[^{11}C](+)-4-*N*-Propyl-,3,4a,5,6,10b-hexahydro-2*H*-naphth[1,2-*b*][1,4]-oxazin-9-ol

[^{11}C](+)-PHNO

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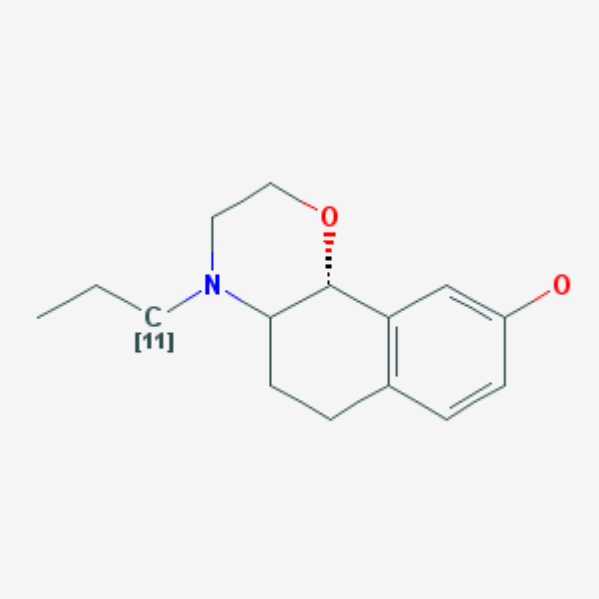
Chemical name:	[^{11}C](+)-4-*N*-Propyl-,3,4a,5,6,10b-hexahydro-2*H*-naphth[1,2-*b*][1,4]-oxazin-9-ol
Abbreviated name:	[^{11}C](+)-PHNO
Synonym:	[^{11}C](+)-4-Propyl-9-hydroxy-1,2,3,4a,5,6-hexahydronaphthoxazine, [^{11}C](+)-4-propyl-9-hydroxynaphthoxazine
Agent Category:	Compound
Target:	Dopamine receptors (D_2 and D_3)
Target Category:	Receptor
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{11}C
Activation:	No
Studies:	• *In vitro* • Rodents • Non-primate non-rodent mammals • Humans



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Background

[PubMed]

Dopamine, a neurotransmitter, plays an important role in the mediation of movement, cognition, and emotion (1, 2). Dopamine receptors are involved in the pathophysiology of neuropsychiatric diseases, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and schizophrenia (3). Five subtypes of dopamine receptors, D_1 through D_5, have been well characterized pharmacologically and biochemically (4). These five subtypes are classified into two subfamilies: D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) dopamine receptors. D_1-like and D_2-like receptors exert synergistic as well as opposite effects at both the biochemical and overall system level. A great majority of striatal D_1 and D_2 receptors are localized postsynaptically on caudate-putamen neurons and to a lesser extent presynaptically on nigrostriatal axons.

Dopamine receptors are G-protein-coupled receptors and exist in high- and low-affinity states, with respect to agonist binding. The two states are interconvertible. The high-affinity state is coupled to G-proteins, whereas the low-affinity state is not. Dopamine has a dissociation constant (K_d) of 7 nM for the high-affinity state (K_{high}) and a K_d of 1,720 nM for the low-affinity state (K_{low}) (5). Under physiologic conditions, dopamine is expected to bind predominately to the high-affinity state, which is ~50% occupied by 10 nM dopamine. The high-affinity state has been suggested to be the functional form of the dopamine receptors.

Substituted benzamides, such as sulpiride, raclopride, and iodobenzamide, are specific ligands with only moderate affinity for the D_2 receptors, making studies of extrastriatal D_2 receptors difficult (6-8). In binding studies, [^{123}I]-labeled epidepride, an analog of isoremoxipride, was found to have high potency and low nonspecific binding, and to be selective for striatal and extrastriatal D_2 receptors (9). Epidepride has marginal binding to D_4 receptors, with little affinity for other known neurotransmitter receptors. (S)-*N*-((1-Allyl-2-pyrrolidinyl)methyl)-5-(3-[^{18}F]fluoropropyl)-2,3-dimethoxybenzamide ([^{18}F]fallypride), an analog of epidepride, was found to be a selective, high-affinity antagonist of $D_{2/3}$ receptors (10), and in positron emission tomography (PET) *in vivo* studies (11-13), it identified extrastriatal $D_{2/3}$ receptors. However, none of these antagonists distinguishes between the high- and low-affinity states of the D_2 receptors. (–)-*N*-Propyl-norapomorphine (NPA), an agonist of the $D_{2/3}$ receptors, was reported to have K_{high} and K_{low} values of 0.07-0.4 and 20-200 nM, respectively (5, 14, 15). This provides a >50-fold selectivity for the high-affinity over the low-affinity receptors. NPA

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has good affinity (K_i = 0.3 nM) for D_3 receptors but not other neurotransmitters (16). [11C]NPA is being developed as a PET agent for the non-invasive study of the high-affinity state of the $D_{2/3}$ receptors in the brain.

(+)-4-Propyl-3,4,4a,5,6,10b-hexahydro-2*H*-naphtho[1,2-*b*][1,4]oxazin-9-ol (PHNO) is an agonist of the D_2 receptors with a reported K_d of 0.56 nM in the canine striatum and a >10,000-fold selectivity for D_2 over D_1 receptors (17). PHNO has a high affinity for human cloned D_2 (K_i = 8.5 nM) and D_3 (K_i = 0.16 nM) receptors (18). [11C]PHNO showed robust specific binding in the striatum of rodents with insensitivity to displacement by D_1, norepinephrine, or serotonin ligands (19). [11C]PHNO is being developed as a PET agent for the non-invasive study of the high-affinity state of the $D_{2/3}$ receptors in the brain.

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Related Resource Links:

- Chapters in MICAD (Dopamine receptors)
- Gene information in NCBI (D_2 receptor,D_3 receptor)
- Articles in Online Mendelian Inheritance in Man (OMIM) (D_2 receptor, D_3 receptor)
- Clinical trials (Dopamine receptors)
- Drug information in Food and Drug Administration (Dopamine receptors)

Synthesis

[PubMed]

Wilson et al. (19) reported a one-pot synthesis of [11C]PHNO in which [11C]propionyl chloride was reacted with (+)-3,4,4a,5,6,10b-hexahydro-2*H*-naphtho[1,2-*b*][1,4]oxazin-9-ol followed by $LiAlH_4$ reduction, with an average radiochemical yield of 10% (based on [11C]CO_2, uncorrected, at the end of synthesis). Specific activities were 33-67 GBq/μmol (900-1,800 mCi/μmol at end of synthesis) after high-performance liquid chromatographic purification, with radiochemical purities >99%. [11C]Propionyl chloride was prepared by reacting [11C]CO_2 with ethylmagnesium bromide, followed by reaction with phthaloyl chloride. The total synthesis time was 40 min.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In studies of binding to dopamine receptors in membranes of canine striatum, [3H]PHNO had an average K_d of 0.56 ± 0.08 nM and a B_{max} of 30.1 ± 2.0 pmol/g tissue (20). Guanilylimidodiphosphate significantly reduced the density of [3H]PHNO binding sites, suggesting that [3H]PHNO was binding mainly to the high-affinity state of dopamine D_2 receptors and not to D_3 receptors.

Animal Studies

Rodents

[PubMed]

Biodistribution studies in rats by Wilson et al. (19) showed high accumulation of radioactivity in the striatum (2% of injected dose(ID)/g) within the first 5 min after injection, followed by a gradual decrease of radioactivity to ~0.7% ID/g at 60 min. Accumulation (~1% ID/g) in all other brain regions was similar to that in the cerebellum at 5 min and was only 0.2% ID/g at 60 min. The striatum specific binding ratios (cerebellum as a reference) were 1.1, 2.8, 3.9, and 4.6 at 5, 15, 30, and 60 min after injection, respectively. Haloperidol (D_2), raclopride (D_2), PHNO, and RTI-32 (dopamine transporter) pretreatment effectively blocked specific binding of [^{11}C]PHNO to the striatum by 73-95%. On the other hand, WAY 100635 (5-HT_{1A}), SCH 23390 (D_1), clonidine (α_2), and MK-912 (α_{2A}) showed little inhibition. Amphetamine (a dopamine releaser) pretreatment revealed dose-dependent inhibition of [^{11}C]PHNO binding to the striatum, indicating that [^{11}C]PHNO binding is sensitive to endogenous dopamine levels in the striatum. At 40 min post injection, 26% of [^{11}C]PHNO radioactivity remained intact in the plasma, with 74% as polar metabolites. However, >98% of the radioactivity in the rat brain was intact [^{11}C]PHNO, suggesting that the polar metabolites cannot cross the blood-brain barrier. [^{11}C]PHNO thus displays uptake and washout kinetics characteristic of reversible radiotracers as well as excellent selectivity toward $D_{2/3}$ receptors.

Other Non-Primate Mammals

[PubMed]

Ginovart et al. (21) reported on [^{11}C]PHNO PET study in cats. [^{11}C]PHNO displayed high uptake in the striatum with a mean striatal binding potential (BP) of 3.95 ± 0.85. Pretreatment with the specific antagonists SCH 23390 (D_1 antagonist), raclopride (D_2 antagonist), haloperidol (D_2 antagonist), and SB-277011 (D_3 antagonist) indicated that [^{11}C]PHNO binding in the striatum is specific to D_2 receptors because raclopride and haloperidol showed >89% inhibition, whereas SCH 23390 and SB-277011 showed no inhibition. The BP of [^{11}C]PHNO in the striatum was 2.5-fold higher than that measured with [^{11}C]NPA. The BP of [^{11}C]raclopride was reduced by 13, 39, and 57% after amphetamine doses of 0.1, 0.5, and 2.0 mg/kg, respectively. The BP of [^{11}C]PHNO was reduced by 21, 67, and 83% after amphetamine doses of 0.1, 0.5, and 2.0 mg/kg, respectively. Amphetamine induced up to 83 ± 4% inhibition of the [^{11}C]PHNO BP, but only up to 57 ± 8% inhibition of the [^{11}C]raclopride BP. Thus, [^{11}C]PHNO was more sensitive to the dopamine-releasing effect of amphetamine than [^{11}C]raclopride. Because raclopride binds to receptors in both the high- and low-affinity states and dopamine binds with high affinity mainly to receptors in the high-affinity state, the percentage reduction is greater when an agonist is used than when an antagonist is used. This large proportion of

high-affinity sites might explain the vulnerability of D_2 radiotracers to competition by endogenous dopamine and is consistent with the reported *in vivo* binding of the agonist radiotracer [^{11}C]PHNO. Scatchard analyses of [^{11}C]PHNO and [^{11}C]raclopride binding in two cats revealed that the B_{max} values obtained with the agonist (29.6 and 32.9 pmol/ml) were similar to those obtained with the antagonist (30.6 and 33.4 pmol/ml).

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

Willeit et al. (22) performed preliminary PET studies of two male and two female healthy volunteers with [^{11}C]PHNO under baseline conditions and after haloperidol pretreatment. The simplified reference tissue method of Lammertsma and Hume (23) was used to derive BPs for each region of interest. The [^{11}C]PHNO BPs in the caudate, putamen, and globus pallidus were 3.00 ± 0.4, 3.10 ± 0.2, and 4.17 ± 1.2, respectively. Small but detectable binding was identified in the substantia nigra/ventral tegmental area. Preliminary test-retest data in two subjects suggested that the BP estimates were reliable. Pretreatment of two subjects with haloperidol (2 mg) reduced the BPs in the caudate, putamen, and globus pallidus by 14-49% with no detectable changes in the cerebellum. [^{11}C]Raclopride binding was studied in two of the subjects. The [^{11}C]raclopride BPs in the caudate, putamen, and globus pallidus were 3.3 ± 0.7, 4.1 ± 0.4, and 1.4 ± 0.2, respectively. [^{11}C]PHNO binding compared with [^{11}C]raclopride binding was lower in the caudate (–8.8%) and putamen (–23.5%) but higher in the globus pallidus (+200%). [^{11}C]PHNO showed favorable kinetics and may allow reliable quantitation of high-affinity $D_{2/3}$ receptors in the brain.

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