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Dopamine D_3 receptor antagonists: The quest for a potentially selective PET ligand. Part 3: Radiosynthesis and in vivo studies

Idriss Bennacef [a], Cristian A. Salinas [a], Thomas A. Bonasera [a], Roger N. Gunn [a], Hélène Audrain [b], Steen Jakobsen [b], Nabeel Nabulsi [c], David Weinzimmer [c], Richard E. Carson [c], Yiyun Huang [c], Ian Holmes [d], Fabrizio Micheli [e,*], Christian Heidbreder [g], Gabriella Gentile [e], Tino Rossi [e], Marc Laruelle [a,f,*]

[a] GlaxoSmithKline Clinical Imaging Centre, Imperial College London, Hammersmith Hospital, Du Cane Road, London, UK
[b] Aarhus PET Centre, Aarhus University Hospital, Aarhus, Denmark
[c] PET Centre, Yale University School of Medicine, New Haven CT, USA
[d] Cancer Therapeutics, Melbourne, Australia
[e] GlaxoSmithKline, Neurosciences Centre of Excellence for Drug Discovery, Verona, Italy
[f] Division of Neurosciences and Mental Health, Imperial College London, Neuroscience Laboratories, Burlington Danes Building, Hammersmith Hospital, UK
[g] Altria Client Service, Richmond VA, USA

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ABSTRACT

Compound **1** is a potent and selective antagonist of the dopamine D_3 receptor. With the aim of developing a carbon-11 labeled ligand for the dopamine D_3 receptor, **1** was selected as a potential PET probe. [^{11}C]**1** was obtained by palladium catalyzed cross coupling using [^{11}C]cyanide and **4** with a specific activity of 55.5 ± 25.9 GBq/μmol (1.5 ± 0.7 Ci/μmol). [^{11}C]**1** was tested in porcine and non-human primate models to assess its potential as a radioligand for PET imaging of the dopamine D_3 receptor. We conclude that in both species and despite appropriate in vitro properties, [^{11}C]**1** does not show any specific signal for the dopamine D_3 receptor.

In 1990, Sokoloff et al. first cloned, characterized and reported the dopamine (DA) D_3 receptor subtype.[1] The expression pattern of D_3 mRNA in rat and human brain are comparable, with high expression primarily in limbic system areas (globus pallidus, caudate, putamen, and medial thalamus). Various anatomical, pharmacological, and genetic observations suggest that the DA D_3 receptor might be involved in Parkinson's disease, schizophrenia, and drug addiction.[2]

Although potent and selective DA D_3 receptor antagonists can be found in the literature[3] and despite tremendous efforts from a number of groups, relatively little progress has been made in identifying a selective DA D_3 radioligand.[4–6] To the best of our knowledge, only [^{11}C]PHNO, a probe firstly described as a DA D_2 radioligand can be used with PET to map the DA D_3 receptor.[7,8] PHNO is an agonist that binds to both the D_2 (pK_i = 8.2) and the D_3 (pK_i = 9.7) receptors.[9] Non-human primate studies have recently demonstrated that it is possible to extract the DA D_3 signal from the analysis of regional [^{11}C]PHNO data which indicated that the ubtantia-(>90%) and globus pallidum (60%) binding potentials were mostly D_3.[10] Although [^{11}C]PHNO is currently the best available tool for imaging the D_3 DA receptor its routine use to examine all D_3 DA rich brain regions is complicated by its adjunct affinity at the D_2 receptor. To use [^{11}C]PHNO as a tool for imaging D_3 receptors throughout the brain would require the use of a selective D_3 blocker, which is difficult. Thus, the identification of a selective DA D_3 PET radioligand would be an important advance in this research field.

A large number of molecules have been identified as part of our research towards a DA D_3 antagonist, some with nanomolar affinity and a good selectivity profile over other dopaminergic receptors. In this Letter, we describe the synthesis and in vitro properties of **1** (Fig. 1), its radiosynthesis and subsequent in vivo evaluation as a PET probe in pig and monkey.[12]

Synthesis of compound **1** is depicted in Scheme 1. 1-(2-Chloroethyl)-2-imidazolidinone **2** was reacted with 4-(trifluoromethyl)

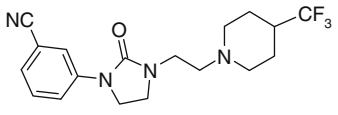

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Figure 1.

* Corresponding authors. Tel.: +39 045 8218515; fax: +39 045 8218196 (F.M.).
E-mail addresses: fm20244@gsk.com (F. Micheli), Marc.A.Laruelle@gsk.com (M. Laruelle).

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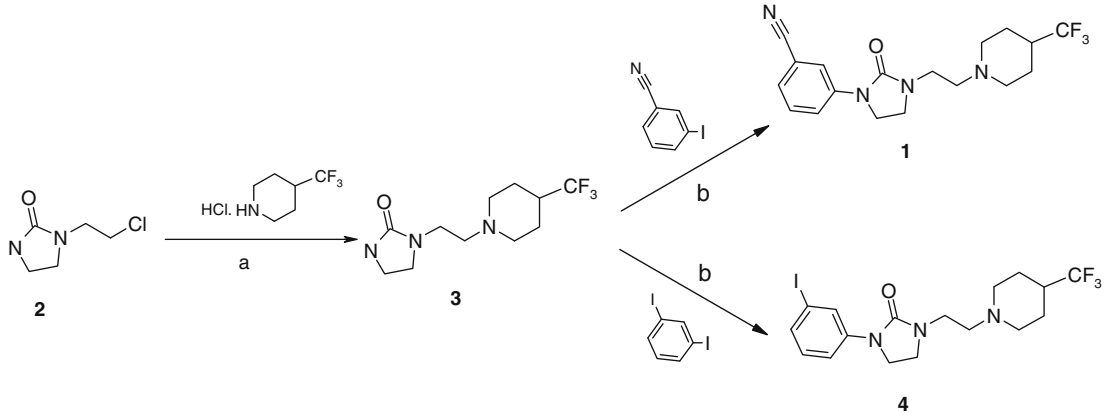


Scheme 1. Reagents and conditions: (a) K_2CO_3, NMP, 100 °C, 2 × 10 min, cooling, 90%; (b) [*trans*-2-aminocyclohexyl]amine, CuI, KOH, toluene, MW, overnight, 25%.

piperidine hydrochloride and potassium carbonate in NMP at 100 °C, affording 1-{2-[4-(trifluoromethyl)-1-piperidinyl]ethyl}-2-imidazolidinone **3** in good yield (90%). Compound **3** was treated with 3-iodobenzonitrile or 1,3-diiodobenzene, [*trans*-2-aminocyclohexyl]amine, copper iodide, and potassium hydroxide in toluene, under microwave irradiation, affording **1** or 1-(3-iodophenyl)-3-{2-[4-(trifluoromethyl)-1-piperidinyl]ethyl}-2-imidazolidinone **4**, which was purified by preparative HPLC.[13]

As reported previously, compound **1** is a potent ($pK_i = 9.1$) antagonist for the DA D_3 receptor.[11] The selectivity profile of **1** was assessed by filtration binding assays on human DA D_3 and D_2 receptors and demonstrated a 100-fold selectivity for the D_3 DA over D_2 DA receptor. Further assays indicated that it was selective (1000-fold) over serotoninergic receptors ($5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{2A}$, $5HT_{2C}$, $5HT_6$, and $5HT_7$) and adrenergic receptors ($alpha_{1A}$ and $alpha_{1B}$).[14] The high affinity for the DA D_3 receptor was retained in rat and pig native tissues (Table 1).[11]

Compound **1** displayed a suitable pharmacokinetic profile for iv administration in rat, with good brain penetration (brain to blood concentration ratio = 6.4) and high free (protein-unbound) fraction (15.1%) in the brain. Binding to plasma protein was low in both rat (72%) and pig (61%).[11]

Lipophilicity measurements at pH 7.4 of **1** were similar as determined by HPLC (CHI-log $D = 2.9$)[15] and by a phosphate buffer (pH 7.4): octanol shake flask method (log $D = 2.7$, $n = 3$; maximum range, +5%). These lipophilicity measures are in the accepted range for CNS PET tracers (log D between 1 and 3).[16]

Overall, compound **1** displays acceptable physico-chemical and in vitro properties necessary for a PET radioligand. Moreover, the cyanide and urea functional groups are suitable sites for labeling with carbon-11. Therefore, radiosynthesis of [^{11}C]**1** was carried out and PET imaging experiments in pigs and monkeys conducted to investigate its potential as a PET ligand to image the D_3 receptor in vivo.

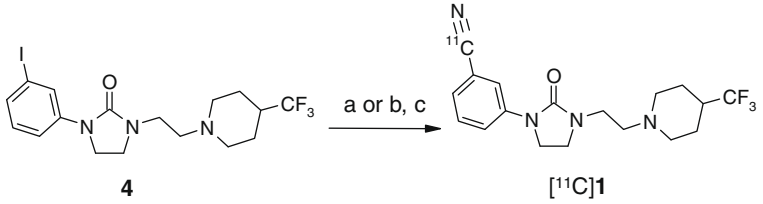
Because of its robustness, radiolabeling with [^{11}C]cyanide labeling route was chosen.[17] Radiosynthesis of [^{11}C]**1** was accomplished via a palladium(0)-catalyzed cross coupling between the iodide **4** and [^{11}C]cyanide using $Pd_2dba_3{\cdot}CHCl_3$/dppf and $KHCO_3$ as base in either NMP (80 °C for 3 min) or DMF (100 °C for 5 min). The crude product was purified by reverse-phase HPLC and the fraction containing the product was diluted with water and concentrated through a C18 SepPak cartridge. Elution of the retained product from the cartridge with ethanol and formulation with saline yielded 899 ± 326 MBq (24.3 ± 8.8 mCi) of [^{11}C]**1** at the end of synthesis (EOS) ($n = 7$). Specific activity at EOS was 55.5 ± 25.9 GBq/μmol (1.5 ± 0.7 Ci/μmol) and both chemical and radiochemical purities were greater than 99%. (Scheme 2).[18]

To assess the binding profile of [^{11}C]**1**, firstly, a series of blocking studies were performed in the Landrace pig ($n = 2$) with SB277011, a high affinity ($pK_i = 8.0$ for D_3) and selective (Ki D_3/D_2 < 100) D_3 antagonist as the blocking agent.[19] After bolus injection of 320 ± 122 MBq (8.7 ± 3.3 mCi), compound [^{11}C]**1** readily entered the brain and a homogeneous distribution was observed at both baseline and following iv administration of SB277011 (Fig. 2). No lipophilic metabolite was observed and the radioligand displayed a moderate rate of metabolism with ~50% of parent remaining in the plasma at 30 min after radioligand injection.[20]

Table 1
DA (human D_2, D_3, rat and pig D_3) receptor affinities, brain penetration, and lipophilicity of **1**

	hpKi DA D_2	hpKi DA D_3	pK_i rat NT	pK_i pig NT	Rat brain to blood level ratio	CHI-log D	Log D
1	6.8	9.1	9.1	9.1	6.4	2.9	2.7

Affinity results. SEM for D_3 and D_2 filtration binding data is ±0.2. NT: native tissue.



Scheme 2. Reagents and conditions: (a) [^{11}C]CN^-, Pd_2dba_3, dppf, $KHCO_3$, NMP, 80 °C, 3 min; or (b) [^{11}C]CN^-, Pd_2dba_3, dppf, $KHCO_3$, DMF, 100 °C, 5 min; (c) semi-preparative HPLC.

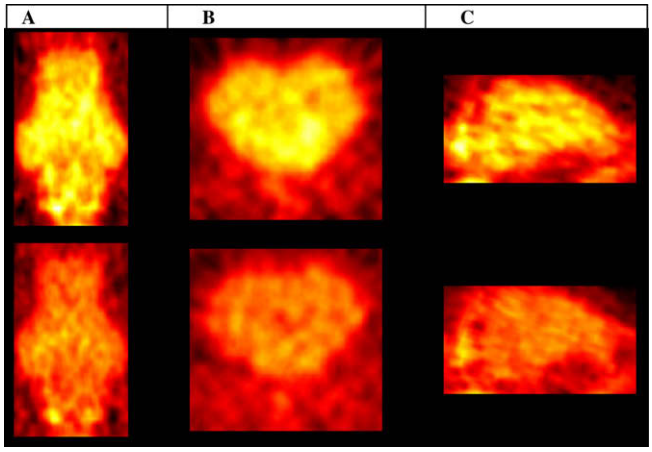


Figure 2. [^{11}C]**1** uptake images in the pig brain, summed from 0 to 90 min post-injection at baseline. Axial (A), coronal (B), and sagital (C) sections (striatum region).

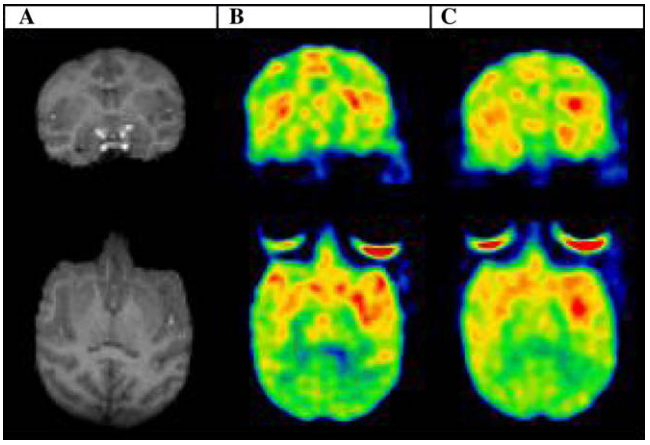


Figure 4. [^{11}C]**1** uptake images summed from 60 to 90 min post-injection at baseline (B) and after SB277011 (C). Corresponding MRI images (A).

Binding potential (BP_{ND}) values were estimated indirectly from total distribution volumes derived from a one tissue compartment model in the D_3 DA target regions of interest (globus pallidus, caudate, putamen, and medial thalamus). Only small values for BP_{ND} were observed at baseline for all target regions (~0.2) and these values were unaffected following administration of SB277011 (Fig. 3). These data did not support the existence of a specific D_3 DA signal with [^{11}C]**1** in the pig.

The candidate radioligand was then evaluated in Rhesus monkeys using the same SB277011 blocking paradigm.[21] In the monkey [^{11}C]**1** also displayed a moderate rate of metabolism with ~50% of parent remaining in plasma after 30 min with no lipophilic metabolites. The plasma free fraction of [^{11}C]**1** was 32 ± 5% (n = 6). Figure 4 shows a set of brain PET images summed from 60 to 90 min at the level of the striatum and globus pallidus and the corresponding anatomical MRI image displaying the same slices. In the brain, equilibrium in regional uptake was reached by about 60 min after injection of [^{11}C]**1**.

Binding potential was calculated by the equilibrium ratio of regions to cerebellum averaged from 60 to 90 min (Fig. 5). Regional BP_{ND} values were small and unaffected by the SB277011 challenge.[22]

As observed for the pig, [^{11}C]**1** does not display any D_3-specific signal in monkey brains.

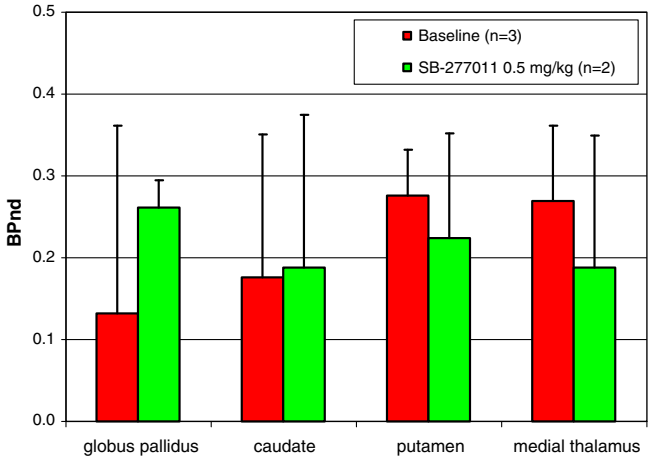


Figure 3. Regional binding potential estimates for [^{11}C]**1** at baseline and following administration of a selective D_3 DA antagonist (SB277011) in pig.

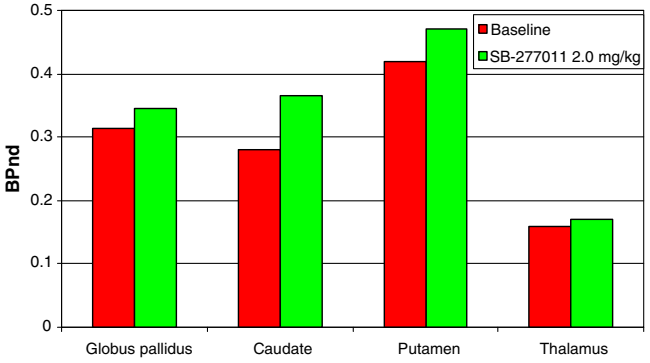


Figure 5. Regional binding potentials from baseline-blocking experiments in rhesus monkey.

To conclude, the synthesis and radiosynthesis of [^{11}C]**1** were robust and straightforward. Physico-chemical and in vitro biological profiles of **1** were assessed and brain PET imaging in pig and non-human primate were performed. Despite displaying appropriate physico-chemical properties, high in vitro binding affinity and selectivity for the DA D_3 receptor, no specific PET signal could be detected in the pig or monkey in vivo. Hence [^{11}C]**1** is not suitable for imaging the DA D_3 receptors. Although the failure of [^{11}C]**1** as a PET radioligand is not completely understood, it indicates that an in vitro pK_i higher than 9.1 for D_3 receptors may be required of future generation of candidate PET tracers, because of the relatively low density of this receptor subtype in the brain.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.055.

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12. The research complied with national legislation and with company policy on the Care and Use of Animals and with related codes of practice.
13. ^{1}H NMR spectra were obtained on Varian INOVA spectrometer (500 MHz). Compound **1** (DMSO-d_6): δ 8.02–7.96 (t, 1H), 7.91–7.83 (dd, 1H), 7.56–7.47 (t, 1H); 7.46–7.38 (d, 1H); 3.86–3.75 (t, 2H); 3.58–3.46 (t, 2H); 3.34–3.26 (t, 2H); 3.04–2.92 (d, 2H); 2.49–2.41 (t, 2H); 2.30–2.16 (m, 1H); 2.01–1.89 (t, 2H); 1.82–1.71 (d, 2H); 1.46–1.31 (dq, 2H). Compound **4** (DMSO-d_6): δ 8.09–7.98 (m, 1H), 7.50–7.41 (d, 1H), 7.34–7.29 (d, 1H); 7.12–7.04 (t, 1H); 3.80–3.70 (dd, 2H); 3.53–3.45 (dd, 2H); 3.35–3.23 (m, 2H); 3.04–2.89 (d, 2H); 2.51–2.39 (t, 2H); 2.30–2.15 (m, 1H); 2.00–1.86 (m, 2H); 1.80–1.71 (m, 2H); 1.48–1.32 (m, 2H). LC/MS analysis proved purity >98% for both compounds.
14. Data not shown.
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18. The amount of [^{11}C]CO_2 at EOB was about 50 GBq (1.35 Ci). In the synthesis of [^{11}C]**1** for the pig studies, the yield of formulated product and specific activity were 2.09 ± 1.71 GBq (56.6 ± 46.2 mCi) and 40.7 ± 11.1 GBq (1.1 ± 0.3 Ci/umol) (n = 5), respectively.
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20. The fraction of unchanged [^{11}C]**1** was measured by radio-HPLC in extracts of plasma from samples taken throughout the scanning session. Free fraction of [^{11}C]**1** in the plasma was determined by ultrafiltration. Brain regions with the highest concentrations of [^{11}C] GSK981352 are shown in yellow, with progressively lower concentrations displayed in orange and red, respectively. PET images are displayed on a common scale.
21. One test–retest study and two control-blocking studies were performed in three different rhesus monkeys using [^{11}C]**1**. Each monkey received two iv injections of [^{11}C]**1**, separated by 3–4 h. A 2-h emission scan was acquired on the High Resolution Research Tomograph (HRRT) scanner for each injection. Brain regions with the highest concentrations of [^{11}C]GSK981352 are shown in red, with progressively lower concentrations displayed in yellow, green, and blue, respectively. PET images are displayed on a common scale. For the test/retest study, the radioligand [^{11}C]**1** was administered to the animal as a bolus. In the blocking studies, solution of SB-277011 (2 mg/kg) was infused for a total of 3 h, starting 1 h before the second scan. Activity in the blood was measured during the scan. Additional blood samples were taken for analysis of [^{11}C]**1** and its metabolites by HPLC. Free fraction of [^{11}C]**1** in the plasma was determined by ultrafiltration.
22. The apparent increase in BP_{ND} is attributed to normal variation in a dataset with little specific binding signal.