RADIOSYNTHESIS OF NO-CARRIER-ADDED ^{75,77}Br-BROMBENPERIDOL

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SUMMARY

A three-step synthesis of the neuroleptic benperidol is presented, and the rapid no-carrier-added (n.c.a.) radiobromination of this compound to form brombenperidol (BBP), a radiolabelled dopaminergic receptor-binding ligand, is described. N.c.a. radiobromination of benperidol using dichloramine-T in trifluoroacetic acid at elevated temperatures results in bromination totally at the benzimidazolinyl ring of the neuroleptic, leaving the molecular characteristics necessary for receptor binding unaltered. Radiopharmaceutical production results in n.c.a. ^{75,77}Br-brombenperidol with a corrected radiochemical yield of 30% and a specific activity exceeding 8,000 Ci/mmol.

Key words: Benperidol, brombenperidol, neuroleptic, dopaminergic receptor, no-carrier-added radiobromination, ^{75}Br, ^{77}Br.

INTRODUCTION

There is currently great interest in the development of positron-emitting, dopamine receptor-binding radiopharmaceuticals for mapping cerebral dopaminergic receptor areas

0362-4803/84/090875-13$01.30

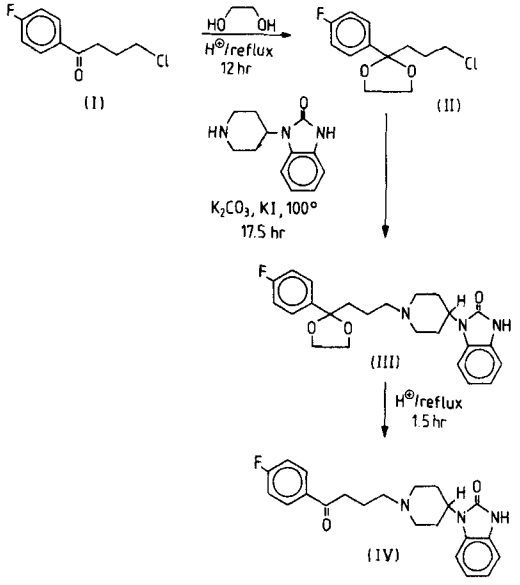
Received March 21, 1984

with positron emission computed tomography (PECT). Neuroleptics are known to bind to cerebral dopamine receptors in vivo (1-3), and these ligands labelled with ^{18}F (4-6), ^{11}C (7-11), and ^{77}Br (12-15) have been proposed as radiopharmaceuticals for cerebral imaging. Unfortunately, useful radiochemical yields at the no-carrier-added (n.c.a.) level are not possible with ^{18}F at this time, and the short half-life and relatively low specific activity of ^{11}C limit the convenience of this nuclide in radiosynthetic and imaging procedures.

Our goal has been to develop a dopamine receptor-binding ligand labelled with the positron-emitter ^{75}Br due to its useful half-life of 1.6 hr and because high radiobromination yields are possible at the n.c.a. level (16). The butyrophenone neuroleptic benperidol was chosen as a radiobromination substrate due to its high affinity for binding to cerebral dopaminergic receptors (K_i = 1.4 nM) and relatively low affinity for binding to competitive cerebral serotonergic (K_i = 3.7 nM) and α-adrenergic (K_i = 7.1 nM) receptors (17). We report here the radiosynthesis of a high specific-activity brominated analogue of benperidol, ^{75}Br- and ^{77}Br-brombenperidol {1-[1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinyl]-1,3-dihydro-2H-bromobenzimidazol-2-one}.

RESULTS AND DISCUSSION

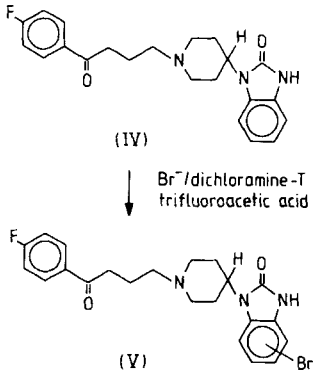
Benperidol bromination substrate was synthesized in a three-step sequence as illustrated in Scheme 1, where ω-chloro p-fluorobutyrophenone was ketalized prior to alkylation of piperidinyl benzimidazolinone.



Scheme 1

Following deketalization and work-up, product benperidol was characterized by ^{1}H-NMR, MS, IR, and melting point, and was chromatographically identical to authentic benperidol.

From the structure-activity relations of neuroleptics, it is known that the 4'-fluoro-4-aminobutyrophenone moiety is essential for cerebral dopaminergic receptor binding (18). Radiolabelling the neuroleptic at a site of the molecular architecture other than this essential region allows for a foreign label such as ^{75}Br or ^{77}Br to be introduced, and relaxes the regiospecific constraints in producing a radiolabelled analogue. This approach was applied in the bromination of benperidol, where electrophilic bromination occurs solely at the benzimidazolinone ring and leaves the essential pharmacophore intact in brombenperidol (BBP, Scheme 2).



Scheme 2

Bromination of the butyrophenone ring does not take place due to the deactivating influence of the fluoro and carbonyl substituents on electrophilic substitution. That bromination at the benzimidazolinone ring occurs is snown by ^{1}H-NMR (loss of one aromatic proton in BBP) and MS (appearance of fragments at m/e = 325/323 and 311/309, brominated N-ethyl and N-methyl pyridinyloximidazolinone fragments) results. Infrared spectroscopy also indicates that bromination occurs on the benzimidazolinone ring, as the infrared spectra show a shift from two absorption bands centered at 750 cm^{-1} for benperidol to two bands centered at 800 cm^{-1} in BBP. These absorption bands are typical for out-of-plane C-H bending modes, the former for 1,2 disubstituted aromatic rings, and the latter for trisubstituted aromatic systems (19,20).

This electrophilic reaction mechanism also worked well using radiobromide at the no-carrier-added level. N.c.a. radiobrominations were performed using benperidol substrate and dichloramine-T (DCT) oxidizing agent in trifluoroacetic acid to enhance the solubility of the neuroleptic as well as to promote formation of ^{75,77}Br bromonium species in the

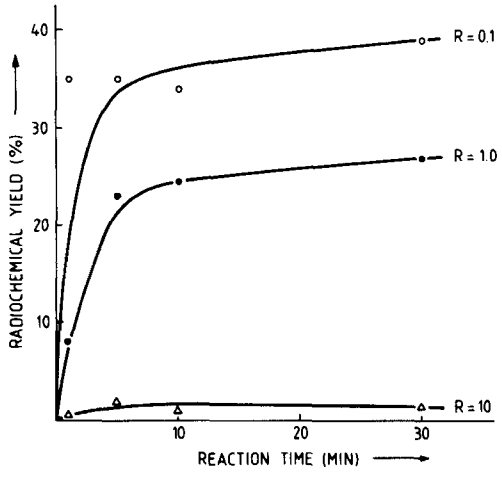


Figure 1. Effect of the dichloramine-T/benperidol molar ratio (R) on the no-carrier-added radiobromination of benperidol.

oxidative labelling reaction (21). The effect of the DCT/benperidol molar concentration on the radiochemical yield of ^{75,77}Br-BBP is shown in Figure 1.

The effect of temperature on the n.c.a. radiobromination reaction is illustrated in Figure 2 for a relative DCT/benperidol molar concentration of 0.1.

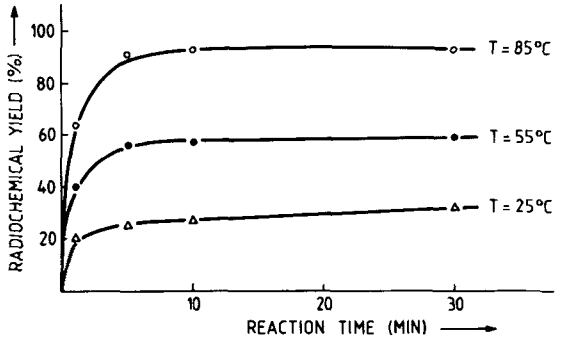


Figure 2. Effect of reaction temperature on the no-carrier-added radiobromination of benperidol.

Reaction yields of ^{75,77}Br-BBP up to 90% were achieved at a DCT/benperidol molar ratio of 0.1, a temperature of 85 ^{o}C, and a reaction time of 30 minutes.

These model study parameters were applied to the radiosynthesis of ^{77}Br-BBP for in vivo applications. Radiopharmaceutical production, including HPLC purification and sterile filtration, results in ^{77}Br-BBP with a specific activity exceeding 8,000 Ci/mmol and an overall corrected radiochemical yield of 30%. ^{77}Br-BBP produced by this method has been used for pharmacokinetic studies in mice (22) and regional cerebral localization experiments in rats (23). Current efforts are devoted toward applying this radiosynthetic method to the high-activity, high specific-activity synthesis of ^{75}Br-BBP for evaluation in primates. In this regard, we are evaluating the use of chloramine-T in aqueous reaction media, in which synthesis times may be shortened because drying of $^{75}Br^{-}$ prior to labelling is unnecessary.

EXPERIMENTAL

4-Chloro-1,1-ethylenedioxy-1-(4-fluorophenyl)butane (II)

A mixture consisting of 4-chloro-(4-fluorophenyl)butyrophenone (6 ml, 36.5 mmol), ethylene glycol (6 ml, 110 mmol), p-toluenesulfonic acid (500 mg) in anhydrous benzene (400 ml) was refluxed for 12 hours with azeotropic distillation of water. The organic phase was successively washed with equal volumes of 5% $NaHCO_3$ and water, dried with Na_2SO_4, and the solvents removed to leave 10.6 g (98%) product.

4-[4-(2-Oxo-1-benzimidazolinyl)-piperidinyl]-1,1-ethylene-dioxy-1-(4-fluorophenyl)butane (III)

A mixture of 4-chloro-1,1-ethylenedioxy-(4-fluorophenyl)-butane (4.5 g, 18.4 mmol), 4-(2-oxo-1-benzimidazolinyl)-piperidine (4 g, 18.4 mmol), potassium carbonate (9 g), and potassium iodide (210 mg) in anhydrous dimethylformamide (75 ml) was stirred at 100 °C for 17.5 hours. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with water, dried with magnesium sulfate, and the solvent removed to leave 6.66 g (85%) product.

1-{1-[4-(4-Fluorophenyl)-4-oxobutyl]-4-piperidinyl}-1,3-dihydro-2H-benzimidazol-2-one (IV; Benperidol)

4-[4-(2-Oxo-1-benzimidazolinyl)-piperidinyl]-1,1-ethylene-dioxy-1-(4-fluorophenyl)butane (5 g, 11.8 mmol) and concentrated hydrochloric acid (5 ml) in methanol (60 ml) was refluxed for 2 hours. The reaction mixture was diluted with ethyl acetate and successively washed with 5% aqueous ammonia and water. The solution was dried with magnesium sulfate and the solvents removed. The residue was recrystallized twice from ethyl acetate to yield a solid product (3.95 g, 88%); m.p. 170-174 °C. IR (KBr): 3400 cm^{-1} (m, br, ν NH); 3090-3010 (m, ν CH aryl); 2940-2820 (m, ν CH alkyl); 1710 (s, ν CO amide); 1680 (s, ν CO aryl); 1590, 1480 (m, ν C=C); 1360-1080 (m-s, ν CF); 830 (s, γ CH p-disubst. Ø); 765, 735 (s, γ CH o-disubst. Ø); ^{1}H-NMR ($CDCl_3$); δ = 1.7-2.6 (m, 13H); δ = 3.0 (t, J = 7 Hz, CO-C<u>H</u>$_2$, 2H); δ = 4.35,(s, N<u>H</u>, 1H); δ = 7.1-7.3 (m, ArH, 6H); δ = 8.15 (dd, J = 3 Hz, 6 Hz, ArH, 2H).

MS: M^+(381), m/e = 363 (40%), 244 (40%), 230 (100%), 165 (10%), 123 (50%), 95 (15%).

Analysis: Calculated for $C_{22}H_{24}N_3O_2F$: C, 69.3%; H, 6.3%. Found: C, 69.7%; H, 6.5%.

The retention of the above product was identical to authentic benperidol on two thin-layer chromatographic systems: Kieselgel Si-60, MeOH: c-C_6H_{12}: AcOEt = 18:33:49, R_f = 0.58; Kieselgel Si-60, EtOH: CCl_4 = 16:84, R_f = 0.51.

1-{1-[4-(4-Fluorophenyl)-4-oxobutyl]-4-piperidinyl}-1,3-dihydro-2H-bromobenzimidazol-2-one (V, Brombenperidol)

1 g (2.6 mmol) benperidol and 360 mg (3 mmol) potassium bromide were dissolved in 30 ml trifluoroacetic acid in a round-bottomed flask equipped with reflux condenser. 3.4 ml of dichloramine-T solution (100 mg/ml CH_2Cl_2; 1.3 mmol) was added and the solution was stirred at 80 °C for two hours. Following this period, the reaction mixture was cooled in an ice bath and neutralized by dropwise addition of 50 ml 33% aqueous NH_3. The organic products were extracted into 200 ml $CHCl_3$, washed twice with 100 ml water, and dried with Na_2SO_4. The solvents were removed by evaporation at reduced pressure to leave 1.19 g solid (theoretical 1.21 g, 98%). Preceeding further analysis, BBP was purified using preparative HPLC (RP-18, MeOH: H_2O: Et_3N = 60:40:0.1, k' = 21) to leave a solid with m.p. 182-185 °C.

IR (KBr): 3340 cm^{-1} (m, br, ν NH); 3080-3010 (m, ν CH aryl); 2960-2800 (ν CH alkyl); 1690 (s, br, ν C=O); 1590, 1480 (s, ν C=C); 1365-1090 (m-s, ν CF, CBr); 825 (m, δ CH p-disubst. Ø); 810, 790 (m, γ CH 1,2,3-trisubst. Ø);

^{1}H-NMR ($CDCl_3$): δ = 1.8-2.6 (m, 13H); δ = 3.1 (t, J = 7 Hz,

CO-C$\underline{H}_2$, 2H); δ = 4.1 (s, N$\underline{H}$, 1H); δ = 7.1-7.3 (m, ArH, 5H); δ = 8.15 (dd, J = 4 Hz, 6 Hz, ArH, 2H).

MS: M^+(462/460); m/e = 444/442 (45%); 325/323 (40%); 311/309 (100%); 165 (10%); 123 (50%); 95 (12%).

Analysis: Calculated for $C_{22}H_{23}N_3O_2BrF$: C, 57.3%; H, 5.0%. Found: C, 57.7%; H, 5.3%.

TLC: Kieselgel Si-60, MeOH: c-C_6H_{12}: AcOEt = 18:33:49, R_f = 0.70; Kieselgel Si-60, EtOH: CCl_4 = 16:84, R_f = 0.58.

Radiobromination Studies

The bromine radionuclides were produced via the $^{75}As(^3He,3n)^{75}Br$ reaction or the $^{75}As(\alpha,2n)^{77}Br$ reaction using the Jülich CV-28 compact cyclotron (24). For synthetic optimization studies, 100-200 μCi of $^{75}Br^-$ or $^{77}Br^-$ was dried in vacuo at 100 °C and stirred for 5 minutes in a sealed reaction vessel after addition of benperidol and 1 ml trifluoroacetic acid. Dichloramine-T as a 0.3 $\underline{M}$ solution in CH_2Cl_2 was then added to begin the oxidative bromination reaction. 100 μl of the reaction mixture was removed following a given reaction time, and the reaction quenched by addition to 2 ml 33% aqueous NH_3 and extraction of the organic products into 1 ml chloroform. The organic phase was washed with 2 ml distilled water, and the organic products determined by eluting a 100 μl sample of the organic phase through an HPLC Si-100 column (10 μm, 1.7 cm x 52 cm) using a mobile phase consisting of chloroform: ethanol = 90:10, flow rate 0.5 ml/min. 0.25 ml fractions were collected and counted in an automated NaI (Tl) well-type scintillation counter. The radiochemical yield of ^{75,77}Br-BBP

was calculated based on the % organic yield resulting from extraction and the fraction of the organic radioactivity which was eluted with the retention time of BBP standard (k' = 5.5).

Preparation of High Specific Activity ^{75,77}Br-BBP for In-Vivo Studies

Based upon the results of the optimization studies, n.c.a. radiobrominations were performed using 5 mg (13 μmol) benperidol and 5 μl (1.4 μmol) dichloramine-T (75 mg/ml CH_2Cl_2) in 0.5 ml trifluoroacetic acid at 85 °C for 30 minutes. Following extraction into 1 ml chloroform as above, the organic solvent was removed using a helium stream. The dried ^{75,77}Br-BBP was then dissolved in 1 ml MeOH and purified using HPLC. Separation of ^{75,77}Br-BBP from unlabelled benperidol substrate as well as chlorinated side-product was achieved using reverse-phase HPLC (7 μm RP-18, 1.3 cm x 26 cm); mobile phase MeOH: H_2O: Et_3N = 60:40:0.1, flow rate 2.0 ml/min). 4 ml fractions were collected, and the fractions corresponding to the elution time of BBP (k' = 21) were combined. The solvents were removed by evaporation at reduced pressure, and the ^{75,77}Br-BBP was redissolved in 0.9% NaCl prior to sterile filtration and animal studies. The overall radiopharmaceutical production yield was 30% (corrected for decay), and the specific activity as determined using a benperidol mass/UV absorption correlation exceeded 8,000 Ci/nmol.

ACKNOWLEDGMENTS

S.M.M. is grateful for NIH/PHS National Research Service Award 1-F32-NS-07047-2 in support of this work. The authors

thank the members of the Jülich CV-28 compact cyclotron team for their numerous irradiations, Dr. F. Wirtz-Peitz of A. Nattermann & Cie, Köln, for performance of ^{1}H-NMR and MS analyses, and Herrn P. Laufer for performing ^{1}H-NMR spectra. The authors are also grateful to Dr. P. Laduron of Janssen Pharmaceutica for the gift of benperidol standard.

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