Synthesis and *in Vitro* Affinity for Dopamine D-2 Receptor of N-Fluorine-Substituted Analogs of Eticlopride

Toshimitsu FUKUMURA,[a] Hideki DOHMOTO,[b] Minoru MAEDA,*[,a] Etsuko FUKUZAWA,[a] and Masaharu KOJIMA[a]

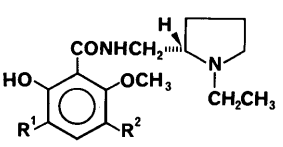
Faculty of Pharmaceutical Sciences, Kyushu University,[a] Maidashi 3-1-1, Higashi-ku, Fukuoka 812, Japan and Life Science Laboratories, Central Research Laboratories, Ajinomoto Co., Inc.,[b] 214 Maeda-cho, Totsuka-ku, Yokohama 244, Japan. *Received October 30, 1989*

With the aim of developing fluorinated benzamides that may be useful as radioligands for positron emission tomography, fluorine analogs of eticlopride, in which the N-ethyl group was replaced by 2-fluoroethyl, 3-fluoropropyl and *p*-fluorobenzyl groups, were synthesized. These derivatives were tested for their ability to displace [^{3}H]spiperone from its specific dopamine D-2 receptor. Their potencies were decreased when compared with eticlopride, but the two fluoroalkylated analogs were in the same order of magnitude as that of haloperidol.

Keywords fluoroalkylated analog; dopamine D-2 receptor; *in vitro* binding affinity; eticlopride; ligand

Positron emission tomography (PET), together with recent development of radioligands labeled with positron emitters, has allowed the study of the distribution and characteristics of neuroreceptors in the living human brain.[1] For the analysis of dopamine D-2 receptors, the butyrophenone neuroleptics and their analogs have been investigated as positron ligands for PET.[2] Their usefulness, however, has not always been optimal. Recently, the substituted benzamides such as raclopride [(*S*)-(−)-3,5-dichloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide],[3] eticlopride [(*S*)-(−)-5-chloro-3-ethyl-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide][4] and YM-09151-2 [*N*-benzyl-2-methyl-3-pyrrolidinyl)-4-chloro-2-methoxy-4-methylaminobenzamide][5] have attracted considerable interest as highly potent and selective antagonists of the dopamine D-2 receptors. These carbon-11 labeled forms have been developed and appear to be the ligands of choice for PET studies at equilibrium conditions.[6-8] The short 20 min half-life of carbon-11, among the positron-emitting radiolabels, has limited the ability to gain understanding of relatively slow receptor ligand kinetics. Fluorine-18 with its longer half-life (109.7 min) may be favored for studies of the kinetic analysis with PET. Thus, fluorine analogs of raclopride and YM-09151-2 have recently been labeled with fluorine-18 as longer-lived alternatives to the carbon-11 labeled forms.[9,10] In our research for better radioligands for PET studies, we become interested in the preparation of fluorine-18 labeled analogs of eticlopride that may be useful as tracers for mapping dopamine D-2 receptors. This paper describes the replacement of the N-ethyl group on the pyrrolidine nitrogen of eticlopride by 2-fluoroethyl, 3-fluoropropyl and *p*-fluorobenzyl groups. The competitive *in vitro* binding of these nonlabeled compounds with the dopamine D-2 receptors was also evaluated in order to select new ligand candidates for fluorine-18 labeling. After the present work had been completed, a preliminary study by PET using a ^{18}F-labeled analog of eticlopride has appeared in abstract form.[11]

The synthetic route for N-substituted [(2-pyrrolidinyl)-methyl]-5-chloro-3-ethyl-6-methoxysalicylamides (**7**, **8** and **10**) is shown in Chart 2, based on the published procedure for analogous benzamides.[12,13] 5-Chloro-3-ethyl-6-methoxysalicylic acid (**1**) was prepared according to the method in the literature.[12a] The acid chloride in crude form, obtained by conducting with thionyl chloride in dry chloroform in the presence of a catalytic amount of *N*,*N*-dimethylformamide, on treatment with the (*S*)-(−)-2-(aminomethyl) N-fluoroalkylated pyrrolidines (**5** and **6**) gave the required fluorine analogs (**7** and **8**) of eticlopride in moderate yields. The preparation of these aminopyrrolidines (**5** and **6**) were started from commercially available (*S*)-(−)-prolinamide (**2**). The hydrochloride salt of **2** was N-fluoroalkylated by 2-fluoro-1-(toluene-*p*-sulfonyloxy)-ethane and 3-fluoro-1-(toluene-*p*-sulfonyloxy)propane in the presence of triethylamine to give the respective N-fluoroalkylated compound (**3** and **4**). These amides were reduced to the requisite (*S*)-(−)-aminopyrrolidines (**5** and **6**) with boran–tetrahydrofuran (THF) complex. The



raclopride $R^1=R^2=Cl$
eticlopride $R^1=C_2H_5$, $R^2=Cl$

Chart 1

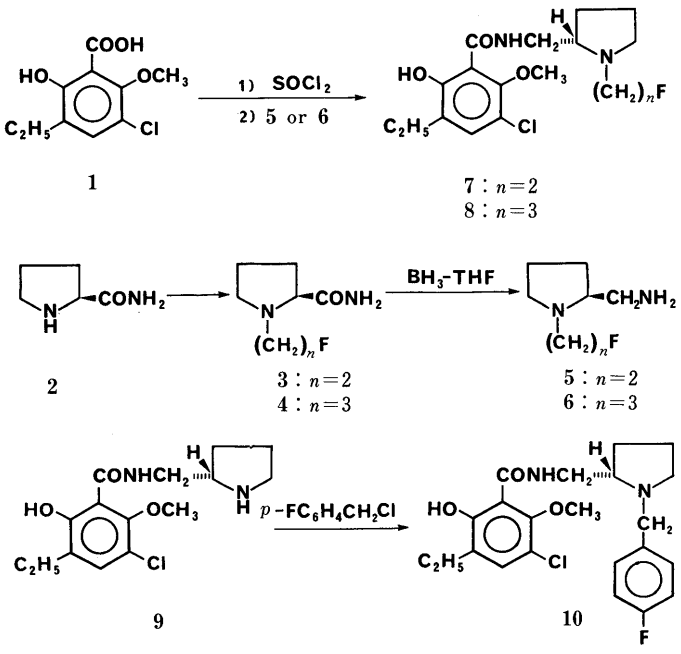


Chart 2

TABLE I. Inhibition of [^{3}H]Spiperone Binding in Striatal Homogenates of Bovine Brain

Compound	[^{3}H]Spiperone binding,[a] IC_{50} (nM)
7	23
8	17
10	4800
Eticlopride	1.4 (0.9)[b]
Haloperidol	15 (12.0)[b]
Sulpiride	590 (210)[b]

a) Homogenates of the bovine striata tissue were incubated with 0.38 nM [^{3}H]spiperone and various concentrations of the indicated compounds. Nonspecific binding was determined in the presence of 10^{-5} M sulpiride. IC_{50} represents the concentration of compound required to inhibit 50% of specifically bound [^{3}H]spiperone. Values represent means of three determinations. *b*) Values in parentheses were taken from ref.,[12b] in which rat striatal membranes *in vitro* were used.

preparation of the N-(*p*-fluorobenzyl) analog (**10**) was achieved by reaction of des-N-ethyl-eticlopride (**9**) with *p*-fluorobenzyl chloride. The structure of the fluoro analogs of eticlopride were confirmed by mass and proton nuclear magnetic resonance (^{1}H-NMR) spectra and their optical purity was not determined.

The IC_{50} of new compounds (**7**, **8** and **10**) for the dopamine D-2 receptors was determined by their ability to displace [^{3}H]spiperone bound to bovine striatal membranes. Nonspecific binding was determined by measuring binding in the presence of 10^{-5} M sulpiride. For comparison, eticlopride, sulpiride and haloperidol were assayed simultaneously. The results are shown in Table I. Unfortunately, all of the compounds tested were found to have lower affinities to the [^{3}H]spiperone binding site than eticlopride itself. In particular, replacement of the N-ethyl group in eticlopride with a *p*-fluorobenzyl group resulted in a compound (**10**) that is virtually inactive. The limited studies of de Paulis *et al.*[13] indicate that, for the class of substituted benzamide compounds, the biological activity is not dependent on the N-alkyl group chain length in the pyrrolidine nucleus. Recent related literature[9] has shown that the replacement of the N-ethyl group in the pyrrolidine ring of raclopride with an N-fluoroethyl group resulted in lower affinity for the dopamine D-2 receptor *in vitro*. It thus appears that, in the benzamide class of D-2 receptor antagonists, fluorine substitution in the N-substituent of the pyrrolidinyl moiety commonly causes reduced binding affinity. Nevertheless, as shown in Table I, the *N*-(2-fluoroethyl) (**7**) and *N*-(3-fluoropropyl) (**8**) still retain high affinities for striatal dopamine receptors in the nanomolar range, comparable to that for haloperidol measured under similar conditions. The compounds **7** and **8**, therefore, were considered to be candidates for further studies. Detailed biological evaluation *in vivo* using the fluorine-18 labeled counterparts is now in progress.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are not corrected. Optical rotations were determined by a JASCO DIP-360 digital polarimeter (cell length: 0.5 dm). ^{1}H-NMR spectra were recorded on JEOL FX-100 and/or GX-270 spectrometer with tetramethylsilane (in $CDCl_3$) or sodium 4,4-dimethyl-4-silapentane-1-sulfonate (in D_2O) as internal reference. Mass spectra (MS) were obtained on a JEOL TMS-D300 spectrometer and infrared (IR) spectra were recorded on a JASCO IRA-1 spectrometer. Column chromatography was carried out with kieselgel 60 (70—230 mesh, Merk). All chemicals and solvents were analytical grade and used without further purification. [^{3}H]Spiperone (71.5 Ci/mmol) was purchased from Amersham International plc, England. Drugs were obtained from the following sources: haloperidol (Sigma), sulpiride (Funai Pharmaceutical), and ketanserin (Ajinomoto). Eticlopride [(*S*)-(−)-5-chloro-3-ethyl-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide] was prepared by published procedure.[12a] Elemental analysis were performed by the staff of the microanalytical section of Kyushu University.

(*S*)-(−)-1-(2-Fluoroethyl)-2-pyrrolidinecarboxamide (3) To a mixture of (*S*)-(−)-prolinamide hydrochloride (1.55 g), triethylamine (1.04 g) in dry acetonitrile (15 ml) was added slowly 2-fluoro-1-(toluene-*p*-sulfonyl oxy)ethane[14] (2.24 g) at room temperature. The reaction mixture was stirred at room temperature for 24 h. After evaporation of the acetonitrile, the residue was chromatographed on silica gel ($CHCl_3$) to give **3** (0.42 g, 25%) as colorless needles: mp 100—102 °C (diisopropyl ether) $[\alpha]_D^{28}$ −103.7° (c=1.1, $CHCl_3$). MS *m/z*: 160 (M^+). IR (Nujol): 1640 cm^{-1} (CONH). ^{1}H-NMR ($CDCl_3$) δ: 7.22 (1H, br, NH_2), 5.35 (1H, br, NH_2), 4.50 (2H, dt, J=49.6, 4.1 Hz, CH_2F), 3.38—1.71 (9H, m). *Anal.* Calcd for $C_7H_{13}FN_2O$: C, 52.49; H, 8.18; N, 17.49. Found: C, 52.43; H, 8.15; N, 17.30.

(*S*)-(−)-1-(3-Fluoropropyl)-2-pyrrolidinecarboxamide (4) Compound (**4**) was prepared from 3-fluoro-1-(toluene-*p*-sulfonyloxy)propane[15] (2.06 g) as described for compound (**3**): colorless needles: yield (0.33 g, 21%): mp 101—102 °C. $[\alpha]_D^{28}$ −118.9° (c=1.0, $CHCl_3$). MS *m/z*: 174 (M^+). IR (Nujol): 1640 cm^{-1} (CONH). ^{1}H-NMR ($CDCl_3$) δ: 7.16 (1H, br, NH_2), 5.63 (1H, br, NH_2), 4.53 (2H, dt, J=47.6, 5.4 Hz CH_2F), 3.82—1.65 (11H, m). *Anal.* Calcd for $C_8H_{15}FN_2O$: C, 55.15; H, 8.68; N, 16.08. Found: C, 55.22; H, 8.71; N, 15.94.

(*S*)-(−)-(2-Aminomethyl)-1-(2′-fluoroethyl)pyrrolidine (5) To a solution of boran–THF complex (1 mmol/ml THF solution) in THF (26.3 ml) was slowly added at 0 °C a solution of **3** (0.52 g) in dry THF (40 ml). After stirring at room temperature for 30 min, the mixture was refluxed for an additional 3 h. After cooling, 1 N HCl was added and the mixture was allowed to stand at room temperature for 30 min. The THF was removed under reduced pressure. The resulting aqueous layer was alkalinized by addition of ammonium hydroxide solution, then extracted three times with CH_2Cl_2. The CH_2Cl_2 layer was dried (Na_2SO_4) and evaporated. To the residue, was then added a solution of D(−)-tartaric acid (0.78 g) in methanol (10 ml). The precipitated solid was recrystallized from water–ethanol to yield di-D(−)-tartrate monohydrate salt of **5** (0.94 g, 64%) as needles: mp 154—155 °C $[\alpha]_D^{30}$ −34.8° (c=1.0, H_2O). ^{1}H-NMR (D_2O) δ: 4.88 (2H, dt, J=51.3, 4.6 Hz, CH_2F), 4.52 (4H, s, CH part of tartaric acid), 3.93—3.19 (7H, m), 2.83—1.87 (4H, m). *Anal.* Calcd for $C_{15}H_{25}FN_2O_{12}\cdot H_2O$: C, 38.80; H, 6.29; N, 6.03. Found: C, 38.77; H, 6.40; N, 6.01.

(*S*)-(−)-(2-Aminomethyl)-1-(3-fluoropropyl)pyrrolidine (6) Compound (**6**) was obtained as di-D(−)-tartarate semihydrate (0.78 g, 42%) salt of **6** from **4** in the same manner described for **5**: mp 167—168 °C. $[\alpha]_D^{31}$ −41.5° (c=1.0, H_2O). ^{1}H-NMR (D_2O) δ: 4.63 (2H, dt, J=52.5, 5.4 Hz, CH_2F), 4.52 (4H, s, CH part of tartaric acid), 3.76—3.19 (7H, m), 2.30—1.84 (6H, m). *Anal.* Calcd for $C_{16}H_{29}FN_2O_{12}\cdot 1/2H_2O$: C, 40.94; H, 6.44; N, 5.97. Found: C, 40.92; H, 6.39; N, 5.96.

(*S*)-(−)-5-Chloro-3-ethyl-*N*-[1-(2′-fluoroethyl)-2-pyrrolidinylmethyl]-6-methoxysalicylamide (7) A mixture of 5-chloro-3-ethyl-6-methoxysalicylic acid[12a] (0.4 g), thionyl chloride (0.19 ml), and *N*,*N*-dimethylformamide (0.014 ml) in dry chloroform (4 ml) was stirred at room temperature for 1 h, and heated at 50 °C for an additional 30 min. The solvent was evaporated, and the residue was dissolved in dry CH_2Cl_2 (3 ml), and again evaporated. This procedure was repeated three times. The final residue was dissolved in dry CH_2Cl_2 (3 ml). A solution of **5**, prepared by CH_2Cl_2 extraction of a solution of the di-D(−)-tartarete monohydrate salt of **5** (0.8 g) in 20% NaOH (10 ml), was added at room temperature. The reaction mixture was stirred at room temperature for 12 h. After the solvent was evaporated, the residue was chromatographed on silica gel ($CHCl_3$) to give **7** (0.37 g, 59%) as colorless oil: $[\alpha]_D^{23}$ −53.5° (c=1.2, $CHCl_3$). MS *m/z*: 358 (M^+). IR (neat): 1640 cm^{-1} (CONH). ^{1}H-NMR ($CDCl_3$) δ: 13.9 (1H, s, OH), 8.87 (1H, br, NH), 7.26 (1H, s, aromatic), 4.56 (2H, dt, J=37.3, 5.1 Hz, CH_2F), 3.88 (3H, s, OCH_3), 3.74 (1H, m, $NHCH_2$), 3.37 (1H, m, $NHCH_2$) 3.20 (1H, m), 2.99—2.24 (6H, m), 2.03—1.67 (4H, m), 1.19 (3H, t, J=7.3 Hz, CH_3). *Anal.* Calcd for $C_{17}H_{23}ClFN_2O_3$: C, 56.90; H, 6.74; N, 7.81. Found: C, 57.02; H, 6.71; N, 7.76.

(*S*)-(−)-5-Chloro-3-ethyl-*N*-[1-(3′-fluoropropyl)-2-pyrrolidinylmethyl]-6-methoxysalicylamide (8) Compound **8** (0.317 g, 56%) was obtained as a colorless oil by the same procedure as described for **7**: $[\alpha]_D^{24}$ −70.7° (c=1.4, $CHCl_3$). MS *m/z*: 372 (M^+). IR (neat): 1640 cm^{-1} (CONH).

^{1}H-NMR ($CDCl_3$) δ: 13.7 (1H, s, OH), 8.84 (1H, br, NH), 7.21 (1H, s, aromatic), 4.53 (2H, dt, J=43.7, 5.6 Hz, CH_2F), 3.87 (3H, s, OCH_3), 3.74 (1H, m, $NHCH_2$), 3.36 (1H, m, $NHCH_2$), 3.16 (1H, m), 2.99—1.56 (12H, m), 1.19 (3H, t, J=7.8 Hz, CH_3). *Anal.* Calcd for $C_{18}H_{26}ClFN_2O_3$: C, 57.98; H, 7.03; N, 7.51. Found: C, 57.86; H, 6.96; N, 7.50.

(*S*)-(−)-5-Chloro-3-ethyl-*N*-[1-(*p*-fluorobenzyl)-2-pyrrolidinylmethyl]-6-methoxysalicylamide (10) To a mixture of *S*(+)-5-chloro-3-ethyl-6-methoxy-*N*-(2-pyrrolidinylmethyl)salicylamide (**9**)[12a] (0.4 g), and triethylamine (0.26 g) in dry acetonitrile (10 ml) was added slowly a solution of *p*-fluorobenzyl chloride (0.22 g) in dry acetonitrile (5 ml). The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated and the residue was chromatographed on silica gel ($CHCl_3$) to give **10** (0.147 g, 32%) as a colorless oil: $[\alpha]_D^{27}$ −86.9° (c=1.2, $CHCl_3$). MS m/z: 420 (M^+). IR (neat): 1640 cm^{-1} (CONH). ^{1}H-NMR ($CDCl_3$) δ: 13.81 (1H, s, OH), 8.94 (1H, br, NH), 7.37—6.90 (5H, m, aromatic), 3.98 (1H, d, J=13.3 Hz, $PhCH_2$), 3.84 (3H, s, OCH_3), 3.80—3.70 (1H, m, $NHCH_2$), 3.44—3.01 (2H, m, $NHCH_2$ and $PhCH_2$), 3.01—2.51 (4H, m), 2.35—2.02 (1H, m), 1.78—1.66 (4H, m), 1.20 (3H, t, J=7.6 Hz, CH_3). *Anal.* Calcd for $C_{22}H_{26}ClFN_2O_3$: C, 62.78; H, 5.23; N, 6.66. Found: C, 62.71; H, 5.21; N, 6.71.

In Vitro* Receptor Binding Assay** The ability of the fluorinated compounds to displace specific [^{3}H]spiperone binding to the membrane preparations from bovine striatal tissue was determined by a modification of a previously described technique.[16] Striata dissected from bovine was homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4) with a Kinematika Polytron PT-10 (setting 7, 20 s). The homogenates were centrifuged (50000 ***g, 10 min) three times, with resuspension of the intermediate pellet in the buffer. The membrane pellets were stored at −80 °C until assayed.

The buffer for [^{3}H]spiperone binding assay consisted of 50 mM of Tris–HCl, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 10^{-7} M ketanserin. The assays were carried out in a mixture of 200 μl of [^{3}H]spiperone (0.38 nM), 200 μl of various concentration of drug, and 200 μl of striatal membrane preparation (0.5 mg wet weight/tube). The assay tubes were incubated for 30 min at 25 °C. Incubation was terminated by vacuum filtration over polyethylenimine-treated glass filters (Whatman GF/B). The filters were washed with 20 ml of ice-cooled Tris–HCl buffer. Radioactivity remaining on the filters were counted by liquid scintillation spectrometry. The nonspecific binding was defined in the presence of 10^{-5} M sulpiride. The IC_{50} values was estimated by log-probit analysis.

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