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The Synthesis of Tritiated 2-Phenyl-4-[4-(2-pyrimidyl)piperazinyl]methylimidazole ([^{3}H] NGD 94-1), a ligand selective for the Dopamine D_4 receptor subtype.

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Summary

A tritiated ligand for the dopamine D_4 receptor subtype, 2-phenyl-4-[4-(2-pyrimidyl) piperazinyl] methylimidazole ([^{3}H] NGD 94-1), was prepared in six steps starting with 3, 5-dichlorobenzonitrile. NGD 94-1 was shown to have a greater than 500 fold specificity for D_4 over other dopamine receptor subtypes.

Key Words: D_4 receptor, subtype specific ligand, NGD 94-1, 2-phenyl-4-[4-(2-pyrimidyl)piperazinyl]methylimidazole, tritium.

INTRODUCTION

Schizophrenia is a psychological syndrome of unknown origin whose many symptoms can include hallucinations, social withdrawal and paranoia. The effectiveness of antipsychotic medications has led to a biochemical hypothesis of the illness. This hypothesis points to a malfunctioning of the dopamine receptor system.[1-3] This postulate is reinforced by a remarkable correlation between the cerebrospinal fluid (CSF) concentration and the affinity for the D_2 receptor subtype of the vast majority of clinically effective antipsychotic agents.[4] An important exception to this correlation is the dibenzo-1,4-diazepine clozapine, whose CSF concentration correlates best with its affinity for the D_4 receptor subtype.[5] This correlation suggests that, unlike most neuroleptic drugs, the antipsychotic effects of clozapine are a result of its interaction with the D_4 receptor population rather than D_2 receptor sites. This distinction is important in light of the observation that the neuroleptics having strong affinity for D_2 receptors also present a high risk for development of extrapyrimidyl motor side effects and tardive dyskinesia, while clozapine is free of

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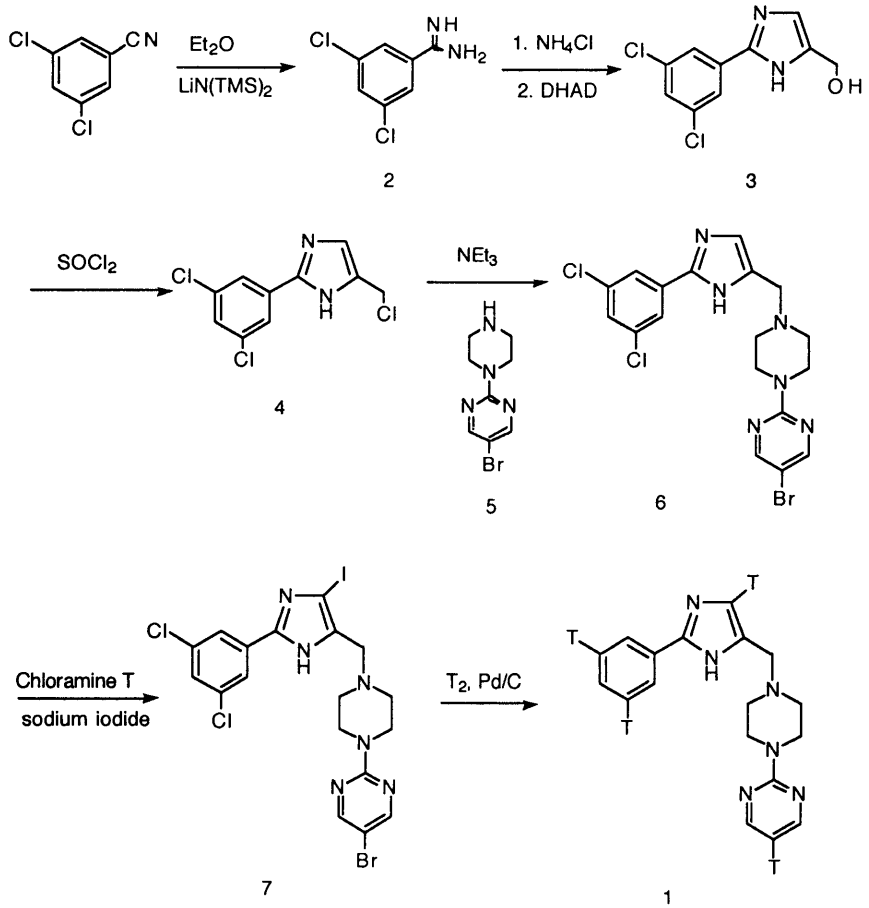
these disturbing problems.[6] Although clozapine is an important antipsychotic agent, it displays an affinity for a wide variety of CNS receptor systems. In order to investigate the role of D_4 receptors in schizophrenia and other neurological disorders it is necessary to have a radiolabelled ligand with high selectivity for this dopamine receptor subtype. This paper describes the preparation of the D_4 selective compound 2-phenyl-4-[4-(2-pyrimidyl)piperazinyl] methylimidazole, hereafter referred to as [^{3}H] NGD 94-1.

CHEMISTRY

The preparation of [^{3}H] NGD 94-1 (1) is outlined in Scheme 1. Benzamidine 2 was prepared by the addition of lithium hexamethysilazane to commercially available 3, 5-dichlorobenzonitrile followed by hydrolysis.[7] The hydrochloride salt of compound 2 was then condensed with dihydroxyacetone dimer (DHAD) to provide 2-(3, 5-dichlorophenyl)-4-hydroxymethyl

Scheme 1. Synthesis of Tritiated 2-Phenyl-4-[4-(2-pyrimidyl)piperazinyl] methylimidazole ([^{3}H]-NGD 94-1).



imidazole (3). Treatment of 3 with thionyl chloride provided the intermediate chloromethyl compound 4 which was condensed with 1-(5-bromo-2-pyrimidyl)piperazine (5) in the presence of triethylamine to give the bromodichloro compound 6. Iodination of 6 using sodium iodide/Chloramine T provided 7, the tetrahalo precursor of [^{3}H] NGD 94-1. Tritiation of 7 over palladium on carbon followed by HPLC purification gave [^{3}H] NGD 94-1 (1), of specific activity 57 Ci/mmol at >99% radiochemical purity as determined by GC-mass spectral analysis.

RECEPTOR BINDING.

Affinity of NGD 94-1 at dopamine receptor subtypes (D_1-D_5) was determined via standard competitive displacement assays using D_2, D_3, and D_5 receptor cloned from the African Green monkey and D_1 and D_4 receptor cloned from human. Each of the receptor clones was independently expressed in the chinese hampster ovary cell, CHO-K1, by the methods of Graham and van der Eb.[8] The competitive ligands used for each of the binding assays and the affinity of NGD 94-1 for each of the receptor subtypes is shown in Table 1. All binding assays were conducted in triplicate.

Table 1. Affinity of NGD 94-1 to Cloned Dopamine Receptor Subtypes.

Receptor Clone	Competitive Ligand	Affinity (nM)
D_1	[^{3}H]SCH 23390	>10000
D_2	[^{3}H]YM 09151	2230 ± 176
D_3	[^{3}H]YM 09151	>10000
D_4	[^{3}H]YM 09151 [9]	4 ± 0.3
D_5	[^{3}H]SCH 23390	>10000

The affinity of NGD 94-1 for the D_4 receptor was found to be greater than its affinity for the other dopamine receptor subtypes by a factor of at least 500. Tritiated NGD 94-1 would therefore serve well as competitive ligand for D_4 receptors and as a probe to determine D_4 receptor distribution in the brain.

EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Elemental analysis of intermediates 2, 3, 6, 7 and unlabelled NGD 94-1 (dimaleate salt) were performed at Robertson Microlabs, Madison, NJ; elemental

analyses were within 0.4% of the theoretical C, H and N. Electron ionization mass spectra were obtain using a Hewlett-Packard 5890 mass spectrometer. ^{1}H NMR spectra were recorded from DMSO-d_6 solutions using a Varian Gemini 300 spectrometer; results are recorded as ppm downfield from the TMS signal. Spectral data for all amines are reported in the free base form.

3,5-dichlorobenzamidine (2).

Commercially available 3, 5-dichlorobenzonitrile (1.0 g, 5.8 mmol) was dissolved in 25 ml of anhydrous diethyl ether and solid lithium hexamethyldisilazide (1.3 g, 1.3 eq.) was added in one portion and the reaction stirred at room temperature for 3 hr. The mixture was then cooled in ice water and treated with 25 mL of 3.0 N hydrochloric acid solution. The organic layer was discarded and the aqueous layer was treated with excess 25% NaOH solution and extracted with 50 mL portions of chloroform. The combined chloroform extracts were dried with potassium carbonate, filtered and concentrated to provide 3, 5-dichlorobenzamidine as a white solid (0.78 g, 71 %), m.p. 105-107 °C.

2-(3, 5-dichlorophenyl)-4-hydroxymethylimidazole (3).

A suspension of 3, 5-dichlorobenzamidine (0.7 g, 3.72 mmol), dihydroxyacetone dimer (0.67 g) and ammonium chloride (0.5 g) in 2 mL of tetrahydrofuran and 4 mL of concentrated NH_4OH solution was refluxed for 45 min and then cooled to room temperature. The resulting heterogeneous mixture was partitioned between water and chloroform. The organic layer was washed once with water, dried (Na_2SO_4) and concentrated. The resultant solid was triturated with ice cold isopropanol and filtered to provide 2-(3, 5-dichlorophenyl)-4-hydroxymethylimidazole (0.61 g, 68 %) as an off-white solid, m.p. 208-209 °C. ^{1}H NMR (DMSO-d_6) 7.91 (s, 2H), 7.53 (bs,1H), 7.15 (s, 1H), 4.39 (m, 2H); a small amount (approx. 10%) of the tautomeric imidazole can also be observed in the ^{1}H NMR with signals at 7.98 (2H), 7.53 (s, 1H), 6.91 (s, 1H), 4.45 (m, 2H).

1-(2-(5-bromo)pyrimidyl)piperazine (5).

To a solution of 15 g (91.5 mmol) of 1-(2-pyrimidyl)piperazine base in 120 mL of methylene chloride cooled to 0 °C was added dropwise a solution of bromine (15 g, 1.02 eq) in 40 mL of methylene chloride. After the addition was complete, the resultant solid was filtered and washed with cold methylene chloride. The solid was transferred to a separatory funnel and partitioned between chloroform and 1 N NaOH solution. The organic layer was dried (Na_2SO_4) and concentrated. The resultant solid was dissolved in 25 mL of hot ethyl acetate and allowed to crystallize overnight to provide 4.2 g of the desired product, m.p. 165-175 °C (dec). ^{1}H NMR (DMSO-d_6) 8.47 (s, 2H), 3.76 (m, 4H), 2.92 (m, 4H).

2-((3,5-dichloro)phenyl)-4-(4-(2-(5-bromo)pyrimidyl)piperazinyl)methylimidazole (6).

2-(3, 5-dichlorophenyl)-4-hydroxymethylimidazole (0.5 g, 2.07 mmol) was dissolved in 4 mL of thionyl chloride and the solution was brought briefly to reflux before being concentrated on a rotary evaporator. The resultant solid was slurried in 5 mL of chloroform and reconcentrated to provide the intermediate chloromethyl hydrochloride as an off-white solid. This material was again slurried in chloroform (5 mL) and a solution of 1-(2-(5-bromo)pyrimidyl)piperazine (0.5 g, 2.07 mmol) in 5 mL of chloroform containing 0.5 mL of triethyl amine was added. The resultant mixture was stirred for 30 min and then washed with 5 mL of 1 N NaOH solution, dried (Na_2SO_4) and concentrated. The resulting solid was triturated with ice cold methanol and filtered to provide the desired product (0.78 g, 81 %), m.p. 223-224 °C. ^{1}H NMR (DMSO-d_6) 8.41 (s, 2H), 7.94 (s, 2H), 7.52 (s, 1H), 7.08 (bs, 1H), 3.64 (m, 4H), 3.47 (s, 2H), 2.43 (m, 4H).

2-((3,5-dichloro)phenyl)-4-iodo-5-(4-(2-(5-bromo)pyrimidyl)piperazinyl)methylimidazole (7).

To a stirred solution of 200 mg (0.43 mmol) of 2-(3, 5-dichlorophenyl)-4-(4-(2-(5-bromopyrimidyl))piperazinyl)methyl imidazole (6) in 5 mL of methanol was added a solution of sodium iodide (77 mg) in 0.5 mL of water. To the resultant mixture was added 116 mg of Chloramine-T hydrate. After 10 min, 8 mL of ethyl acetate was added and the mixture was washed with 2 mL of saturated sodium bisulfate solution. The stirring was stopped and the mixture allowed to stand at room temperature for 45 min after which time the resultant solid was collected by filtration to provide 57 mg (25 %) of the desired product as a white solid (m.p. 218-221 °C). A further 96 mg of product was obtained by concentration of the filtrate and purification of the resultant material on preparative TLC (10% $CH_3OH/CHCl_3$, R_f = 0.59). ^{1}H NMR (DMSO-d_6) 8.42 (s, 2H), 7.95 (s, 2H), 7.59 (s, 1H), 3.64-3.75 (m, 4H), 3.48 (s, 2H), 2.40-2.45 (m, 4H).

Tritiated 2-phenyl-4-[4-(2-pyrimidyl)piperazinyl]methylimidazole (1, NGD 94-1).

A slurry of 10 mg of 7 and 15 mg of 10% Pd/C in 2 mL of methanol containing 0.1 mL of triethyl amine was stirred and exposed to an atmosphere of tritium (T_2) gas for a period of 60 min. The mixture was then filtered through a small pad of celite to remove the catalyst before being concentrated under a stream of nitrogen. The resulting material was taken up in 1 ml of chloroform and washed with 0.5 mL of 1 N NaOH solution. The organic layer was dried by passing through a small amount of anhydrous sodium sulfate. The chloroform was then removed under a stream of nitrogen. The

residue was purified by reverse phase high pressure liquid chromatography using an ODS column (20 cm) eluting with acetonitrile/water to afford 49 mCi (27 %) of **1** in >99% radiochemical purity as determined by TLC scanner. A specific activity of 57 Ci/mmol was determined by GC-MS analysis.

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