on the same in vitro model with endogenous neurokinin B (NKB), the senktide itself, the known potent synthetic antagonists SB 223412[4] and SB 222200,[4b,10] and finally with endogenous neurokinin A (NKA), which exhibits preferential binding to the NK-2 receptors (Table 2). The position of the halogen atoms was crucial for affinity, and the presence of a methoxy instead of a hydroxy group at C3 was found advantageous. Compounds (*S*)-**6c** and (*S*)-**7c** bearing both the fluorine at C8 and the iodine at C7 displayed K_i values close to those of the references. An inversion of the halogens onto the positions 7 and 8 [isomers (*S*)-**6b** and (*S*)-**7b**] led to a considerable loss of binding properties, especially for the hydroxy compound (*S*)-**7b**. Compounds (*S*)-**6a** and (*S*)-**7a**, halogenated at C6 and C7, exhibited a 5−15-fold lower affinity.

The dihaloquinolines (*S*)-**6c** and (*S*)-**7c** showed no affinity at NK-1r, determined by specific displacement of [^{125}I]-Bolton−Hunter-substance P binding (Table 2). Thus, the selectivity for h-NK-3r over h-NK-1r was retained after substitution by a fluorine and an iodine atom at C8 and C7, respectively.

In conclusion, a new series of vic-dihalogenated 2-phenylquinoline-4-carboxamides was prepared using regioselective DoM strategy. These compounds were evaluated as selective NK-3 ligands, and binding properties were found strongly dependent on the position of the halogen atoms. Compound (*S*)-**6c**, substituted by a fluorine atom at C8 and by an iodine at C7, displayed an affinity and a selectivity comparable to that of SB 223412, the unsubstituted reference. Thus, (*S*)-**6c** is a promising candidate as radioligand for NK-3 receptor imaging studies by both PET and SPECT.

TABLE 2. Binding Affinities of Reference Compounds and Quinoline Carboxamides 6−7 at Cloned Human NK-3 and NK-1 Receptors

	binding affinities (K_i, nM; mean ± SEM)	
compd	hNK-3[a]	hNK-1[b]
References		
NKB	28 (±3)	450 (±40)
Senktide	22 (±1)[c]	*d*
SB 223412	27 (±9)	>10000
SB 222200	31 (±5)	>10000
NKA	>1000	*d*
SP	*d*	4.7 (±1.6)
Dihaloquinolines		
(*S*)-**6a**	140 (±23)	*d*
(*S*)-**6b**	553 (±63)	*d*
(*S*)-**6c**	45 (±7)	>10000
(*S*)-**7a**	510 (±56)	*d*
(*S*)-**7b**	>1000	*d*
(*S*)-**7c**	78 (±5)	>10000

[a] Ability to displace [^{3}H]senktide binding from h-NK-3-CHO membranes. [b] Ability to displace [^{125}I]-Bolton−Hunter-substance P binding from h-NK-1-CHO cells. [c] K_d = 12 (±2) nM. [d] Not determined.

Experimental Section

Representative Procedure for Metalation Reactions. At −78 °C, *n*-BuLi (1.0 mmol) in hexanes (0.63 mL), and 15 min later, (*S*)-*N*-(1-phenylpropyl)-fluoro-3-methoxy-2-phenylquinoline-4-carboxamide **2** (0.10 g, 0.25 mmol) in THF (2 mL) were added to 2,2,6,6-tetramethylpiperidine (0.13 mL, 0.11 g, 0.76 mmol) in THF (1 mL). After 5 h at −78 °C, iodine (0.19 g, 0.76 mmol) in THF (2 mL) was quickly added. The mixture was stirred at −78 °C for 1 h, and ammonium chloride (0.1 g, 2 mmol) and then water (0.5 mL) were added. The mixture was allowed to reach room temperature, and sodium thiosulfate was added until discoloration. After the addition of AcOEt, the organic layer was washed with citric acid (5%), water, brine, and then dried over $MgSO_4$, filtrated, and concentrated under vacuum. The crude product was purified by chromatography on silica gel and crystallized from pentane to afford (*S*)-**6**.

(*S*)-*N*-(1-Phenylpropyl)-6-fluoro-7-iodo-3-methoxy-2-phenylquinoline-4-carboxamide (*S*)-6a. The quinoline (*S*)-**6a** (78 mg, 61% yield) was obtained from (*S*)-*N*-(1-phenylpropyl)-6-fluoro-3-methoxy-2-phenylquinoline-4-carboxamide (*S*)-**2a** (0.10 g, 0.25 mmol) and was isolated, after purification by chromatography on silica gel (eluent CH_2Cl_2) and recrystallization from pentane, as a white solid: mp 152 °C; $[\alpha]_D = -29.6$ (c = 0.5 MeOH); ^{1}H NMR ($CDCl_3$) δ 8.58 (d, J = 6.2 Hz, 1H), 7.95−7.92 (m, 2H), 7.57 (d, J = 9.0 Hz, 1H), 7.43−7.26 (m, 8H), 6.58 (d, J = 8.5 Hz, 1H), 5.23 (q, J = 7.5 Hz, 1H), 3.48 (s, 3H), 2.01−1.97 (m, 2H), 1.04 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 164.2, 156.3 (d, J = 248 Hz), 153.8, 148.3, 142.1, 140.9, 140.4, 136.2, 132.6, 129.0, 128.7, 128.3, 127.9, 127.2, 126.4, 125.5 (d, J = 8.9 Hz), 107.6, (d, J = 27.0 Hz), 84.5 (d, J = 30.0 Hz), 61.6, 55.2, 28.6, 10.5; ^{19}F NMR δ -(94.2−94.5) (m); IR (KBr) ν 3244, 2964, 2933, 1633, 1548, 1473, 1451, 1383, 1345, 1232, 1195, 1160, 1030, 697 cm^{-1}. Anal. Calcd for $C_{26}H_{22}FIN_2O_2$: C, 57.79; H, 4.10; N, 5.18; found: C, 57.54; H, 4.26; N, 5.12.

Representative Procedure for Demethylation Reactions. Boron tribromide (93 mg, 0.37 mmol) in CH_2Cl_2 (1 mL)

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was added dropwise for 30 min at −78 °C to (*S*)-*N*-(1-phenylpropyl)-fluoroiodo-3-methoxy-2-phenylquinoline-4-carboxamide (*S*)-**6** (50 mg, 0.093 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at −78 °C for 1 h and allowed to reach room temperature. After 3 h at this temperature and cooling to −78 °C, EtOH (3 mL) was added. The mixture was allowed to reach room temperature and was then concentrated under vacuum. EtOH (3 mL) was added to the residue and then evaporated. This was repeated twice. The residue was diluted in CH_2Cl_2, and the organic layer was washed with $NaHCO_3$ (5%), dried over $MgSO_4$, filtrated, and concentrated under vacuum. The crude product was purified to afford (*S*)-**7**.

(*S*)-*N*-(1-Phenylpropyl)-6-fluoro-3-hydroxy-7-iodo-2-phenylquinoline-4-carboxamide (*S*)-7a. The quinoline (*S*)-**7a** (35 mg, 71% yield) was obtained from (*S*)-*N*-(1-phenylpropyl)-6-fluoro-7-iodo-3-methoxy-2-phenylquinoline-4-carboxamide (*S*)-**6a** (50 mg, 0.093 mmol) and isolated, after recrystallization from CH_2Cl_2/pentane, as a light brown solid: mp 162 °C; $[\alpha]_D = -5.6$ ($c = 0.5$ MeOH); ^{1}H NMR ($CDCl_3$) δ 11.05 (s, 1H), 8.56 (d, J = 6.3 Hz, 1H), 8.04−8.01 (m, 2H), 7.59 (d, J = 9.5 Hz, 1H), 7.50−7.31 (m, 8H), 6.62 (d, J = 7.0 Hz, 1H), 5.22 (q, J = 6.9 Hz, 1H), 2.16−1.98 (m, 2H), 1.02 (t, J = 7.2 Hz, 3H); ^{13}C NMR δ 167.8, 160.4 (d, J = 247 Hz), 152.5, 152.4 (d, J = 2.9 Hz), 136.6, 130.1, 130.0, 129.6, 128.7, 128.6, 127.1, 124.9 (d, J = 9.0 Hz), 115.9, 115.8 (d, J = 4.3 Hz), 106.4 (d, J = 27.7 Hz), 81.4 (d, J = 28.6 Hz), 56.8, 29.4, 11.3; ^{19}F NMR δ −93.2 (dd, J = 6.3, 9.5 Hz); IR (KBr) ν 3566, 1654, 1636, 1628, 1624, 1618, 1544, 1540, 1534, 1522, 700 cm^{-1}; EI-MS (220 °C, 70 eV, 300 mA) m/z 526 ($M^{+\cdot}$, 34), 408 (72), 391 (55), 363 (23), 360 (18), 208 (17), 119 (100); HRMS calcd for $C_{25}H_{20}FIN_2O_2$: 526.05536, found: 526.05693.

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Supporting Information Available: Experimental procedures and spectral data for dihaloquinolines (*S*)-**6b**, (*S*)-**6c**, (*S*)-**7b**, and (*S*)-**7c** and detailed experimental procedures for the in vitro evaluation of compounds **6**−**7**. This material is available free of charge via the Internet at http:// pubs.acs.org.

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