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Research Article

Synthesis of [^{123}I]-3-(4-iodobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one, a potential dopamine D_4 ligand for SPECT studies

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Summary

Schizophrenia is a devastating mental disorder characterized by relapsing psychotic episodes accompanied with emotional, professional and social decline. The classical dopamine hypothesis of schizophrenia postulates that hyperactivity of dopaminergic neurotransmission is responsible for the positive symptoms of the disorder, more exactly hyperactivity of the dopamine D_2-like receptors. One of these receptors is the D_4 receptor which is thought to be involved in the motor side-effects caused antipsychotics. However, research into the specific role of this receptor has been hampered by the lack of specific ligands. Therefore, a new ^{123}I-labelled compound was developed which may allow *in vivo* visualization of the D_4 receptor by SPECT. [^{123}I]-3-(4-iodobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one was prepared by electrophillic aromatic substitution of the tributylstannyl derivative. The radiochemical yield was $68 \pm 3\%$ ($n = 5$) and the specific activity was > 2.96 Ci/μmol. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: dopamine; D_4 receptor; SPECT; radiotracer

Introduction

The interest in dopamine D_4 receptors and their role in schizophrenia was primarily sparked by two observations. The first observation was the fact that the atypical antipsychotic clozapine has been reported to have a ten-fold

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higher affinity for the dopamine D_4 receptor than for the dopamine D_2 receptor and that this affinity correlated well with its antipsychotic activity.[1,2] Furthermore, reports indicated that clozapine occupied only about 40% of D_2 receptors in therapeutical doses (extra pyramidal symptoms occurring at about 80% or higher occupancy).[3,4] Secondly, it has been reported that there is a six-fold increase in dopamine D_4 receptor density in the brain tissue of schizophrenics which also suggests an important role for the dopamine D_4 receptor in the pathophysiology of schizophrenia.[5,6] However, later observations could not confirm higher numbers of D_4 receptors in schizophrenics.[7,8] It also became clear that occupation of the D_2 receptor by clozapine was higher than initially thought (85%).[9] It is now assumed that D_4 blockade by itself is not sufficient to alleviate positive symptomatology of schizophrenia and that D_2 receptor blockade is probably indispensable. However, D_4 receptor blockade is thought to be responsible for lower propensity of extrapiramidal side-effects.[2]

A limiting factor in the study of the dopamine D_4 receptor is the lack of specific radiotracers. The best known technique for *in vivo* visualization of the D_4 receptor at this point subtracts the binding of nemonapride and raclopride, the first binds to all D_2-like receptor while the second binds only to D_2 and D_3 receptors. However, there have been reports of a raclopride insensitive dopamine D_2 receptor making this system all but ideal.[2]

The aim of this study was to synthesise a tracer for SPECT studies of the dopamine D_4 receptor. In order to develop a tracer for these receptors a number of demands have to be met. First of all the tracer needs to have high affinity for the dopamine D_4 receptor due to the low density of these receptors. Furthermore, the selectivity over the other dopamine receptor subtypes and other receptor types in general needs to be good. Finally, the lipophilicity should not be too high in order to avoid non-specific binding. The selection of [^{123}I]-3-(4-iodobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one was based upon an article published by Unangst *et al.*[10] In this article it was shown that 3-(4-chlorobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one the corresponding chlorine derivative has high affinity for the dopamine D_4 receptor (pKi = 8.61) and shows a more than 1000-fold selectivity over D_2 (pKi = 5.55) and more than 100-fold selectivity over D_3 (pKi = 6.50) receptors.[10] From these data it was assumed that the iodinated derivative would also show selectivity for the D_4 receptor.

Experimental

General

All reagents were purchased from Sigma-Aldrich (St Louis, MO, USA) and used without further purification. [^{123}I] Sodium iodide (in 0.05 M NaOH) was

purchased from Bristol-Myers Squibb Pharma (Brussels, Belgium). Proton NMR spectra were recorded on a 300 MHz FT-NMR (Varian Mercury, Palo Alto, USA) spectrometer (Department of Medicinal Chemistry, Ghent University, Belgium). Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in either chloroform-d_3, acetone-d_6 or methylsulfoxide-d_6. Mass spectra and exact masses were obtained using a time of flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface (Rega Institute, KUL, Belgium). Chromatographic purification of unlabelled compounds was performed on silica gel using the solvent systems indicated in the text. For mixed solvent systems, ratios are given with respect to volumes. HPLC purification and analysis of the radioligand was performed using a Waters 515 HPLC pump, a Waters 2487 UV detector (320 nm) (Waters, Milford, USA), and a Ludlum model 2200 scalar ratemeter (Ludlum Measurements Inc., Sweetwater, USA). The columns, mobile phases and flow rates used are indicated in the text below.

*Synthesis of 1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-c]pyridin-5-one (***1***).* To resorcinol (3.303 g, 30 mmol), methyl-4-oxo-3-piperidinecarboxylate.HCl (4.685 g, 30 mmol) is added and this mixture is cooled to 0°C. 30 ml 80% concentrated sulphuric acid in water is added dropwise over a period of 1 h. The mixture is then stirred for 62 h at room temperature. Afterwards the mixture is slowly added to 100 g of ice and 15 ml of concentrated ammonium hydroxide. More ammonium hydroxide is added till a pH of 10–11 is reached. The mixture is then stirred until the initial sticky precipitate becomes granular. This precipitate is now filtered and washed with 3% sodium hydroxide and 10% methanol. The precipitate is analysed by TLC (dichloromethane/methanol/triethylamine: 89/10/1) (R_f 0.24). The mixture is then purified on a silica column (silica, 500 × 25 mm) using dichloromethane/methanol/triethylamine (94/5/1) as starting eluent. Once 750 ml have passed through the column the methanol concentration is changed to 10%. The eluted fractions are collected and the solvent evaporated *in vacuo*. A yellowish solid is obtained (0.687 g yield = 9.5%).

^{1}H-NMR (d_6-DMSO): 7.4–7.6 (d, 1 H, Ar*H*), 6.8–6.9 (d, 1 H, Ar*H*), 6.7–6.8 (s, 1 H, Ar*H*), 3.5–3.6 (s, 2 H, NH-CH_2-C), 2.9–3 (t, 2 H, NH-CH$_2$-CH_2), 2.6–2.8 (m, 2 H, NH-CH_2–CH$_2$). ESI-MS *m*/*z*: 216.6 ([M-H]$^-$).

*Synthesis of 3-(4-bromobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-c]pyridin-5-one and 3-(4-iodobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-c]pyridin-5-one (***2*** *and* ***4***).* An amount of 1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one (**1**) and a 1.1-fold excess of 4-bromobenzaldehyde or 4-iodobenzaldehyde are dissolved in a mixture of THF (3.8 ml/mmol of

1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one) and 1,3-dimethyl-2-imidazolidinone (equimolar) under a nitrogen atmosphere. Acetic acid is added (equimolar) and the mixture is stirred at room temperature for 10 min. Subsequently, sodiumtriacetoxyborohydride (1.5-fold excess) is added in small portions over a 30 min period. The mixture is then stirred at room temperature for another 18 h. The reaction mixture is now added to 300 ml of ice-cold water. The resulting precipitate is filtered and washed with 300 ml of water. The precipitate is analysed by TLC (dichloormethane/methanol/triethylamine: 89/10/1) (R_f 0.40 or 0.50 for **2** or **4**, respectively). The mixture is then purified on a silica column (silica, 500 × 25 mm) using dichloromethane/triethylamine (99/1) as starting eluent and adding 1% of methanol each time 200 ml have passed through the column until the methanol concentration is 10%. The eluted fractions are collected and the solvent evaporated *in vacuo*. A yellowish solid is obtained (yield = 34.64% for **2** and 51.33% for **4**).

^{1}H-NMR (d_6-DMSO): 7.4–7.6 (d, 1 H, Ar*H*), 7.2–7.6 (dd, 4 H, parasubst Ar*H*), 6.8–6.9 (d, 1 H, Ar*H*), 6.7–6.8 (s, 1 H, Ar*H*), 3.6–3.7 (s, 2 H, NH-CH_2-C), 3.1–3.3 (s, 2 H, N-CH_2-Ar), 2.9–3.1 (t, 2 H, NH-CH_2-CH_2), 2.6–2.8 (m, 2 H, NH-CH_2-CH_2). ESI-MS *m*/*z*: 386.0 ($[M+H]^+$)(**2**).

^{1}H-NMR (d_6-DMSO): 7.4–7.6 (d, 1 H, Ar*H*), 7.1–7.7 (dd, 4 H, parasubst Ar*H*), 6.7–6.8 (dd, 1 H, Ar*H*), 6.6–6.7 (d, 1 H, Ar*H*), 3.6–3.7 (s, 2 H, NH-CH_2-C), 3.1–3.3 (s, 2 H, N-CH_2-Ar), 2.7–2.9 (t, 2 H, NH-CH_2-CH_2), 2.6–2.7 (t, 2 H, NH-CH_2-CH_2). Exact mass *m*/*z*: 432.0084 ($[M-H]^-$)(**4**).

*Synthesis of 3-(4-(tributylstannyl)benzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-c]pyridin-5-one (**3**).* 3-(4-(tributylstannyl)benzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one (**3**) is produced by a 'stille'-type coupling. 3-(4-bromobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one (**2**) (0.420 g, 1.091 mmol) is dissolved in dry toluene (18 ml). Hexabutylditin (10.44 g) and a catalytic amount (± 3 mg) of tetrakistriphenylphosfinepalladium were added and the mixture is refluxed under nitrogen for 15 h. The reaction mixture is analysed by TLC (dichloormethane/methanol/triethylamine: 89/10/1) and shows a newly formed product (R_f 0.82). The mixture is then purified on a silica column (silica, 500 × 25 mm) using hexane/ethylacetate (95/5) as starting eluent, each time 300 ml have passed through the column the ethylacetate concentration is increased (7.5, 10, 15, 20 and 25%). The eluted fractions are collected and the solvent evaporated *in vacuo*. A yellowish oily substance is obtained (0.100 g yield = 15.35%).

^{1}H-NMR (d_6-DMSO): 10.4–10.5 (s, 1 H, O*H*), 7.5–7.6 (d, 1 H, Ar*H*), 7.2–7.5 (dd, 4 H, parasubst Ar*H*), 6.7–6.9 (d, 1 H, Ar*H*), 6.6–6.7 (s, 1 H, Ar*H*), 3.6–3.7 (s, 2 H, NH-CH_2-C), 3.1–3.3 (s, 2 H, N-CH_2-Ar), 2.8–3.0 (m, 2 H, NH-CH_2-CH_2), 2.6–2.8 (t, 2 H, NH-CH_2-CH_2), 1.4–1.6 (m, 6 H, Sn-CH_2-CH_2-CH_2-CH_3), 1.1–1.4 (m, 6 H, Sn-CH_2-CH_2-CH_2-CH_3), 0.9–1.1 (t, 6 H, Sn-CH_2-

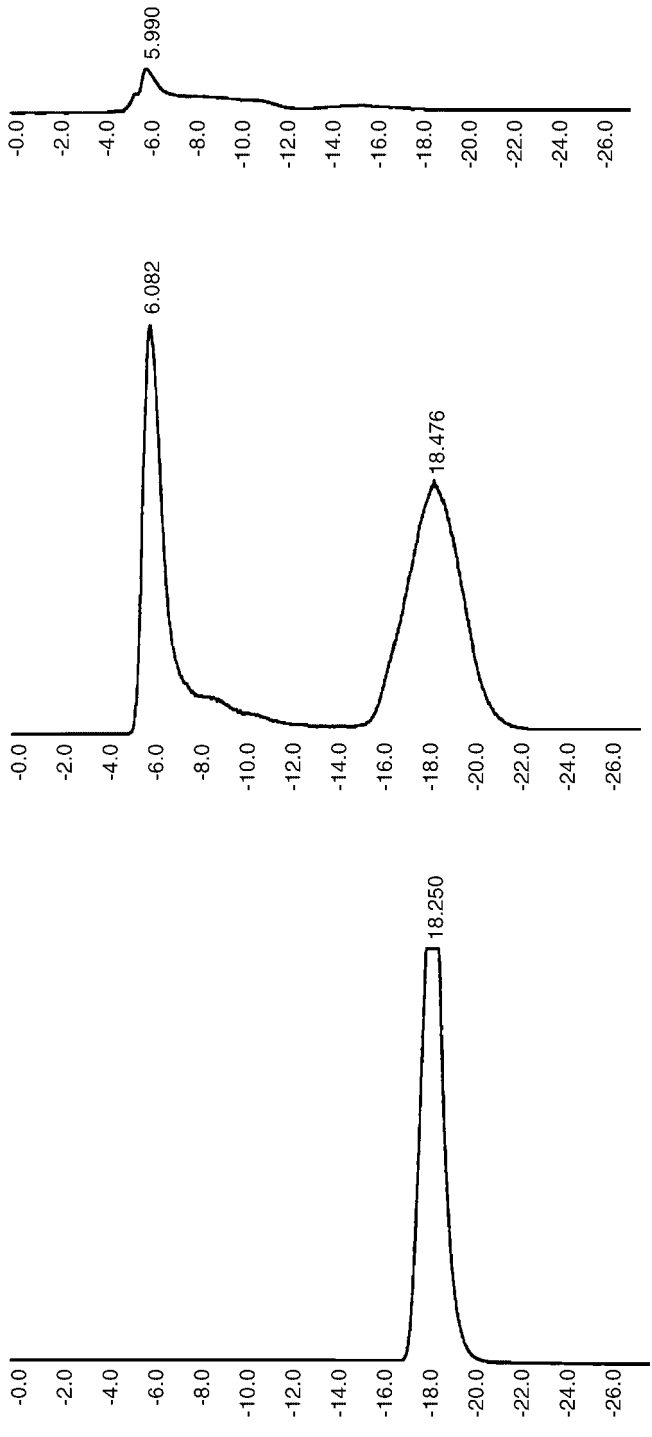


Figure 1. Radioactive (middle) and UV (upper) chromatograms of [^{123}I]-3-(4-iodobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one purification and UV (lower) chromatogram of cold product

CH_2-CH_2-CH_3), 0.7–0.9 (t, 9 H, Sn-CH_2-CH_2-CH_2-CH_3). Exact mass *m/z*: 596.2191([M-H]$^-$).

*Synthesis of [^{123}I]-3-(4-iodobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-c] pyridin-5-one (^{123}**1-4**).* The iodination is conducted by electrophillic aromatic substitution of the tributylstannyl derivative. 3-(4-(tributylstannyl)benzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one (**3**) (299 µg, 0.5 µmol) is dissolved in ethanol (150 µl) and cooled to 0°C. n.c.a. [^{123}I]NaI in sodium hydroxide solution (0.01 M), 2 µl of chloramine T solution (56.4 µg, 0.2 µmol) and acetic acid glacial (5 µl) are added. The mixture is stirred and left to react for 2 min at room temperature. Afterwards an aqueous solution of sodium metabisulphite (3 µl, 57 µg, 0.3 µmol) is added to quench the reaction. After dilution to 500 µl with eluent the mixture is purified by HPLC on a Waters XTerra® MS C_{18} column (250 × 3.6 mm; 3.5 µm) with ethanol/acetate buffer (0.05 M, pH 5) (55/45) as mobile phase at a flow rate of 0.5 ml/min (Figure 1). The radiolabelled product is collected (R_t: 18.4 min) and analysed with an HPLC system consisting of an Alltech Alltima C_{18} column (250 × 3.6 mm; 5 µm) with acetonitrile/acetate buffer (0.025 M, pH 6), (50/50) as mobile phase at a flow rate of 1 ml/min (R_t: 25.4 min).

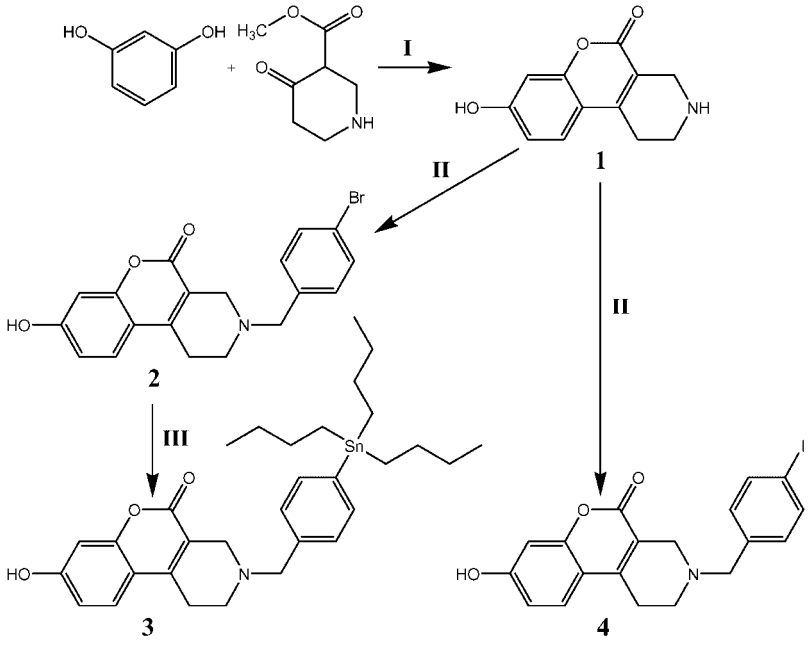
Determination of lipophilicity

Lipophilicity (Log *P*) was determined by adding 100 µCi of the labelled compound to a mixture of 100 ml water and 100 ml octanol. Consequently the mixture was shaken vigorously and left to allow phase separation. 50 ml of octanol is then removed and added to 50 ml of water. The mixture was shaken again and after phase separation 1 ml of both octanol and water was counted for radioactivity. The Log *P* was calculated by dividing the counts in the octanol by those in the water.

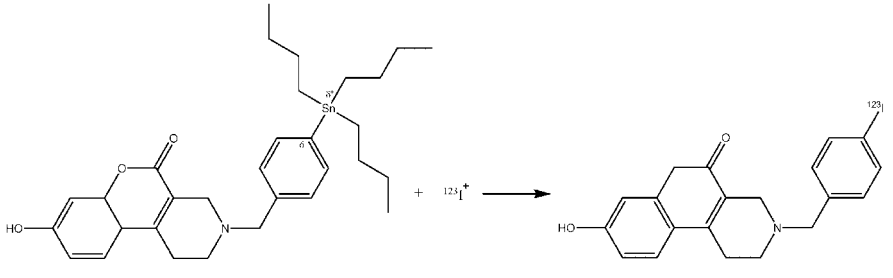
Results and discussion

Synthesis of the precursor molecule and the cold product is shown in Scheme 1. These products are synthesized starting from methyl-4-oxo-2-3-piperidine carboxylate and resorcinol to form 1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one. This is then coupled to the appropriate halogen benzaldehyde to obtain either the unlabelled product or the bromine derivative. The iodinated product was synthesized in an overall yield of about 4.88%. Further reaction of the bromine compound with hexabutylditin in the presence of tetrakistriphenylphosfinepalladium gave the tributylstannyl precursor. The overall yield for the precursor synthesis was about 0.51%.

The radiolabelling of the precursor molecule was conducted by electrophilic iododestannylation of the tributylstannyl precursor as shown in Scheme 2 and gave a good radiochemical yield of 68 ± 3% ($n = 5$). The radiochemical purity



Scheme 1. Synthesis of 3-(4-(tributylstannyl)benzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*] pyridin-5-one and 3-(4-iodobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one. (I) Resorcinol, 4-oxo-3-piperidinecarboxylate.HCl and sulphuric acid, (II) bromo- or iodobenzaldehyde, 1,3-dimethyl-2-imidazolidinone, acetic acid and THF (III) hexabutylditin, tetrakistriphenylphosfinepalladium and toluene



Scheme 2. synthesis of [^{123}I]-3-(4-iodobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one. (IV) [^{123}I]-NaI, chloramine T, acetic acid, sodiummetabisulphite and ethanol

of the collected fraction was $>95\%$. Since no UV signal was obtained from the synthesized product specific activity was obtained by determination of the detection limit of the UV detector, this showed the specific activity to be >2.96 Ci/μmol. Stability was tested by leaving the product in solution at room

temperature and reinjecting it into the HPLC system. Up to 48 h after synthesis the radiochemical purity remained >95%. The total amount of radioactivity obtained was up to 10 mCi, sufficient to perform SPECT studies in human volunteers. The Log *P* was determined to be 3.8 this might be a problem for *in vivo* testing since this could result in a large degree of aspecific binding.

Conclusion

We reported the synthesis and radiolabelling of [^{123}I]-3-(4-iodobenzyl)-1,2,3,4-tetrahydro-8-hydroxychromeno[3,4-*c*]pyridin-5-one as a potential radiotracer for *in vivo* visualization of the dopamine D_4 receptor. The compound was labelled in a good yield (68 ± 3%) and high specific activity (>2.96 Ci/μmol) and will be evaluated by means of *in vitro* and *in vivo* experiments.

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