Synthesis and β-Adrenergic Activities of R-Fluoronaphthyloxypropanolamine

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Purpose. Many biogenic amines where an aromatic proton is substituted with fluorine have exhibited pharmacological properties that are dependent on the position of fluorine on the aromatic ring. For example, 6-fluoroepinephrine is selective for α-adrenergic receptors whereas the 2-fluoroisomer is selective for β-receptors. Aryloxypropanolamines are β-receptor agonists or antagonists, depending on the aryl group and its substituents. We therefore hypothesized that fluorine substitution on the aromatic ring could lead to significant biological effects in this class. A target with fluorine on naphthyl group of a known β-antagonist was chosen for investigation.

Methods. Synthesis of the target compound began with fluoronaphthalene and involved introduction of 4-hydroxy group by Friedel-Crafts acylation followed by Baeyer Villiger oxidation. The side chain was introduced stereoselectively using the chiral synthon (2R)-glycidyl 3-nitrobenzenesulfonate, a Sharpless epoxidation technique. The epoxide was opened with t-butyl amine. HPLC methods were used to characterize %ee of the enantiomer.

Results. The target compound was synthesized in several hundred milligram quantity, and in good yield and high enantiomeric excess, showing practicality of the synthetic scheme. It exhibited potent binding activities on β-adrenergic receptors, and was found to be two times selective for β_2-receptors over β_1.

Conclusions. The current report demonstrates that aromatic fluorine substitution on β-adrenergic ligands can be achieved, and that such can be used to obtain binding selectivity between β receptors.

KEY WORDS: fluoroaromatic; naphthyloxypropanolamine; aryloxypropanolamine; enantioselective synthesis; β-adrenoceptor ligands.

INTRODUCTION

There is current and growing interest in incorporation of fluorine into various organic molecules (1). A recent Tetrahedron Symposium in Print and an American Chemical Society (ACS) Monograph were dedicated to this topic (2). Some of the reasons for this interest include the small size of fluorine, hence steric effects are kept to a minimum, its strong electron withdrawing properties, and the possibility of use of such fluorocompounds in various spectroscopic methods such as positron emission tomography (^{18}F) and nuclear magnetic resonance (^{19}F) (3). Furthermore, there are many biomolecules where substitution of a proton with fluorine has led to profound biological activities. The more known examples are the antineoplastic agent 5-fluorouracil, the anti-inflammatory corticosteroid dexamethasone, the Krebs cycle inhibitor fluoroacetic acid and several pheromones (3). However, the fluorine in all these cases were incorporated into non-aromatic compounds. Interest in the fluoroaromatic compounds have been increasing. We have been involved in incorporating fluorine into molecules of biological interest. Many biogenic amines where an aromatic proton is substituted with fluorine have exhibited pharmacological properties that are dependent on the position of fluorine on the aromatic ring (4–11). For example, 6-fluoroepinephrine was found to be selective for α-adrenergic receptors whereas the 2-fluoro isomer was selective for β-adrenergic receptors (6), following the same trend observed for norepinephrine earlier (4). To further study the effects of fluorine substitution on the aromatic groups of adrenergic compounds, we chose to investigate the aryloxypropanolamine class. This is because compounds in this class can be β-adrenergic receptor agonists or antagonists, depending on the aryl group. We therefore hypothesized that fluorine perturbation of the aromatic ring could lead to significant biological effects in this class. For the agonists investigated, again, the 2-fluoro-isomer was selective for β-adrenergic receptors whereas the 6-isomer was practically devoid of β-adrenoceptor activities. However, the compounds synthesized were racemic mixtures as were the fluoroepinephrines and previous fluorocompounds, hence contribution to biological activitites by each of the enantiomers was not clear. This made it necessary to investigate synthesis and adrenergic activities of individual fluoro-enantiomers. The current report is on synthesis and β-adrenergic activities of an enantiomer of a fluoroaryloxypropanolamine. The aryl group was naphthyl and the non-fluorinated parent compound is a β-antagonist.

CHEMISTRY

Synthesis of the target, enantiomer is shown in Scheme 1. The key intermediate in the synthesis was 4-fluoronaphthol *3*. Though this is a previously reported compound (12), we now report a more efficient synthesis which also avoids the potentially explosive decomposition of a diazonium salt in a Schiemann reaction. The new method can also be used to synthesize large quantities. This method consisted of conducting a Friedel-Crafts acylation (13) on commercially available fluoronaphthalene to give a methyl ketone. The fluorine directs the acyl group predominantly to the para position. The ketone was then subjected to Baeyer-Villiger oxidation (12,14) followed by hydrolysis to give the desired naphthol in 60% yield from the fluoronaphthalene. The side chain was introduced stereoselectively using the Sharpless epoxidation technique (15,16) by use of the commercially available chiral synthon (2R)-glycidyl 3-nitrobenzenesulfonate. Opening of the side chain epoxide with t-butyl amine then gave the desired enantiomer. HPLC methods were used to characterize the %ee of the enantiomer after derivatizing with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC). It was obtained in 50% yield from the naphthol and 98%ee.

PHARMACOLOGY

The pharmacological evaluations were carried out by NOVASCREEN Corporation under the National Institute of Mental Health (NIMH)/NOVASCREEN Psychotherapeutic Drug Discovery Program. The activities on β-adrenergic receptors were evaluated following the methods of Kalaria and coworkers (17) and Minneman and coworkers (18). The experiments carried out were *in vitro* binding experiments on β-

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adrenergic receptors on rat cortical membranes and the K_i, IC_{50} and % inhibition were obtained. The results are shown in Table 1.

DISCUSSION

The target compound was synthesized in several hundred milligram quantity, and in good yield and high enantiomeric excess, showing the practicality of the synthetic scheme. It exhibited potent binding activities on β-adrenergic receptors, comparable to isoproterenol in both β_1 and β_2-receptors. It was found to be two times selective for β_2-receptors over β_1. The current report demonstrates that fluorine substitution on ligands can be used to obtain binding selectivity between β-adrenergic receptors. This is consistent with observations in the agonist class. For example, selectivity was observed on the β-receptors when fluorine was introduced to the aromatic ring of the clasical agonist 3-(tert-butylamino)-1-(3,4-dihydroxyphenoxy)-2-propanol (9). The 6-fluoro compound was found to be devoid of activity unlike the 2-fluoro analog which retained both binding and agonist activities but was slightly selective for β_2-receptors. Another example in the agonist class was demonstration of selectivity for β_2-receptors observed for trimetoquinol (7,10). Further pharmacological evaluations of compound *5* such as β-adrenergic antagonist activity, potency on β_3 receptors, and syntheses of other compounds in this class are being explored to obtain a more accurate picture of the effects of fluorine substitution on the biological activities of this class.

EXPERIMENTAL SECTION

General

Melting points were determined on a Mel temp II capillary melting point apparatus. IR spectra were recorded on a Perkin-Elmer 457 spectrophotometer. ^{1}H NMR spectra were recorded on Varian EM 390 (90 MHz) spectrophotometer with TMS as internal standard and $CDCl_3$ as solvent. Some ^{1}H NMR spectra were also recorded on Brucker AC250 (250 MHz) spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using a 1 dm length quartz cell. MS spectra were obtained by electrospray method using the Finnigan TSQ-700 mass spectrometer. HPLC studies were performed on a Varian vista 5500 and Varian DS651. Flash chromatography was performed on silica gel 60 (70–230 mesh). Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN and observed values were within +/− 0.4% of theoretical values.

4-Fluoro-1-acetylnaphthalene 1

To a heterogeneous solution of aluminum chloride (0.7 g, 5.25 mmol) in dichloromethane (4 ml) at room temperature was added acetyl chloride (0.28 g, 3.57 mmol) and then fluoronaphthalene (0.48 g , 3.27 mmol), both dropwisely. The addition of fluoronaphthalene was exothermic and the reaction became yellow. The reaction was refluxed for 4 h by which time the fluoronaphthalene could not be detected on TLC (hexane: CH_2Cl_2; 1:1). The reaction was quenched by addition of ice and 1 N HCl. The dichloromethane was then removed by rotary evaporation and the residue was taken up in ethyl ether. It was then washed three times with water and dried over Na_2SO_4. After removal of the ether by rotary evaporation, the residue was purified on silica gel (hexane: CH_2Cl_2; 7:3) to give a slightly yellow oil (13) (0.495 g, 80% yield), IR (KBr): 1690–1640; ^{1}H NMR ($CDCl_3$) δ 8.85–8.70 (m, 1H, aryl H), 8.10–7.40 (m, 4H, aryl H), 6.95 (dd, 1H, aryl H), 2.6 (s, 3H, $COCH_3$).

4-Fluoro-1-oxyacetylnaphthalene 2

A solution of 4-fluoro-1-acetylnaphthalene (4.00 g, 0.021 mole) and *m*-chloroperbenzoic acid (MCPBA, 20 g, 0.058 mole) in 40 ml of CH_2Cl_2 was heated at reflux for 24 h during which time a solid precipitated from the solution. CH_2Cl_2 was removed by evaporation and the residue was dissolved in ethyl acetate, washed three times with 10% sodium bicarbonate and one time with a saturated aqueous NaCl solution and then dried over anhydrous Na_2SO_4. Removal of the AcOEt gave 10 g of a crude product that was purified by flash chromatography (hexane: CH_2Cl_2: Et_2O; 9:1:0.2). After partial removal of the solvent, 2.35 g of white crystals and 1.35 g of slightly yellow solid as a second crop were obtained. Yield: 3.70 g (84%); mp 67–68°C; IR. (KBr); 3520(w), 3100–2900(w), 1750, 1640(w); ^{1}H NMR ($CDCl_3$) δ 8.2–8.10 (m, 1H, aryl H), 7.60–7.50 (m, 1H, aryl H), 7.20–7.10 (m, 2H, aryl H), 2.43 (s, 3H $OCOCH_3$).

4-Fluoro-1-naphthol 3

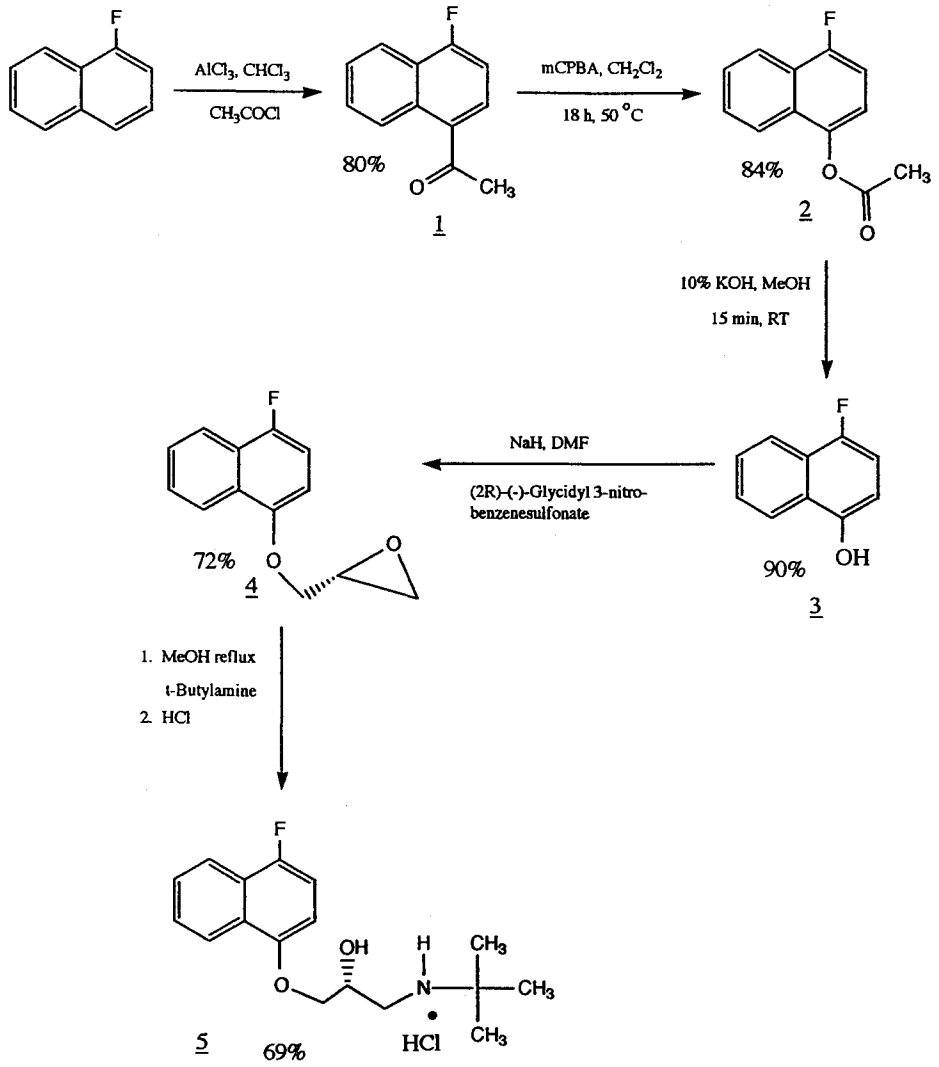
A solution of 4-fluoro-1-oxyacetylnaphtalene (0.394 g) in potassium hydroxide 10% (2 ml) and methanol (6 ml) was stirred for 30 min at room temperature. TLC (hex: CH_2Cl_2:Et_2O; 9:1:0.2) showed that there was no more of the ester after 15 min. The mixture was then acidified with HCl until pH = 3 at 0°C. It was then extracted with ethyl acetate and the combined extracts were washed three times with saturated brine and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave 284 mg (90%) of the naphthol as a brownish solid. The solid was crystallized from hexane to give colorless crystals; mp 124–125°C (lit[12] 120–122°C); IR (KBr); 3575, 3300; ^{1}H NMR ($CDCl_3$) δ 8.20–7.40 (m, 4H, aryl H), 6.9–7.0 (dd, 1H, aryl H), 6.66–6.76 (dd, 1H, aryl H). 5.10–4.95 (s,1H, OH).

(2R)-3-(4-fluoro-1-naphthyloxy)-1,2-epoxypropane 4

A solution of 4-fluoro-1-naphtol (595 mg, 3,67 mmol) in dimethylformamide (30 ml) was stirred at 0°C under N_2

Table 1. Table Showing the β-Adrenergic Activities of Compound 5

Receptor	Reference compound	K_i (nm)		IC_{50} compd. *5*	Initial % inhibition
		Ref. compd.	Compd. *5*		
β_1	Isoproterenol	626.61	431	448	107.5
β_2	Isoproterenol	182.89	213	223	105.7



Scheme 1.

atmosphere and then NaH (176 mg, 7.33 mmol) was added. The reaction was stirred for 30 min at 0°C and it turned from red to a greenish color. After this, 587 mg of (2R)(−) glycidyl 3-nitro benzene sulfonate (1g, 3.86 mmol) was added and the reaction was stirred at room temperature for 18 h. After TLC (CH_2Cl_2: hex; 1:1) indicated that the reaction was complete, the mixture was extracted with ethyl ether and washed three times with water. The ethereal extracts were dried over $MgSO_4$ and after rotary evaporation it gave 1.5 g of a crude oil. Purification by flash chromatography (CH_2Cl_2: hex; 1:1) gave 580 mg (72%) of a pale yellow oil. This was used in the next reaction without further characterization.

(2R)-3-(4-fluoro-1-naphtyloxy)-1-(tert*—butyl-amino)-2-propanol.HCl* 5

A solution of (2R)-3-(4-fluoro-1-naphtyloxy)-1,2-epoxy-propane (580 mg, 2.66 mmol) and *tert*-butylamine (4.0 ml, 37.9 mmol) in methanol (16 ml) was stirred at reflux for 24 h. After complete disappearance of the epoxide on TLC (MeOH:Et_2O; 7:3), the solvent was removed by rotary evaporation. The residue was taken up in ethyl ether and wash three times with water. The organic layer was dried over $MgSO_4$ and the solvent was removed by rotary evaporation to give an oil which was crystallized from hexane to give 600 mg (77%) of a white solid of (2R)-3-(4-fluoro-1-naphthyloxy)-1-(*tert*—butyl-amino)-2-propanol; mp 56–57°C; $[\alpha]^{25}D = 3.71$ (c = 1.96 , EtOH); ^1H NMR ($CDCl_3$) δ 8.27–7.40 (m, 4H, aryl H), 7.00–7.10 (t, 1H aryl H), 6.75–6.65 (dd, 1H aryl H), 4.10 (s, 2H,C**H**$_2$CHOH), 2.95–2.40 (m,3H,C**H**OHC**H**$_2$N), 1.11 (s, 9H C(C**H**$_3$)$_3$.

Then 300 mg of this solid was dissolved in 100 ml of ethyl ether and HCl gas was bubbled until a white solid precipitated. After filtration, 300 mg of a white solid (89%) was obtained: mp 165–166°C; $[\alpha]^{25}D = +13.2°$ (c = 0.81, EtOH); MS 292 (M+1), 236 (M+1–56, base); Anal ($C_{17}H_{23}NFO_2Cl$) CHNF.

Determination of the enantiomeric purity by LC: 1 mg of the amine was dissolved in 2.5 ml of acetonitrile and 2 mg of GITC was dissolved in 2.5 ml of acetonitrile. 1 ml of each solution were mixed together and allowed to react at room temperature for 30 min. The chromatogram gave one major

peak at 3.7 min for the R-enantiomer (98%) and a very minor peak at 3.15 min for the S-enantiomer. Mobile phase was 65% acetonitrile and 35% ammonium phosphate monobasic buffer 0.02 M PH = 4.6. The column was spherisorb ODS2 5 mm RP18 150 mm *4.6 mm. The flow rate was 2 ml/min and detection was by UV at 254 nm.

PHARMACOLOGY

The compound was tested through the NIMH/NOVASCREEN Psychotherapeutic Drug Discovery and Development Program. Briefly, competitive binding assays were performed using rat cortical membranes. Reactions were carried out in 50 mM TRIS-HCl (pH 7.5), containing 150 mM NaCl, 2.5 mM $MgCl_2$, and 0.5 mM ascorbate for 30 minutes at 37°C. The reaction was terminated by rapid vacuum filtration over Whatman glass fiber filters. Radioactivity trapped on the filter was determined by liquid scintillation spectroscopy and compared to control values in order to ascertain any interactions between the test compound and the receptors. Isoproterenol was used as the positive control and the radioligand was [^{125}I](−) Iodopindolol. The degree of specific binding was determined with 100 uM isoproterenol and found to be 85 ± 8% for the β_1 receptors and 80 ± 7% for β_2. The K_d (binding activity) and B_{max} (receptor number) were found to be 5.4 nM and 7.7 pmol/mg protein respecively for β_1 receptors. The corresponding numbers for β_2 were 4.4 nM and 5.9 pmol/mg protein. The values and error margins are consistent with previously reported values (17,18). The values being reported represent the average of duplicate tubes at the particular concentration. Inhibition constants were determined by the Cheng-Prusoff equation.

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