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Synthesis and Affinity of a Possible Byproduct of Electrophilic Radiolabeling of [^{123}I]IBZM

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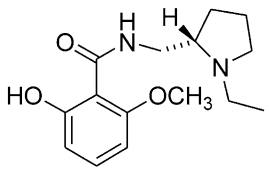
Abstract—The iodobenzamide neuroleptic analogue (*S*)-*N*-(1-ethylpyrrolidin-2-ylmethyl)-2-hydroxy-5-iodo-6-methoxybenzamide (5-IBZM) was synthesized stereospecifically and its pharmacological properties were compared with the 3-iodo isomer (IBZM) used for imaging D_2 receptors in vivo. The isomer 5-IBZM had 100-fold lower affinity than IBZM and migrated with similar retention time as the byproduct formed during electrophilic iodination of BZM.

The dopamine D_2 receptor is implicated in the pathogenesis of Parkinson's disease and in psychiatric disorders including schizophrenia and mania. Measurements of occupancy of this receptor in vivo help to better understand the pathophysiology and pharmacology or such disorders as well as to monitor illness progression and effects of treatment. One of the first and still most widely used radioligands for this receptor is (*S*)-*N*-(1-ethylpyrrolidin-2-ylmethyl)-2-hydroxy-3-iodo-6-methoxybenzamide (IBZM, **2**) labeled with iodine-123 for in vivo studies.[1,2]

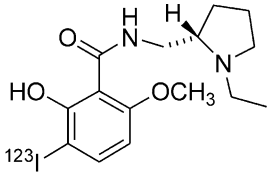
Usually [^{123}I]IBZM (**2**) is prepared by electrophilic iodination of the desiodophenol BZM (**1**) with [^{123}I]NaI in the presence of peracetic acid.[1] This preparation led to the synthesis of a major derivative (IBZM) plus a minor slightly more polar compound. BZM (**1**) has two active labeling sites, *ortho* and *para* to the phenol, so in addition to IBZM (**2**), there is the possibility of producing the byproduct (*S*)-*N*-(1-ethylpyrrolidin-2-ylmethyl)-2-hydroxy-5-iodo-6-methoxybenzamide (**3**) during the radiolabeling reaction. This byproduct might represent a problem for in vivo imaging studies if it competes for the D_2 receptor or if its pharmacokinetics are not appropriate.

To identify this possible byproduct of radiosynthesis, we synthesized 5-iodo-BZM (**3**) by an unambiguous regiospecific method, tested its affinity, and compared it with the byproduct formed during electrophilic iodination of BZM.



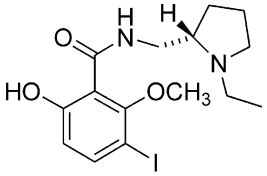
BZM

1



[^{123}I]IBZM

2



5-Iodo-BZM

3

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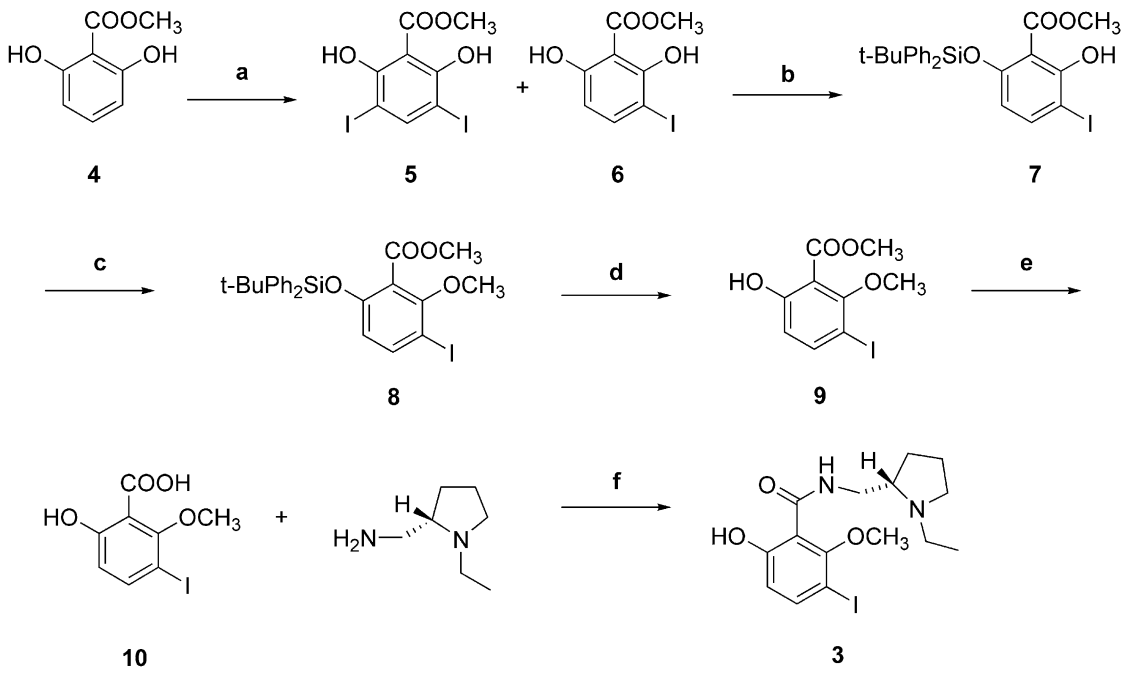

a. ICl, CCl_4, b. t-$BuPh_2SiCl$, CH_2Cl_2, 76%, c. CH_3I, Acetone,K_2CO_3, 72%, d, Bu_4NF, THF, 95%. e.2M KOH, Dioxane, 74%, f. DCC, HOBt, 42%

The key step in obtaining **3** was the synthesis of 6-hydroxy-3-iodo-2-methoxybenzoic acid (**9**). To achieve differentiation of the two phenolic groups, we used a bulky protecting group to avoid iodination of the phenyl ring at the *ortho* position. The adamantanyl carbonyl group, which was initially chosen for this purpose, did not lead to iodination *ortho* to the methoxy group. To achieve the desired differentiation of reactivity of the two phenols, we changed to *tert*-butyldiphenyl silyl ether.

The synthesis began with 2,6-dihydroxybenzoic acid methyl ester (**4**). Two iodination methods were applied to synthesize 2,6-dihydroxy-3-iodo-benzoic acid methyl ester (**6**). Iodine and HgO gave a mixture of 2,6-dihydroxy-3,5-diiodo-benzoic acid methyl ester (**5**) and 2,6-dihydroxy-3-iodo-benzoic acid methyl ester (**6**) (ratio 40/60),[3] then treatment of the mixture of **5**/**6** with *tert*-butyldiphenylsilyl chloride provided 6-(*tert*-butyldiphenylsilyloxy)-2-hydroxy-3-iodobenzoic acid methyl ester (**7**) in 5% yield (two steps) exclusively.[4] Use of iodine monochloride as iodinating agent led to an increase in the ratio of **6**/**5** from 60/40 to 90/10, and the total yield of the first two steps increased from 5 to 26%. Although this overall yield is moderate, this route allowed us to obtain sufficient amount of intermediate. Compound **7**, produced from **6**, was treated with methyl iodide to afford 6-(*tert*-butyldiphenylsilyloxy)-3-iodo-2-methoxybenzoic acid methyl ester (**8**) in 72% yield.[5] Deprotection of **8** with Bu_4NF gave 6-hydroxy-3-iodo-2-methoxy-benzoic acid methyl ester (**9**).[6] The structure of **9** was confirmed by NOE experiment. Saponification of ester **9** led to 2-hydroxy-5-iodo-6-methoxybenzoic acid (**10**).[7] The desired product **3** was synthesized by coupling **10** and (*S*)-(1-ethyl-pyrrolidin-2-yl)-methylamine in the presence of dicyclohexylcarbodiimide and HOBt.

Table 1. Potency of iodinated-BZM derivatives compared to raclopride (mean $IC_{50} \pm SE$, nM) and reported vales for brominated analogues at D_1 and D_2 receptors in rat caudate-putamen tissue homogenates. Reported affinity[1] of 3-IBZM (**2**) $IC_{50} = 1.2$ nM

Compound	Dopamine D_2	Dopamine D_1
3-IBZM (**2**)	1.71 ± 0.35	> 10,000
5-IBZM (**3**)	196 ± 18	> 10,000
(−)-(*S*)-Raclopride	7.2 ± 1.3	> 10,000
3-BrBZM[8]	21	—
5-BrBZM[8]	56	—

The retention time of **3** on reverse-phase HPLC (C_{18}, $MeOH/H_2O/Et_3N$: 75/25/0.1, 1.0 mL/min) was 5.9 min, which corresponded to that of the byproduct observed during electrophilic iodination of BZM. 5-IBZM was evaluated for potency (indicated by inhibition constant K_i) in competitive radioreceptor assays with tissue homogenates of rat forebrain, compared with common ligands IBZM and raclopride (Table 1). Preliminary comparison indicated that 5-IBZM (**3**) was about 100 times *less* potent than IBZM (**2**) at D_2; neither isomer showed appreciable affinity for D_1 receptor sites. The disparity in D_2 binding between the two iodo positional isomers is surprising since it has been reported that the bromo analogues (3-Br and 5-Br) present similar D_2 affinity.[8]

In summary, we accomplished regiospecific synthesis of (*S*)-*N*-(1-ethyl-2-methyl-pyrrolidin-2-ylmethyl)-2-hydroxy-5-iodo-6-methoxybenzamide (**3**). Results of HPLC coinjection of 5-IBZM (**3**) with products of electrophilic substitution of BZM confirm that 5-IBZM (**3**) was the byproduct obtained during direct electrophilic iodination. Since 5-IBZM (**3**) had a 115-fold lower affinity for D_2 than IBZM (**2**), this compound should not interfere with quantification of D_2 receptor density with

preparations of IBZM (**3**) if present in small amounts after purification of electrophilic iodination of BZM.

Experimental

2,6-Dihydroxy-3-iodo-benzoic acid methyl ester (6). To a solution of 200 mg (1.19 mmol) of 2,6-dihydroxybenzoic acid methyl ester in 3 mL of CCl_4 was added 213 mg (1.31 mmol) of ICl. The mixture was stirred at rt for 48 h, then purified by flash chromatography on silica gel with 80:20 hexane–Et_2O to give 120 mg (34% yield, 90% purity) of **6**. ^{1}H NMR (CD_2Cl_2) δ 7.69 (d, 1H, $J=8.8$ Hz), 6.31 (d, 1H, $J=8.8$ Hz), 4.01 (s, 3H).

6-(*tert*-Butyldiphenylsilyloxy)-2-hydroxy-3-iodobenzoic acid methyl ester (7). To a solution of 115 mg of 2,6-dihydroxy-3,5-diiodo-benzoic acid methyl ester and 2,6-dihydroxy-3-iodo-benzoic acid methyl ester (10:90) in 5 mL of CH_2Cl_2 was added 107 mg (0.391 mmol) of TBDPSiCl followed by 26 mg (0.391 mmol) imidazole. The mixture was stirred at rt for 5 h, then purified by flash chromatography on silica gel with 70:30 hexane–CH_2Cl_2 to give 160 mg (76% yield) of **7**, mp 134–136 °C. ^{1}H NMR (CD_2Cl_2) δ 7.79 (m, 4H), 7.23 (m, 6H), 7.04 (d, 1H, $J=8.8$ Hz), 5.92 (d, 1H, $J=8.8$ Hz), 3.48 (s, 3H), 1.15 (s, 9H).

6-(*tert*-Butyldiphenylsilyloxy)-3-iodo-2-methoxybenzoic acid methyl ester (8). To 350 mg (0.660 mmol) of 6-(*tert*-butyldiphenylsilyloxy)-2-hydroxy-3-iodobenzoic acid methyl ester in 10 mL of acetone was added 187 mg (0.660 mmol) of MeI followed by 100 mg (0.726 mmol) $KHCO_3$. The mixture was heated to reflux for 24 h, then purified by flash chromatography with 60:40 hexane–CH_2Cl_2 to give 260 mg (72% yield) of **8**. ^{1}H NMR ($CDCl_3$) δ 7.84 (m, 4H), 7.24 (m, 6H), 7.18 (d, 1H, $J=8.8$ Hz), 6.06 (d, 1H, $J=8.8$ Hz), 3.81 (s, 3H), 3.72 (s, 3H), 1.17 (s, 9H).

6-Hydroxy-3-iodo-2-methoxybenzoic acid methyl ester (9). To a solution of 260 mg (0.475 mmol) of 6-(*tert*-butyldiphenylsilyloxy)-3-iodo-2-methoxybenzoic acid methyl ester in 5 mL of THF was added 0.48 mL (0.48 mmol) of 1.0 M TBAF solution in THF. The mixture was stirred at rt for 2 h, and then purified by flash chromatography on silica gel. Elution with 90:10 hexane–Et_2O afforded 140 mg (95% yield) of **9** as an oil. ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 1H, $J=8.8$ Hz), 6.64 (d, 1H, $J=8.8$ Hz), 4.03 (s, 3H), 3.81 (s, 3H).

(*S*)-*N*-(1-ethylpyrrolidin-2-ylmethyl)-2-hydroxy-5-iodo-6-methoxybenzamide (3). To a solution of 44 mg (0.34 mmol) of (*S*)-(1-ethyl-pyrrolidin-2-yl)methylamine in 5 mL of CH_2Cl_2 under a nitrogen atmosphere was added 90 mg (0.31 mmol) of 6-hydroxy-3-iodo-2-methoxybenzoic acid followed by 75 mg (0.34 mmol) of DCC and 45 mg (0.33 mmol) of 1-hydroxy-benzotriazole hydrate. The mixture was stirred at room temperature for 18 h. Solids were removed by filtration, the solvent was removed on a rotary evaporator, and the residue was purified by flash chromatography on silica gel 50:50 hexane–Et_2O–5% Et_3N afforded 53 mg (42% yield) of **3**. ^{1}H NMR ($CDCl_3$) δ 7.69 (d, 1H, $J=8.8$ Hz), 6.61 (d, 1H, $J=8.8$ Hz), 3.82 (s, 3H), 3.76 (qd, 1H), 3.34 (dq, 1H), 3,24 (m, 1H), 2.88 (m, 1H), 2.65 (m, 1H), 2.26 (m, 2H), 1.95 (m, 1H), 1.77–1.60 (m, 3H), 1.13 (t, 3H, $J=7.2$).

Binding assays

Radioreceptor competitive binding was measured as follows: for D_1, radioligand [^{3}H]SCH-23390 (0.3 nM) in presence of Na^+ (150 mM), using *cis*-flupenthixol (300 nM) to define blank; for D_2, radioligand [^{3}H]nemonapride (0.075 nM), using haloperidol (10 mM) to define blank.[9]

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