Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVIII, No. 9

Synthesis of [^{18}F]-1-(3-Fluoropropyl)-4-(4-cyanophenoxymethyl)-piperidine: A Potential Sigma-1 Receptor Radioligand for PET

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Summary

[^{18}F]-1-(3-Fluoropropyl)-4-(4-cyanophenoxymethyl)piperidine has been prepared as a potential sigma-1 receptor ligand for PET. The unlabeled ligand was found to be selective *in vitro* for the sigma-1 receptor [$Ki(\sigma_1) = 4.3$ nM] when tested in a variety of neuroreceptor binding assays. Furthermore, the ligand is selective for sigma-1 over sigma-2 receptors *in vitro*, having only moderate affinity for the sigma-2 receptor [$Ki(\sigma_2) = 144$ nM)]. The lipophilicity of the ligand ($\log P_{7.5} = 2.8$) is appropriate for good brain uptake and low non-specific binding. Radiofluorination was accomplished using nucleophilic fluorination methods using an alkylmesylate precursor and the specific activity of the product was >74,000 MBq/µmole. The average radiochemical chemical yield after purification was 62.3 ± 5.2% (56-70% EOB; n = 5) and the radiochemical purity of the product was >99%. The average time of synthesis and purification was 80 minutes.

Key Words: sigma receptor, PET, fluorine-18, radiotracers, schizophrenia,

Introduction

Sigma (σ) receptors have generated recent interest due to their implicated role in certain neurological and immunological processes (1). Most of the attention has been given to sigma-1 and sigma-2 subtypes and recently a sigma-3 receptor has been postulated (2). Sigma receptors are distributed heterogeneously throughout the central nervous system and possible endogenous neurotransmitters have been identified (3-5). It has been shown from post-mortem examination that there are changes in sigma receptor densities in the cerebral cortex of schizophrenic patients (6-9) although there is conflicting evidence on this point perhaps due to the use of non-selective ligands in these studies. Our efforts have focused on the preparation of agents for *in vivo* tomographic

CCC 0362–4803/96/090785–10

Received 11 January 1996
Revised 22 April 1996

assessment of sigma receptors in humans. Such agents would allow for the determination and direct comparison of sigma receptor subtype densities in normal humans and in subjects with various neurological disorders, most notably schizophrenia (10).

The preparation of several sigma receptor ligands labelled with isotopes of carbon, fluorine, iodine and hydrogen has been reported. These include $[^{18}F]$-α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol (11), $[^{123}I]$-iodobenzamides (12), $[^{125}I]$-iodobenzovesamicol (13), $[^{125}I]$-iodophenyl-3-(adamantyl)guanidine (14), ^{18}F-haloperidol (15), and N-^{11}C-benzyl-N-normetazocine (16, 17). One tritiated compound, $[^{3}H]$-1-(cyclopropylmethyl)-4-(2'-(4"-fluorophenyl)-2'-oxoethyl)piperidine ($[^{3}H]$-DuP 734), was reported as having good brain uptake and high affinity and selectivity for sigma receptors in mice (18). However, DuP 734 also binds to serotonin $5HT_2$ receptors (Ki = 15 nM) *in vitro*. The synthesis and characterisation of a series of more selective DuP 734 analogs has also been reported (19). Of these compounds, 1-cyclopropylmethyl-4-(4-cyanophenoxymethyl)piperidine, **1**, interacts selectively with the sigma receptor *in vitro*. However, in contrast to DuP 734, **1** exhibited negligible affinity for serotonin $5HT_2$ receptors when examined in receptor binding assays (20) (Figure I). Based in part on this information, we have prepared a series of novel compounds that are selective for the sigma receptor and that can be labelled with common PET and SPECT radioisotopes. The first of these radioligands to be characterised, $[^{123}I]$-1-(*trans*-3-iodopropen-2-yl)-4-(cyanophenoxymethyl)piperidine, is selective for sigma-1 receptors *in vitro* [Ki (σ-1)/Ki (σ-2) = 0.02] and exhibits specific binding to sigma receptors *in vivo* (20, 21). We report here the synthesis and *in vitro* characterisation of 1-(3-fluoropropyl)-4-(4-cyanophenoxymethyl)piperidine, **2**, and the synthesis of the corresponding ^{18}F-labelled compound as a potential PET radioligand for the assessment of sigma-1 receptor densities.

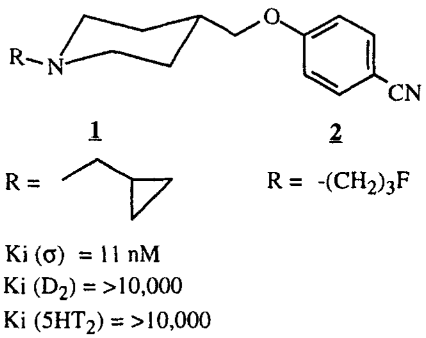


Figure I. Alkylated 4-(4-Cyanophenoxymethyl)piperidine Derivatives.

Experimental

Proton NMR spectra were recorded on a JEOL 400 MHz FT-NMR spectrometer. Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in deuterochloroform and coupling constants (J) are reported in Hz. High resolution fast atom bombardment mass spectroscopy (HRMS) was performed using a ZAB-EQ mass spectrometer at the Department of Chemistry, The University of Tennessee (Knoxville, TN). Melting points were recorded on a GallenKamp melting point apparatus and are uncorrected. Elemental analysis was performed by Atlantic Microlabs Inc. (Norcross Georgia). Gravity chromatography was performed using silica gel (Fluka, 70-230 mesh, ASTM) using the solvent systems indicated in the text. For mixed solvent systems, the ratios are given with respect to volumes.

All reagents were purchased from commercial sources and were used without further purification. [^{18}F]Fluoride was obtained from the National Medical Cyclotron (Sydney, Australia) as an aqueous solution. HPLC analysis of the radioligand was performed using a Spectraphysics P1000 HPLC pump, a Spectraphysics UV 1000 detector, and a EG&G NaI scintillation detector connected to a model NS276 photomultiplier preamplifier and an EG&G 925-SCINT-S Ace Mate amplifier and bias supply. The columns used was a reverse-phase base-deactivated column [Activon, Goldpak Exsil, ODS B, 10 µm, 4.6 x 250 mm (analytical) or 10 x 250 mm (semiprep)] and the mobile phases used are indicated in the text below. 4-(4-Cyanophenoxymethyl)-piperidine was prepared as previously described (19, 21).

1-(3-fluoropropyl)-4-(4-cyanophenoxymethyl)piperidine, **2**: To ethanol-free dichloromethane (6 ml) was added 4-(4-cyanophenoxymethyl)piperidine (225 mg, 1.04 mmol), potassium carbonate (575 mg, 4.16 mmol) and 3-bromo-1-fluoropropane (158 mg, 1.09 mmol). The resulting solution was stirred at room temperature for 24 hours. The reaction mixture was then diluted with water (100 ml) and the product extracted into dichloromethane (2 x 20 ml). The organic extracts were combined, dried over magnesium sulfate and the solvent removed *in vacuo* to provide the crude product as a yellow oil. The product was purified by column chromatography (silica gel; ethyl acetate / ethanol (9:2)) to provide a white solid (242 mg, 0.88 mmol, 84%), mp 55-56 °C; ^{1}H NMR: δ 1.36-1.49 (m, 2H), 1.75-2.03 (m, 7H), 2.50 (t, 2H, J = 7.15), 3.00 (t, 2H, J = 6.10), 3.85 (d, 2H, J = 6.10), 4.50 (dt, 2H, JHF = 47/50, JHH = 5.90), 6.94 (d, 2H, J = 9.0), 7.58 (d, 2H, J = 9.00); MS m + H/z: 277.5; Anal. calcd for $C_{16}H_{21}N_2OF$: C, 69.56; H, 7.66; N, 10.14. Found: C, 69.62; H, 7.69; N, 10.08.

1-(3-Hydroxypropyl)-4-(4-cyanophenoxymethyl)piperidine, 4: To ethanol-free dichloromethane (20 ml) was added 4-(4-cyanophenoxymethyl)piperidine (900 mg, 4.16 mmol), potassium carbonate (1.70 mg, 12.48 mmol) and 3-bromo-1-propanol (416 µl, 4.37 mmol). The resulting solution was stirred at room temperature for 24 hours. The reaction mixture was then diluted with water (100 ml) and the product extracted into dichloromethane (2 x 20 ml). The organic extracts were combined, dried over sodium sulfate and the solvent removed *in vacuo* to provide a yellow oil. The product was purified by column chromatography (silica gel, 100% ethanol) to provide a white solid (600 mg, 0.88 mmol, 53%), mp 61-62 °C; ^{1}H NMR: δ 1.50-1.65 (m, 2H), 1.83-2.02 (m, 5H), 2.20 (t, 2H, J = 7.0), 2.77 (t, 2H, J = 5.90), 3.27 (d, 2H, J = 6.10), 3.85 (t, 2H, J = 5.19), 3.89 (d, 2H, J = 6.10), 6.95 (d, 2H, J = 9.0), 7.59 (d, 2H, J = 9.00); HRMS FAB m/z (obs.) = 274.168; m/z (calc. for $C_{16}H_{22}N_2O_2$) = 274.168.

1-[(3-Methanesulfonyl)propyl]-4-(4-cyanophenoxymethyl)piperidine, 5: 1-(3-Hydroxypropyl)-4-(4-cyanophenoxymethyl)piperidine (300 mg, 1.09 mmol) was dissolved in ethanol-free dichloromethane and to this was added anhydrous triethylamine (607 µl, 4.36 mmol) and methanesulfonyl chloride (95 µl, 1.20 mmol). The resulting solution was stirred for 30 minutes at room temperature and a white precipitate formed over time. The reaction mixture was concentrated *in vacuo* and loaded directly onto a silica column. The product was eluted with ethyl acetate (Rf = 0.15) and the solvent removed *in vacuo* to provide a clear, colorless oil (310 mg, 88%) which solidified upon standing to a white, crystalline solid, mp 67-68 °C; ^{1}H NMR: δ 1.65-1.80 (m, 2H), 2.12 (d, 2H, 12 Hz), 2.28-2.40 (m, 1H), 2.75-2.88 (m, 5H), 3.60-3.75 (m, 2H), 3.93 (d, 2H, J = 6.10), 4.05 (d, 2H, J = 10.0), 4.39 (t, 2H, J = 5.50 Hz), 4.50 (t, 2H, J = 5.50), 6.95 (d, 2H, J = 9.0), 7.59 (d, 2H, J = 9.00); HRMS FAB m/z (obs.) = 352.144; m/z (calc. for $C_{17}H_{24}N_2O_4S$) = 352.146.

Synthesis of [^{18}F]-1-(3-Fluoropropyl)-4-(4-cyanophenoxymethyl)piperidine: Deionized water (100 µl) and acetonitrile (1 ml) were added to the Kryptofix-potassium carbonate reagent RBI K-107 and the vial shaken until all solids had dissolved. The resulting solution was transferred to a 5 ml Reacti-vial followed by [^{18}F]fluoride (5 mCi). The vial was placed into an aluminum block well that was pre-heated to 85 °C. The volatile materials were evaporated under a stream of nitrogen gas.

Acetonitrile (anhydrous, 1 ml) was added and the solvent was evaporated once again. This procedure was repeated 4 times. Next, the mesylate **5** (2 mg), dissolved in acetonitrile (anhydrous, 2 ml), was added and the mixture heated at 85°C for 15 minutes. The reaction progress was monitored by radio-TLC analysis to determine the formation of ^{18}F-**2** over time. The amount of unsolubilized [^{18}F]fluoride was not determined during these measurements. Silica gel plates (Merck) were used and the plates were developed with ethyl actetate/ethanol (1:1, v/v). The retention factor of ^{18}F-**2** was 0.45 under these conditions.

It was necessary to destroy the excess mesylate to allow for efficient purification of ^{18}F-**2** by HPLC. Aqueous sodium hydroxide (100 µl, 0.1 N) was added and heating continued for an additional 10 minutes. The addition of aqueous sodium hydroxide served to destroy remaining mesylate precursor and effected dissolution of the solids contained in the reaction mixture. The vial was allowed to cool to room temperature and the reaction mixture was injected directly onto the HPLC system. The product was purified using a reverse-phase semi-preparative column (Exsil ODS-B, 10 µm, 10 x 250 mm) using a mobile phase of acetonitrile and 0.1 M ammonium acetate buffer (20:80 v/v). The flow rate was 5.0 ml / min and the retention time of the ^{18}F-labeled product (1.10 mCi, 60% EOB) was 35 minutes.

An aliquot of the collected peak was spiked with **2** and re-injected onto a separate analytical HPLC column. The radioactive peak co-eluted with the authentic compound with a retention time of 27.5 minutes.

Specific Activity Determinations: The specific activity of the product was determined by HPLC analysis using an analytical reverse-phase base-deactivated column and a mobile phase consisting of acetonitrile and ammonium acetate buffer (20:80 v/v, 0.1 M), with a flow rate of 1.0 ml / min. The limit of detection of **2** was determined by plotting the mass of **2** injected versus UV detector response at 247 nm. The detection limit was determined to be the response of the detector providing a peak height 2.5 times the noise level. From extrapolation, this response corresponded to 1.8×10^{-11} mol of **2** injected. Upon analysis of 40 µCi (50 µL, n = 3) of the radioligand, the UV peak corresponding to **2** was below the limit of detection. The specific activity of the product was thus determined to be >74,000 mBq / µmol.

Lipophilicity Estimations: The lipophilicity of **2** was examined by determination of the log $P_{7.5}$ value using a HPLC method previously described (22). Briefly, samples were analysed using a C18 column (Goldpak Exsil 10 µm, 4.6 x 250 mm) and a mobile phase of MeOH and phosphate buffer (85:15 v/v, pH = 7.5) at 1.0 ml/min. The lipophilicity of 2 was determined by comparison of the retention time of the compound to that of standards having known log P values. The standards used in our study were catechol, aniline, benzene, bromobenzene, ethyl benzene, trimethylbenzene and hexachlorobenzene dissolved in an appropriate solvent. Relative retention times, RRT (to catechol), were calculated, and a calibration curve of log P vs. log RRT was generated. The calibration equations were polynomial with r^2 of 0.994 or greater. All sample injections were done in triplicate and the results averaged to provide the final values. Using this method, the log $P_{7.5}$ value for 2 was found to be 2.8.

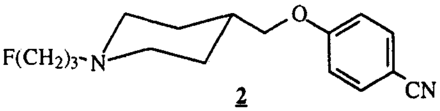
Ligand Binding Assays: Compound 2 was tested through the NIMH/NovaScreen Drug Discovery & Development Program (Contract No. NIMH-2003). Briefly, competitive binding assays were performed in either 250 or 500 µl volumes containing, by volume, 80% receptor preparations, 10% radioligand and 10% of 2 (non-specific binding determinant / 4% DMSO (total binding determinant)). All compounds were solubilised in neat DMSO and diluted with water to a final concentration of 0.4% DMSO for use in the assay. Assays were terminated by rapid vacuum filtration over glass fiber filters (Whatman) followed by rapid washing with cold buffer. Radioactivity was determined by either liquid scintillation or gamma spectrometry. Data was reduced by a software program proprietary to NOVASCREEN. Details of methods used in the *in vitro* receptor binding assays are provided in references 25-34.

Results and Discussion

The synthetic methods used to prepare the target compound, 1-(3-fluoropropyl)-4-(4-cyanophenoxymethyl)piperidine, were straightforward. 4-(4-Cyanophenoxymethyl)piperidine, 3, was prepared in five steps from ethyl isonepecotate as previously described (19, 21). Alkylation of 2 with 3-bromo-1-fluoropropane in the presence of potassium carbonate provided 2 in good yield. Likewise, compound 4 was prepared by alkylating 3 with 3-bromo-1-propanol (Scheme I). The alkyl mesylate precursor 5 was synthesised by treating 4 with methanesulfonyl chloride in the presence of triethyl amine.

Once synthesized, compound 2 was characterized *in vitro* in selected receptor binding assays (Table I). It was determined that 2 binds sigma-1 receptors with high affinity [Ki (σ-1) = 4.3 nM] and is selective for sigma-1 over sigma-2 receptors [Ki (σ-1)/Ki (σ-2) = 0.03]. Furthermore, 2 did not exhibit significant binding to other neuroreceptors examined, including phencyclidine (PCP), serotonin, dopamine, muscarinic or N-methyl-D-aspartate (NMDA) receptors. Furthermore, the lipophilicity of this compound (log $P_{7.5}$ = 2.8) is appropriate for good brain uptake and low non-specific binding in contrast to compounds having high log P values (ie log P > 4.0) (23, 24). In view of these results, the ^{18}F-labeled analogue was synthesized.

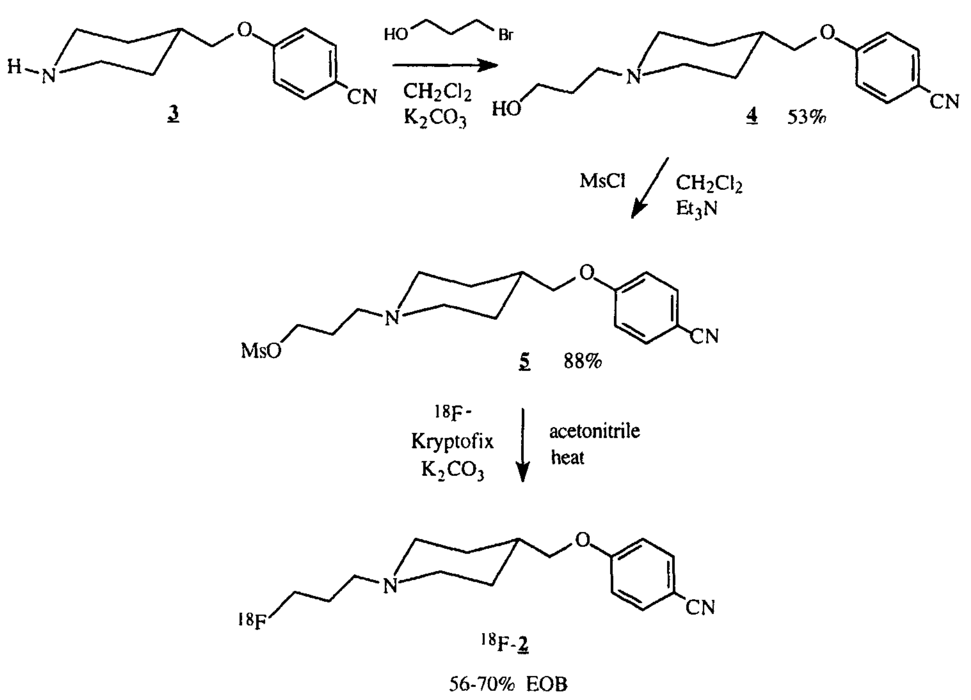
[^{18}F]-1-(3-fluoropropyl)-4-(4-cyanophenoxymethyl)piperidine was synthesized by heating 5 in a solution of [^{18}F]fluoride and Kryptofix-potassium carbonate mixture in acetonitrile. The reaction yield was optimised by observing the effects of temperature, time and amount of 5 used in the synthesis on the yield of ^{18}F-2 as observed by radio-TLC analysis of the crude reaction mixture. Reaction temperature was found to be important and 70-80% of the solubilized portion of the [^{18}F]fluoride was incorporated into the product within 15 minutes at 85 °C. However, it should be noted that the TLC results were not corrected for the amount of [^{18}F]fluoride that might not have been in solution.



2

	Reference
Ki (σ-1) = 4.3 nM	25
Ki (σ-2) = 144 nM	26
Ki (D_2) = >10,000 nM	27
Ki ($5HT_2$) = >10,000 nM	28
Ki (PCP) = >10,000 nM	29
Ki (NMDA) = >10,000 nM	30
Ki (M_1) = >10,000 nM	31
Ki (M_2) = >10,000 nM	32
Ki (M_3) = >10,000 nM	33
Ki (alpha) = >10,000 nM	34

Table I: *In Vitro* Characterisation of 1-(3-Fluoropropyl)-4-(cyanophenoxymethyl)-piperidine



Scheme I. Synthesis of $[^{18}F]$-1-(3-Fluoropropyl)-4-(4-cyanophenoxymethyl)-piperidine

It was also determined that 2 mg of 5 provided reasonable yields, whereas the yield dropped off when lesser amounts were used. To simplify purification, it was necessary to destroy any remaining mesylate precursor by adding to the reaction mixture a small amount of aqueous sodium hydroxide. This addition also effected the solubilisation of all components of the reaction mixture and allowed the resulting solution to be injection directly onto the HPLC system.

Purification of ^{18}F-2 was accomplished using a semi-preperative reversed phase HPLC column and eluting the radioligand with a mixture of acetonitrile and ammonium acetate buffer (20:80 v/v) at 5 ml / minute. The average product yield after purification was 62.3 ± 5.2% EOB (n = 5) and the range in yield was 56-70% EOB (22-29% EOS). The specific activity of the product was determined by HPLC analysis to be >74,000 MBq / μmol and the radiochemical purities were >99%. The average time for radioligand synthesis and HPLC purification was 80 minutes.

Conclusions

1-(3-Fluoropropyl)-4-(4-cyanophenoxymethyl)piperidine is a selective sigma-1 receptor ligand *in vitro* having appropriate lipophilicity for use as a neuroreceptor imaging agent. The corresponding PET radioligand, [^{18}F]-1-(3-fluoropropyl)-4-(4-cyanophenoxymethyl)piperidine, has been prepared in high specific activity and good radiochemical purity in sufficient quantities to permit *in vitro* and *in vivo* evaluations.

Acknowledgments

The authors would like to thank the staff at the National Medical Cyclotron and Royal Prince Alfred Hospital (Sydney, Australia) for preparing the no-carrier-added [^{18}F]fluoride used in this study. The authors are also indebted to NIMH/NovaScreen Drug Discovery & Development Program for performing the *in vitro* characterisation of **2**. Finally, the authors thank Mrs. Filomena Mattner for obtaining the ^{1}H NMR spectra of the compounds described in this paper.

References

1. Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R. De Costa, B. and Rice, K. C.-Pharmacol. Rev. 42:355 (1990).
2. Wyrick, S. D.; Booth, R. G; Meyers, A. M. *et al.*-J. Med Chem. 36:2542 (1993).
3. Sonders, M.; Cambell, B. and Weber, E.-Neurosci. Abst. 12:172 (1986).
4. Contreras, P. C.; Contreras, M. L; O'Donohue, T. L and Liar, C. C.-Synapse 2:240-243 (1988).
5. Conner, M. A. and Chavki, C.-Mol. Pharmacol. 42:471 (1992).
6. Shibuya, H.; Mori, H. and Toru, M.-Neurochem Res. 17:983 (1992).
7. Weissman, A. D.; Su, T.-P.; Hefreen, J. C. and London, E. D.-J. Pharmacol. Exp. Ther. 247:29 (1988).
8. Weissman, A. D.; Casonova, M. F.; Kleinman, J. E.; Londen, E. D. and Desouza, E. B.-Biol. Psychiatry 29:41 (1991).
9. Shibuya, H.; Mori, H. and Toru, M.-Neuropharmacology 10:839S (1994).
10. Tam, W.; Steinfels, G. F.; Gilligand, P. J.; Schmidt, W. K. and Cook, L.-J. Pharmacol. Exp. Ther. 263:1167 (1992).
11. Ding Y-U, Fowler F. S., Dewey S. L., Wolf A. P., Logan J., Gatley S. J., Volkow N. D., Shea C. and Taylor D. P. - J. Nucl. Med. 34:246 (1993).
12. John C. S., Bowen W. D., Saga T., Kinuya S., Vilner B. J., Baumgold J., Paik C. H., Reba R. C., Neumann R. D., Varma V. M. and McAfee J. G. - J. Nucl. Med. 34:2169 (1993).
13. Jung Y.-W, Mukhopadhay S., Sherman P.S., Frey K. A. and Wieland, D. M. - J. Nucl. Med. abst. 35:93p (1994).
14. Kimes A. S., Wilson A. A., Scheffel U., Campbell B. G. and London E. D. - J. Med. Chem. 35:4683 (1992).
15. Schlyer D. J., Volkow N. D., Fowler J. S., Wolf A. P., Shiue C. Y., Dewey S. L., Brendriem B, Logan J., Raulli R., Hitzemann R., Brodie J., Alavi A. A. and MacGregor R. R. - Synapse 11:10 (1992).

16. Musachio J., Mathews W., Ravert H., Carroll F. and Dannals R. - J. Labelled Compds. Radiopharm. 34:49 (1993).
17. Masachio, J. L.; Scheffel, U.; Stathis, M.; Ravert, H. T.; Mathews, W. B. and Dannels, R. F.- Life Sciences 55:225 (1994).
18. Wantanabe, M.; Rominger, D.; Hurt, S. D.; De Souza, E. B. and Tam, W. S.- J. Pharmacol. Exp. Ther. 266:1541 (1993).
19. Gilligan J. P., Cain G. A., Christos T. E., Cook L., Drummond S., Johnson A. J., Kergaye A. A., McElroy J. F., Rohrbach K. W., Schmidt W. K. and Tam W. S. - J. Med. Chem. 35:4344 (1992).
20. Waterhouse, R. N.; Collier, T. L.; Mardon, K. and O'Brien, J.-J. Nucl. Med. 36:124P (1995).
21. Waterhouse, R. N.; Collier, T. L. and O'Brien, J. C.-J. Labelled Compds. Radiopharm. (in press).
22. Brent D. A., Sabatka J. J., Minick, D. J. and Henry D. W. - J. Med. Chem. 26:1014 (1983)
23. Coats E. A. - Radiopharmaceuticals: Structure-Activity Relationships; Spencer R. P. ed., Grune and Stratton, New York; p. 3-22 (1981).
24. Eckleman, W. C. In Nuclear Imaging in Drug Discovery, Development and Approval: Burns, D. H.; Gibson, R. E. Dannals, R. F. and Siegl, P. K. eds. Birkhauser:Boston. p113 (1993).
25. Karbon E.W., Naper K. and Pontecorvo M.J.-Eur. J. Pharmacol. 193:21 (1991).
26. Leysen J. E., Niemegeers C. J., Van Nueten J. M. and Laduron, P. M.-Mol. Pharmacol. 21:301 (1982) with modifications.
27. Lummis S. C. R. and Kilpatrick G. J.-Eur. J. Pharmacol. 189:223 (1990) with modifications.
28. Peroutka, S. J. and Snyder S. H.-Mol. Pharacol. 16:687 (1979) with modifications.
29. Luthin G. R. and Wolfe B. B.-Mol. Pharmacol. 26:164 (1984).
30. Hanack D. and Pfeiffer A.-Digestion 45:196 (1990).
31. Lehmann J., Hutchinson A. J. et al.-J. Phamacol. Exp. Ther. 246:65 (1988) with modifications.
32. Vignon J. et al.-Brain Research 280:194 (1983) with modifications.
33. Anderson P. H., Gronvald, F. C. et al.- Eur. J. Pharmacol. 219:45 (1992).
34. Riva M. and Creese I-Mol. Pharmacol. 36:211 (1989) with modifications.