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Synthesis of Two Dopamine D4 Receptor Ligands: ^{11}C Labelled Chromeno[3,4-c]pyridin-5-ones

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Summary

The synthesis of two ^{11}C labelled chromeno[3,4-c]pyridin-5-ones for the visualisation of the dopamine D4 receptor subtype has been developed. The production entailed an *O*-methylation of the *O*-desmethyl precursor with [^{11}C]iodomethane in the presence of tetrabutylammonium hydroxide. Subsequent purification by RP-HPLC and formulation by tracer enrichment on a C18 Sep Pak provided a solution which was suitable for human iv injection. Specific activity of the tracer averaged 37 GBq/μmol at EOS and the radiochemical yields were 65% (decay-corrected, based on [^{11}C]CH_3I). Total activity obtained was 5.6 – 7.4 GBq. The preparations have been demonstrated to be chemically and radiochemically pure by HPLC.

Introduction

Schizophrenia is a complex disease affecting approximately 1% of the world population (1). Although many neurotransmitter systems have been implicated in the pathophysiology of schizophrenia, most widely used therapeutics in the treatment of this disease are those that block transmission at dopamine receptors (2). Recent advantages in molecular biology lead to the classification of dopamine

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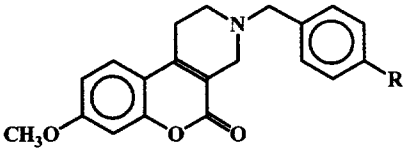
receptors in two categories (D1-like and D2-like) with at least 5 receptor subtypes (D1 and D5 for the D1-like family, and D2, D3, and D4 for the D2-like family) (3). From the historical point of view, pharmaceutical industries showed interest in antagonists of the D2-like family because of their antipsychotic potency in the treatment of schizophrenia (2). These classical antipsychotics cause extrapyramidal side effects (4) and tardive dyskinesias (5). These side effects have been linked to a blockade of the D2 receptor subtype in the striatal regions. Autoradiographic studies (6) and studies based on mRNA distribution (7) suggest that the D4 receptor subtype is preferentially located in the hippocampus, enthorhinal and prefrontal cortex and that no receptors are present in the striatum. Furthermore, it has been postulated that D4 receptor subtype levels are elevated in schizophrenia (8,9), although the evidence for this observation is currently controversial (10).

The development of a labelled selective ligand suitable for the visualisation of the D4 receptor subtype *in vivo* in the living human, may be useful to better understand the mechanism of schizophrenia.

Recently the syntheses of two selective ^{18}F labelled 1-phenyl-3-(aminoethyl)pyrol derivatives were described (11,12). The only *in vivo* PET study that provides evidence of the presence of the D4 receptor subtype was reported by Boy, et al. (17). They performed their studies with the D1/D4 antagonist ^{11}C-SDZ GLC 756 and made estimation on the distribution of the D4 receptor subtype by blocking studies with SCH 23390 and raclopride.

Selective D4 receptor antagonists have recently been reported. These include the imidazoles (13), benzamides (14), isochromanes (15) and chromenopyridines (16).

We hereby report the synthesis of two ^{11}C labelled selective D4 receptor subtype ligands with the chromeno[3,4-c]pyridin-5-one structure (figure 1), namely 3-(4-chlorobenzyl)-8-[^{11}C]methoxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one and 3-(4-trifluorobenzyl)-8-[^{11}C]methoxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one.



R = Cl **1**
R = CF_3 **2**

Figure 1: Chemical structure of chromeno[3,4-c]pyridin-5-ones with affinity for the D4 receptor subtype

Both 1 and 2 show nanomolar affinity and good selectivity for the D4 receptor subtype (16) (Affinities for the cloned human D4 receptor subtype are respectively 8.7 and 8.2 nM, each at least 100-fold selective versus the other dopamine receptor subtypes). Further more, these compounds have no appreciable affinity (K_i > 700 nM) for rat brain α_1-adrenergic, α_2-adrenergic, 5HT-1_A and 5HT-2_A receptors. Compound 1 still shows an intrinsic activity of 26.8% and is considered as a partial agonist, while compound 2 shows no intrinsic activity at all.

Results and Discussion

The radiosynthesis of ^{11}C-labelled 1 or 2 is a one pot reaction and is shown in figure 2. [^{11}C]Methylation of the *O*-desmethyl precursor 5 or 6 with [^{11}C]-iodomethane in the presence of tetrabutylammonium hydroxide gives ^{11}C-labelled 1 or 2, respectively

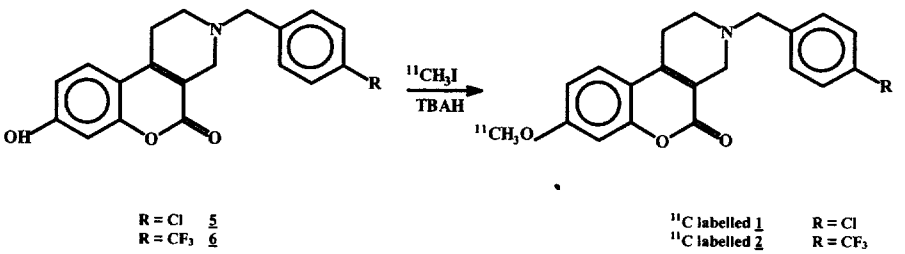


Figure 2: Radiosynthesis of ^{11}C-labelled 1or 2.

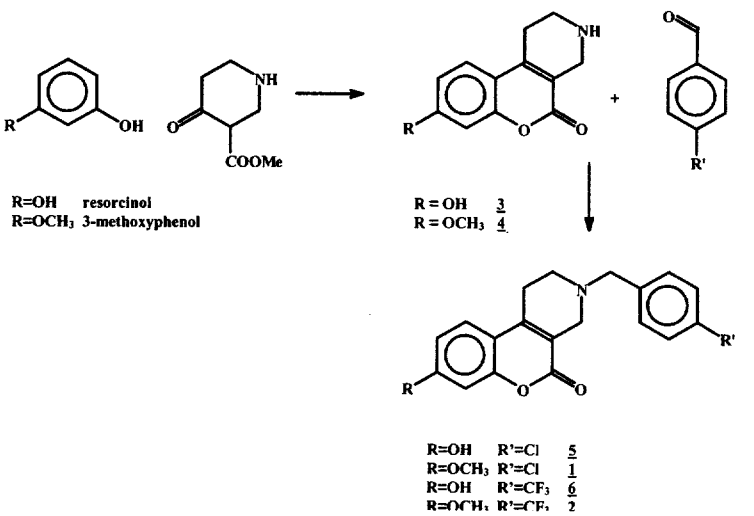


Figure 3: Synthesis of reference 1 and 2 and their *O*-desmethyl precursors 5 and 6.

As no products were commercially available, reference 1 and 2 and their *O*-desmethyl precursors were synthesized as described (14). The synthesis of 1 and 2 and their *O*-desmethylprecursor 5 and 6 is outlined in figure 3.

Intermediates 3 and 4 are obtained by condensation of resorcinol or 3-methoxyphenol with methyl 4-oxo-3-piperidine-carboxylate. Reaction of 3 or 4 with the appropriate substituted benzaldehydes in the presence of sodium triacetoxyborohydride gives the desired products.

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^{11}C-labelled 1 and 2 were purified by reversed phase HPLC using ethanol/water as mobile phase. Product isolation from HPLC mobile phase was done by solid phase extraction (SPE). In order to obtain complete retention of ^{11}C-labelled 1or 2 on the C18 Sep Pak the isolated fraction had to be diluted with phosphate buffer pH 7. If dilution of the isolated fraction was done with water, only 55% of the activity was retained by the Sep Pak. After elution with 750 µL ethanol, a recovery of 94% was found and the procedure took only 5 min.

The total synthesis time, including HPLC purification and formulation was 35 min. The radiochemical yield was 65% (decay-corrected). For medical applications, 5.6 - 7.4 GBq ^{11}C-labelled 1 or 2 were produced. Identification of ^{11}C-labelled 1 or 2 was done by spiking with reference 1 or 2.

Quality control was done by RP-HPLC. Radiochemical purity was better than 99% and a specific activity of 37 GBq/µmol was obtained at EOS. 320 nm was the UV wavelength for the detection of *O*-desmethyl precursor 5 and 6. Only a small peak of 5 or 6 was detected corresponding to a mass amount of 2.4 nmol. Taken into account the total mass of 1 or 2 (approximately 124 nmol) chemical purity is higher than 98.5%.

Experimental

Material and Methods

Tetrabutylammonium hydroxide (TBAH) (40% w/v solution in water) and dimethylformamide (DMF) were purchased from Acros. Absolute ethanol was obtained from UCB. All other chemicals were obtained from Aldrich or Labscan.

DMF was dried over molecular sieves (3 Å). HPLC was performed with a Waters 510 pump, a Pye-Unicam 4110 UV-detector and a NaI(Tl) detector or a GM-tube. Chromatograms were recorded on a dual channel integrator (Shimadzu CR5-A).

Preparation of 1,2,3,4-tetrahydro-8-hydroxy-5H-[3,4-c]pyridin-5-one 3

A mixture of resorcinol (2.86 g, 26 mmol) and methyl 4-oxo-3-piperidinecarboxylate hydrochloride (5 g, 26 mmol) was cooled on ice and treated over one hour with 60 mL of 72% v/v sulphuric acid. The mixture was then stirred at room temperature for 64 hours. Ice/water was added, followed by ammonium hydroxide, until the pH of the mixture was 9.0. The crude product was filtered and washed with water. Yield 79%. m.p. > 290°C with decomposition. ^{1}H NMR (DMSO-d_6) δ= 7.6 (1H, d, 10-H); 6.8 (2H, m, 7-9H); 3.7 (2H,s, 4-H); 3.2 (2H, t, 2-H); 2.9 (2H, t, 1-H). ESI-MS: m/z 218 $(MH)^+$.

Preparation of 1,2,3,4-tetrahydro-8-methoxy-5H-[3,4-c]pyridin-5-one 4

Prepared from 3-methoxyphenol (3.21g, 26 mmol) and methyl 4-oxo-3-piperidinecarboxylate hydrochloride (5 g, 26 mmol) as described for 3. Yield 94%. m.p. 179 - 180 °C (Lit. 179 - 183 °C, ref. 18). ^{1}H NMR (DMSO-d_6) δ= 7.65 (1H, d, 10-H); 7.0 (2H, m, 7-9H); 3.9 (3H,s, OCH3); 3.6 (2H,s, 4-H); 3.15 (2H, t, 2-H); 2.8 (2H, t, 1-H). ESI-MS: m/z 232 $(MH)^+$.

Preparation of 3-(4-chlorobenzoyl)-8-hydroxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one 5

A solution of 3 (300 mg, 1.4 mmol) and 4-chlorobenzaldehyde (248 mg, 1.77 mmol) in 4 mL of THF and 1.4 mL of 1,3-dimethyl-2-imidazolidinone was treated with acetic acid (50 µL). The mixture was stirred for 10 min and sodium triacetoxyborohydride (640 mg, mmol) was added. the mixture was stirred for 18 h and added to 60 g of ice/water. The precipitated solid was filtered, washed with water and purified by column chromatography (Silica gel 50-200 µm, 20 cm x 2.5 cm, eluent: dichloromethane:methanol: conc. aqueous ammonia (87.5:13:5 v/v). Yield 37 %. m.p. 166-167°C. ^{1}H NMR (DMSO d_6): 7.55 (4H, d, 10-H); 7.35 (4H, dd, Phenyl); 6.75 (2H, m, 7-9H); 3.7 (2H,s, 4-H); 3.2 (2H, s, CH_2Ph); 2.85 (2H,t,2-H); 2.70 (2H,t,1-H). ESI-MS: m/z 342 - 344 (MH, ^{35}Cl – ^{37}Cl)$^+$. Exact Mass: calculated 342.0896, found 342.0898 $(MH)^+$.

Preparation of 3-(4-chlorobenzyl)-8-methoxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one 1

Prepared from 4 (324 mg, 1.4 mmoles) and 4-chlorobenzaldehyde (248 mg, 1.77 mmol) as described for 5. Yield 44%. m.p. 139 - 141°C (Lit. 140 - 142°C, ref. 16). ^{1}H NMR (DMSO d_6): 7.6 (4H, d, 10-H); 7.40 (4H, dd, Phenyl); 7.0 (2H, m, 7-9H); 3.9 (3H,s, OCH_3); 3.7 (2H, s, 4-H); 3.25 (2H, s, CH_2Ph); 2.9 (2H, t, 2-H); 2.75 (2H, t, 1-H). ESI-MS: m/z 356 - 358 (MH, $^{35}Cl - ^{37}Cl)^+$.

Preparation of 3-(4-trifluoromethylbenzyl)-8-hydroxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one 6

Prepared from 3 (300 mg, 1.4 mmoles) and 4-trifluoromethylbenzaldehyde (241 mg, 1.77 mmoles) as described for 5. Yield 19%. m.p. 207-208°C. ^{1}H NMR (DMSO d_6): 7.70-7.60 (4H, dd, Phenyl); 7.55 (1H, d, 10-H); 6.75 (2H, m, 7-9H); 3.8 (2H, s, 4-H); 3.20 (2H, s, CH_2Ph); 2.9 (2H, t, 2-H); 2.75 (2H, t, 1-H). ESI-MS: m/z 376 $(MH)^+$. Exact Mass: calculated 376.1160, found 376.1155 $(MH)^+$.

Preparation of 3-(4-trifluoromethylbenzyl)-8-methoxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one 2

Prepared from 4 (324 mg, 1.4 mmoles) and 4-trifluoromethylbenzaldehyde (241 mg, 1.77 mmoles) as described for 5. Yield 33%. m.p. 174 - 176°C (Lit. 173 - 174°C, ref. 16). ^{1}H NMR (DMSO d_6): 7.70-7.60 (4H, dd, Phenyl); 7.55 (1H, d, 10-H); 6.75 (2H, m, 7-9H); 3.8 (2H,s, 4-H); 3.20 (2H, s, CH_2Ph); 2.9 (2H, t, 2-H); 2.75 (2H, t, 1-H). ESI-MS: m/z 390 $(MH)^+$.

Preparation of ^{11}C- labelled 1 or 2

[^{11}C]Iodomethane was trapped in a cooled (-20°C) reaction vial containing 5 or 6 (3 µmol) in dry DMF (150 µl) and 10 µl of a 0.3 M TBAH solution in DMSO. The vessel was sealed and heated for 4 min at 120°C. After cooling, 100 µL of water was added to the mixture. With a Valco injection system (C6W) and a 250 µL loop, the reaction mixture was brought onto a semi-preparative Econosil RP-C18 column. (250 x 10 mm, 10 µm particle size). Elution was performed using water:ethanol (30:70 v/v) at a flow rate of 4 mL/min. The effluent was monitored

simultaneously by UV (320 nm) and radioactivity detection (GM tube). The fraction containing ^{11}C-labelled 1 or 2 ($t_R \cong 10$ min) was collected, diluted with approximately 30 mL phosphate buffer (50 mM, pH 7.0) to reach a final ethanol concentration of 10% and passed through a C18 Sep Pak (conditioned with 5 mL of water). After a washing with water (10 mL) ^{11}C-labelled 1 or 2 was eluted from the C18 Sep Pak with ethanol (750 µL) and diluted with water up to 7.5 mL. The solution was filtered through a 0.22 µm membrane into a sterile and pyrogen-free vial in order to obtain an injectable solution.

Quality control and determination of specific activity

Chemical and radiochemical purity of ^{11}C-labelled 1 and 2 were determined by HPLC using a 50 µL loop and a RP-C18 Alltima column (250 mm x 4.6 mm, 5µm particle size) with water/ethanol (30/70) as mobile phase. (flow rate 1 mL/min). Detection was performed by UV (320 nm) and NaI(Tl).

Conclusion

This paper describes the synthesis of potential radioligands for visualization of the D4 receptor subtype, 3-(4-chlorobenzyl)-8-[^{11}C]methoxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one and 3-(4-trifluorobenzyl)-8-[^{11}C]methoxy-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one using a *O*-methyl-ation with [^{11}C]iodomethane. The radiochemical yields were 65%. The radioligand was found to be chemically and radiochemical pure with a specific activity of 37 GBq/µmol. Biodistribution studies directed at assessing the usefulness of these radioligands for *in vivo* studies using PET are in progress.

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