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MINIREVIEW

25CN-NBOH: A selective agonist for in vitro and in vivo investigations of the serotonin 2A receptor

Emil M. Rørsted, Anders A. Jensen and Jesper L. Kristensen*[a]

Abstract:

4-[2-[(2-hydroxybenzyl)amino]ethyl]-2,5-dimethoxybenzonitrile (25CN-NBOH) was first reported as a potent and selective serotonin 2A receptor (5-HT2A) agonist in 2014, and it has since found extensive use as a pharmacological tool in a variety of in vitro, ex vivo and in vivo studies. 25CN-NBOH is readily available from a synthetic perspective using standard chemical transformations, and displays favorable physicochemical properties in terms of stability and solubility. Due to its superior selectivity for 5-HT2A, 25CN-NBOH has been used to investigate the effects of selective 5-HT2A activation in vivo, and has thus become an important pharmacological tool for the exploration of 5-HT2A signaling in a range of animal models. In the present review, we outline the discovery of 25CN-NBOH, its pharmacological profile and major findings from studies where it has been used.

Introduction

The serotonin (5-hydroxytryptamine) 2A receptor (5-HT2A) has been the focus of significant therapeutic interest for decades. Antagonists of the receptor include marketed antipsychotic drugs such as clozapine, pimavanserin and risperidone, which are prescribed as therapeutics for schizophrenia, bi-polar disorder and potentially other indications.[1] In contrast, exploration of the therapeutic potential of 5-HT2A agonists has historically been restricted by the psychotoxic properties exhibited by agonists of the receptor; most prominently the canonical psychedelics lysergic acid diethylamide (LSD) and psilocybin, the active constituent of “magic mushrooms”. In the past decade, the scientific community has revisited the classic psychedelics as putative therapeutics. Several pivotal clinical studies into the therapeutic potential of these molecules have substantiated previous findings from the 1950-60’s and underlined that psychedelics could hold the potential to revolutionize the current treatment paradigm of psychiatric diseases.[2-4] The efficacy of psychedelic-assisted psychotherapy is currently being investigated for numerous indications, including major depression disorder, treatment-resistant depression, addiction, obsessive-compulsive disorder and general anxiety.[5]

The overall pharmacological profiles of the classical psychedelics differ somewhat, but they share a key components: 5-HT2A agonist activity. A substantial amount of evidence points to this component as key for their characteristic acute effects and efficacy in drug-assisted psychotherapy.[6-14] Activation of the 5-HT2A has been proposed to lead to the induction of a profoundly altered state of consciousness and down-regulation of the activity of the brains default-mode network, the latter effect is believed to be crucial for the observed beneficial clinical effects.[6] All canonical members of this drug-class are relatively non-selective for the 5-HT2A, e.g. LSD exhibits potent agonist activity at a plethora of dopaminergic and serotonergic receptors. Psilocin (the active metabolite of psilocybin) mediates equipotent agonist activity at numerous serotonin receptors.[15] The renewed therapeutic interest in classical psychedelics has prompted the search for agonists with selectivity for this receptor in order to facilitate studies of the physiological functions mediated by 5-HT2A as well as to explore the isolated therapeutic potential in activation of this receptor.

Selectivity at the 5-HT2A

While several agonists display selectivity for the 5-HT2 receptors over other serotonin and monoaminergic receptors, 5-HT2A subtype-selectivity in agonists is relatively rare due to the high degree of conservation across the orthosteric sites in the three 5-HT2 receptor subtypes: 5-HT2A, 5-HT2B and 5-HT2C. Historically, 5-HT2C agonists developed for the treatment of obesity and metabolic disorders have routinely been counter-screened at 5-HT2A due to the psychotoxic effects of agonists of this receptor, and thus 5-HT2 agonists have most often been screened at these two receptor subtypes. However, selectivity of 5-HT2A agonists towards the 5-HT2A subtype is equally important. 5-HT2A plays a crucial role in healthy cardiovascular function and the development and maintenance of heart tissue.[16] The 5-HT2A contributes to regulating biomechanical function of, and signaling within, the ventricle and heart valve, and is responsible for the regulation and change in the tissues implicated in the development of heart valve disease.[16,17] Thus, high selectivity for the 5-HT2A is not only a desirable characteristic in tool compounds applicable for investigations of the physiological functions of the native receptors, but also in connection with drug discovery and development.

5-HT2A agonist tool compounds

Historically, the predominant pharmacological tool used to study the in vivo effects of 5-HT2A activation has been 1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine (DOI, see Scheme 1). The potent 5-HT2A agonist activity present in the amphetamine class has been thoroughly characterized, and the bioavailability and CNS exposure of DOI upon systemic administration has rendered

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It the 5-HT₂₅R agonist of choice for animal investigations. As the compound contains an iodine atom at the 4′-position the structure also lends itself to radiolabeling, and the more bioactive R enantiomer has been used as a radioligand for autoradiography studies in rat brain. However, although easily accessible and pharmacologically well-characterized, DOI only displays modest selectivity towards the recombinant 5-HT₂₅R and 5-HT₂C-R in binding and functional assays (Table 1), a characteristic that occasionally is overlooked in interpretations of the in vivo effects of DOI.

In the quest for more subtype selective tool agonists for the 5-HT₂₅R medicinal chemistry exploration has yielded several subtype selective scaffolds most notably the Benzylpiperidines and the N-Benzylphenethylamines. 2-(2,5-dimethoxy-4-bromobenzyl)-6-(2-methoxyphenyl)piperidine (DMBMPP, see Scheme 1.) represents the former and is one the most selective agonists reported to date, with high binding affinity to 5-HT₂₅R (Kᵢ = 2.5 nM) and 124-fold selectivity towards the 5-HT₂C-R, although functional data is yet to be reported. DMBMPP is not widely used as a pharmacological tool, presumably due to its relative inaccessibility from a synthetic standpoint.

Another chemotype has emerged from derivatization of the phenethylamine scaffold. Specifically, the 2,5-dimethoxy phenethylamine motif, originally derived from natural product and prototypical phenethylamine, mescaline. Alkylation of the amino moiety with simple alkyl groups is known to reduce activity at 5-HT₂₅R, but benzylolation infers increased binding affinity and agonist potency at the 5-HT₂₅R.

The first NBPEAs were first reported by Glennon in 1995, and later by Ralf Heim in 2003, then further explored and characterized by Nichols in 2006 and eventually made their way to the recreational drug market where they were introduced as new psychoactive substances in 2010. They quickly gained notoriety after being implicated in multiple cases of acute toxicity, several with lethal outcomes. This caused the compound class to be scheduled as controlled substances by the US Drug Enforcement Administration.

Of particular distinction of the numerous synthesized NBPEAs are the 4-chloro and 4-ido analogs, 2-(4-chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine (25CN-NBOH, see Figure 1) and 2-(4-ido-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine (25I-NBOMe, see Figure 1), respectively. These compounds have been used extensively as recreational drugs and several reports on fatal intoxications in humans have been reported. Various modifications to the 2-methoxy benzyl motif have also been reported including, but not limited to, 2,3-methylenedioxy benzene, 2-flouro benzene and 1-hydroxy, 2-methyl benzene as well as various ring constraints of the 1 and 2 position substituents on the benzene ring.

The NBPEAs have been used frequently as preclinical and clinical research tools, for example in the field of neuroimaging. Of particular importance is 2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine (25B-NBOMe, see Scheme 2.), also known as CIMBI-36, which was developed as a positron emission tomography (PET) tracer for visualization of 5-HT₂₅R in the human brain. This radioligand has been used to image and quantify 5-HT₂₅R receptor levels in the human brain, and it has been used in several PET studies in both animals and humans.

With its high 5-HT₂₅R binding affinity and moderate selectivity for this receptor over the homologous 5-HT₂₅R and 5-HT₂C-R, the NBPEAs have been investigated in several structure-activity relationship studies eventually leading to the development of series of potent and increasingly selective 5-HT₂₅R agonists. One of the most 5-HT₂₅R-selective NBPEA to date, 25CN-NBOH, was discovered in 2014 through a structure-activity-relationship study of the NPEAs and variations over their 4′-position substituent and different benzyl motifs (see Scheme 3.).

25CN-NBOH is decorated with a nitrile at the 4′-position and an ortho-hydroxy on the N-benzyl moiety. This combination apparently confers a unique binding mode at the 5-HT₂₅R compared to 5-HT₂₅R and yields a highly selective agonist while retaining modest potency. The tendency of the 4′-cyano substitution conferring 5-HT₂₅R selectivity has previously been observed in 4-(2-aminopropyl)-2,5-dimethoxybenzonitrile, the α-methyl counterpart of the parent phenethylamine, while varying the 2′-position substituent of the benzyl moiety revealed that
exchanging the anisole for a phenol yielded the most selective combination of the series.\textsuperscript{[25,26]}

25CN-NBOH displays high binding affinity to the 5-HT\textsubscript{2A}R and robust selectivity for 5-HT\textsubscript{2A}R over 5-HT\textsubscript{2B}R and 5-HT\textsubscript{2C}R in various radioligand binding assays and functional assay, see detailed discussion below.\textsuperscript{[25,28,30,38,45, 46]}

Synthesis and availability

25CN-NBOH is commercially available from several vendors, but also accessible through simple chemical transformations from inexpensive starting materials. The original procedure was recently improved, providing access to the compound from the commercially available 2,5-dimethoxyphenethylamime (2C-H) in 5 steps, as illustrated in Scheme 4.\textsuperscript{[47,48]}

\begin{center}
\textbf{Scheme 4.} Synthetic overview for synthesis of 25CN-NBOH.
\end{center}

Metabolism

The NBPEAs are characterized by low oral bioavailability in man, which has been ascribed to pronounced first pass metabolism.\textsuperscript{[49]}

The metabolic degradation of 25CN-NBOH has been investigated in vitro and various routes of metabolism have been identified including demethylation, hydroxylation, imination, benzylation, glucuronidation and combinations thereof as is observed for NBPEAs in general.\textsuperscript{[50-52]}

In vivo animal studies of the pharmacokinetic properties of 25CN-NBOH in rats have shown that the compound rapidly crosses the blood brain barrier and that it reaches peak concentration in the brain within approximately 15 min, a concentration maintained for approximately 30 min (see Figure 1).\textsuperscript{[33]}

\begin{center}
\textbf{Figure 1.} Time resolved brain and plasma concentrations of 25CN-NBOH.\textsuperscript{[7]}
\end{center}

Physiochemical Properties

Physicochemical properties of 25CN-NBOH have been investigated and reported. Solubility at pH 7.4 was determined to be 405 \(\mu\)g/mL. Stock solutions for in vivo investigation were prepared with concentrations up to 3 mg/mL in physiologic saline (0.9% NaCl) and up to 6 mg/mL with 5% dimethyl sulfoxide. Upon storage at 5°C some precipitation was seen but sonication caused the compound to re-dissolve. 25CN-NBOH is stable for at least several weeks when stored and handled in this way.

Pharmacological characteristics of 25CN-NBOH at recombinant 5-HT\textsubscript{2} receptors

25CN-NBOH display high binding affinity to the 5-HT\textsubscript{2A}R (K\textsubscript{D} values between 0.81 and 1.7 nM), with 37-fold 5-HT\textsubscript{2A}R/5-HT\textsubscript{2B}R selectivity and 52-100-fold 5-HT\textsubscript{2A}R/5-HT\textsubscript{2C}R selectivity in various radioligand binding competition assays (Table 1). In various functional assays, the compound exhibit EC\textsubscript{50} values from 0.41 nM to 2.4 nM at 5-HT\textsubscript{2A}R with 49-fold and 19-150-fold selectivity over 5-HT\textsubscript{2A}R and 5-HT\textsubscript{2C}R, respectively (Table 2). In comparison, DOI displays K\textsubscript{D} values ranging from 0.7 nM to 27 nM at 5-HT\textsubscript{2A}R and 2.6-29-fold selectivity toward 5-HT\textsubscript{2A}R and 2.7-7.5-fold selectivity towards 5-HT\textsubscript{2C}R in various radioligand binding competition assays (Table 1). In various functional assays, DOI has exhibited EC\textsubscript{50} values from 0.15 nM to 6.3 nM with 5-HT\textsubscript{2A}R/5-HT\textsubscript{2B}R and 5-HT\textsubscript{2A}R/5-HT\textsubscript{2C}R selectivity ratios of 2.4-8.3-fold and 3.8-44-fold, respectively. Both 25CN-NBOH and DOI display partial agonism at the 5-HT\textsubscript{2A}R with R\textsubscript{max} values in various functional assays ranging from 77-86% and 85-95% (of R\textsubscript{max} for serotonin) for 25CN-NBOH and DOI, respectively (Table 2). In summary, 25CN-NBOH consistently exhibits higher degrees of selectivity for 5-HT\textsubscript{2A}R over the two other 5-HT\textsubscript{2}Rs than DOI in various binding and functional assays (Table 1).

The overall selectivity profile of 25CN-NBOH at targets other than the 5-HT2Rs has also been assessed in detail.\textsuperscript{[33,54]}

The compound has been found to exhibit negligible activity at a wide range of neurotransmitter receptors, transporters, ion channels, enzymes and kinases, mostly tested in radioligand binding assays. Notably, 25CN-NBOH did exhibit considerable activity at the hERG channel (IC\textsubscript{50} = 2.7 \(\mu\)M), which could indicate that 25CN-NBOH is not suitable as a therapeutic. A toxicology screening of the compound for acute cellular toxicity showed no effects, indicating an otherwise very benign cellular toxicological profile.\textsuperscript{[7]}

Based on these data and its high brain uptake, it appears that 25CN-NBOH is a superior tool for in vitro and in vivo studies of 5-HT\textsubscript{2A}R-mediated functions compared to DOI.\textsuperscript{[7]}

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Table 1. Summary of reported binding properties for 25CN-NBOH and DOI at recombinant human 5-HT₂A, 5-HT₂B and 5-HT₂C receptors in the literature.

<table>
<thead>
<tr>
<th>Assays</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]ketanserin (2A), [3H]mesulergine (2C)</td>
<td>[23]</td>
</tr>
<tr>
<td>[3H]LSD</td>
<td>[3]</td>
</tr>
<tr>
<td>[3H]25CN-NBOH</td>
<td>[24]</td>
</tr>
<tr>
<td>[3H]LSD</td>
<td>[55]</td>
</tr>
<tr>
<td>[3H]Cimbi-36</td>
<td>[20]</td>
</tr>
<tr>
<td>[3H]DOI (2A, 2C), [3H]5-HT (2B)</td>
<td>[26]</td>
</tr>
<tr>
<td>[3H]DOI</td>
<td>[19]</td>
</tr>
</tbody>
</table>

The reported binding affinities of the two agonists at the three receptors are given as Kᵢ values (in nM) with Kᵢ²B/Kᵢ²A and Kᵢ²C/Kᵢ²A ratios given for 5-HT₂B and 5-HT₂C as measures of the compounds selectivity for 5-HT₂A over the two other subtypes.
## Table 2. Summary of reported functional properties for 25CN-NBOH and DOI as agonists at recombinant human 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R in the literature.

<table>
<thead>
<tr>
<th></th>
<th>5-HT_{2A}R</th>
<th>5-HT_{2B}R</th>
<th>5-HT_{2C}R</th>
<th>2B/2A</th>
<th>2C/2A</th>
<th>Assays</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>25CN-NBOH</td>
<td>EC_{50}</td>
<td>R_{max}</td>
<td>EC_{50}</td>
<td>R_{max}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>86</td>
<td>170</td>
<td>86</td>
<td>83</td>
<td></td>
<td>IP accumulation</td>
<td>[3,25]</td>
</tr>
<tr>
<td>2.4</td>
<td>77</td>
<td>350</td>
<td>114</td>
<td>150</td>
<td></td>
<td>IP-One</td>
<td>[3]</td>
</tr>
<tr>
<td>1.2</td>
<td>77</td>
<td>59</td>
<td>23</td>
<td>99</td>
<td>49</td>
<td>Ca^{2+} mobilization</td>
<td>[3]</td>
</tr>
<tr>
<td>0.41</td>
<td>80</td>
<td>52</td>
<td>93</td>
<td>130</td>
<td></td>
<td>Ca^{2+} mobilization</td>
<td>[3]</td>
</tr>
<tr>
<td>0.59</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G_{i} dissociation (BRET 2)</td>
<td>[55]</td>
</tr>
<tr>
<td>0.38</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G_{i} dissociation (BRET 2)</td>
<td>[55]</td>
</tr>
<tr>
<td>5.48</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>β-Arrestin dissociation (BRET 1)</td>
<td>[55]</td>
</tr>
<tr>
<td>0.41</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G_{i} dissociation (BRET 2)</td>
<td>[55]</td>
</tr>
<tr>
<td>(+/-)DOI</td>
<td>2.9</td>
<td>93</td>
<td>28</td>
<td>100</td>
<td>9.7</td>
<td>IP accumulation</td>
<td>[3,25]</td>
</tr>
<tr>
<td>6.3</td>
<td>87</td>
<td>46</td>
<td>119</td>
<td>7.3</td>
<td></td>
<td>IP-One</td>
<td>[3]</td>
</tr>
<tr>
<td>0.85</td>
<td>91</td>
<td>2.0</td>
<td>94</td>
<td>3.3</td>
<td>96</td>
<td>2.4 Ca^{2+} mobilization</td>
<td>[3]</td>
</tr>
<tr>
<td>0.15</td>
<td>85</td>
<td>6.6</td>
<td>85</td>
<td>44</td>
<td></td>
<td>Ca^{2+} mobilization</td>
<td>[3]</td>
</tr>
<tr>
<td>(R)-DOI</td>
<td>0.58</td>
<td>95</td>
<td>4.8</td>
<td>94</td>
<td>2.2</td>
<td>101 Ca^{2+} mobilization</td>
<td>[18]</td>
</tr>
</tbody>
</table>

The agonist potencies and maximal responses of the two agonists are given as EC_{50} values (in nM) and R_{max} (in % of the R_{max} for 5-HT), respectively, with EC_{50}^{2B}/EC_{50}^{2A}(2B/2A) and EC_{50}^{2C}/EC_{50}^{2A}(2C/2A) ratios given for 5-HT_{2A}R and 5-HT_{2C}R as measures of the compounds selectivity for 5-HT_{2A}R over the two other subtypes.
Binding mode of 25CN-NBOH to 5-HT$_{2A}$R

The molecular basis for the agonist activity of 25CN-NBOH at 5-HT$_{2A}$R was recently elucidated down to the atomic level in a published cryo-electron microscopy (cryo-EM) structure of a 25CN-NBOH/5-HT$_{2A}$R/min-G$_{q}$q complex. [56] The cryo-EM structure reveals the determinants for receptor-G-protein interactions and the conformational rearrangements involved in the transition of the receptor to its active states. 25CN-NBOH binds to the 5-HT$_{2A}$R in a unique binding mode compared to prototypic 5-HT$_{2A}$R agonist LSD and the inverse agonist methiothepin. This pose included a positioning of the 2-hydroxy phenyl moiety of 25CN-NBOH into a previously undescribed hydrophobic pocket within the receptor, creating a hydrophobic interaction between the ligand and receptor pocket, mediated by an edge-to-face π–π interaction with W336. [48] As illustrated by the close proximity of the afore mentioned amino acid to the benzyl moiety (see Figure 2.B). Furthermore, the placement within this cavity enables the formation of a hydrogen bond between the 2-position hydroxyl with S159. [56] These unique binding mode interactions most likely contribute significantly to its subtype-selectivity preference of 25CN-NBOH for the 5-HT$_{2A}$R over other biogenic amine receptors. This structure further calls for a renewed evaluation of 25CN-NBOH the in comparison to other ligands used to investigate the 5-HT$_{2A}$R

Synthesis and applications of radiolabeled 25CN-NBOH

The binding characteristics of tritiated 25CN-NBOH as a radioligand has also been characterized. In agreement with its binding profile at recombinant 5-HT$_{2A}$Rs [3] 25CN-NBOH was found to display substantial specific, ketanserin-sensitive binding to cortex in rat brain slices in autoradiography experiments (see Figure 3.), and as such the radioligand constitutes a novel tool for native 5-HT$_{2A}$R expression studies. [23] The synthetic route to the tritiated compound is summarized in Scheme 3.

Furthermore the tritiated ligand was used to investigate binding properties of [3] 25CN-NBOH at the 5-HT$_{2A}$R, 5-HT$_{2C}$R and 5-HT$_{2B}$R which were in concordance with binding characteristics of the unlabelled compound. Notably, 5 nM concentrations of [3] 25CN-NBOH induced close to full saturation binding at the 5-HT$_{2A}$R but gave no specific binding to the two subtype homologues indicating that the radioligand is indeed selective for the 5-HT$_{2A}$R.
Table 3. Discovery and applications of 25CN-NBOH (2014-2020)

<table>
<thead>
<tr>
<th>Year</th>
<th>Title</th>
<th>Summary</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>“Synthesis and Structure–Activity Relationships of N-Benzyl Phenethylamines as 5-HT2A/2C Agonists”</td>
<td>Discovery of 25CN-NBOH through a SAR investigation of NBOMe class of compounds</td>
<td>[25]</td>
</tr>
<tr>
<td>2015</td>
<td>“Hallucinogen-like effects of 2-[(4-cyano-2,5-dimethoxyphenyl) ethylamino]methylphenol (25CN-NBOH), a novel N-benzylphenethylamine with 100-fold selectivity for 5-HT2A receptors, in mice”</td>
<td>In vivo characterization of the head-twitch response effects and drug discrimination studies of 25CN-NBOH in mice.</td>
<td>[26]</td>
</tr>
<tr>
<td>2016</td>
<td>“Effect of 5-HT2A and 5-HT2C Receptors on Temporal Discrimination by Mice.”</td>
<td>Investigation of the effect of 5-HT2A agonism on temporal discrimination tasks in mice. 25CN-NBOH was compared to DOI. Both compounds showed significant effects on temporal discrimination.</td>
<td>[27]</td>
</tr>
<tr>
<td>2017</td>
<td>“Functional neuroimaging using dynamic radial 3D UTE pulse sequences.”</td>
<td>25CN-NBOH was administered to non-human primates in order to induce cognitive changes measurable by radial 3D UTE pulse sequences—demonstrating the validity of this technique.</td>
<td>[28]</td>
</tr>
<tr>
<td>2018</td>
<td>“Regulatory Mechanism of CCN2 Production by Serotonin (5-HT) via 5-HT2A and 5-HT2B Receptors in Chondrocytes.”</td>
<td>Investigation of 5-HT2A agonism on cartilage growth factors found significant increase in cartilage growth factor CCN2 when 25CN-NBOH was administered along with a 5-HT2A antagonist.</td>
<td>[29]</td>
</tr>
<tr>
<td>2019</td>
<td>“Chronic Treatment with a Metabotropic mGlu2/3 Receptor Agonist Diminishes Behavioral Response to a Phenethylamine Hallucinogen.”</td>
<td>Investigation of the influence of metabotropic glutamate receptor mGlu2mGlu3 activation on 5-HT2A signalling. Response to 25CN-NBOH administration was significantly reduced by pretreatment with the mGlu2mGlu3 agonist LY379268.</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>“The Serotonin 2A Receptor Agonist 25CN-NBOH Increases Murine Heart Rate and Neck-Arterial Blood Flow in a Temperature-Dependent Manner.”</td>
<td>25CN-NBOH was shown to increase heart rate and neck-arterial blood flow in mice.</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>“Correlation between the Potency of Hallucinogens in the Mouse Head-Twitch Response Assay and Their Behavioral and Subjective Effects in Other Species”</td>
<td>Investigation of the correlation between head-twitch responses in mice, reported drug-discrimination studies in rats and the reported hallucinogenic effect in humans for known 5-HT2A agonists, including 25CN-NBOH, found significant correlation between data sets.</td>
<td>[33]</td>
</tr>
<tr>
<td></td>
<td>“Acute Serotonin 2A Receptor Activation Impairs Behavioral Flexibility in Mice.”</td>
<td>Investigation of 5-HT2A agonist on decision making. 25CN-NBOH administration was shown to impair probabilistic reversal learning.</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>“Acute Lysergic Acid Diethylamide Does Not Influence Reward-Driven Decision Making of C57BL/6 Mice in the Iowa Gambling Task.”</td>
<td>Investigation of 5-HT2A agonist on reward-driven decision making. No correlation between administration of LSD or 25CN-NBOH and performance in decision making tasks.</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td>“The Chronic Treatment With 5-HT2A Receptor Agonists Affects the Behavior and the BDNF System in Mice.”</td>
<td>Chronic treatment with 25CN-NBOH showed significant influence on the functioning of the BDNF system.</td>
<td>[36]</td>
</tr>
<tr>
<td></td>
<td>“Serotonin Receptor 2A Activation Promotes Evolutionarily Relevant Basal Progenitor Proliferation in the Developing Neocortex.”</td>
<td>25CN-NBOH administration was shown to increase basal progenitor proliferation in endogenous fetal human neocortex.</td>
<td>[37]</td>
</tr>
</tbody>
</table>
Applications of 25CN-NBOH

25CN-NBOH has found application as a tool compound in a wide range of different experimental set-ups and has been mentioned in several scientific publications since its discovery. The number of published studies using 25CN-NBOH has risen significantly since its discovery in 2014 (Table 3).

Due to its high functional selectivity for 5-HT2A over other serotonin receptors, 25CN-NBOH has been utilized as a tool compound in several in vitro investigations including the connection between 5-HT2A activation and basal progenitor proliferation in the developing neocortex and the implications of 5-HT2A activation in connective tissue growth factor regulation.[50,68]

Other in vivo applications include studies involving animal models of hallucinogenic effects, such as investigations of the respective contributions of 5-HT2A and 5-HT2C to temporal discrimination in mice and of the head-twitch responses induced by the compound in comparison with other phenethylamine derived psychedelics.[50,60-62]

Head-twitch response.

In vivo investigations in rodents have shown that 25CN-NBOH, like DOI, induces head twitch response (HTR), the prototypical phenotypic behavior arising from 5-HT2A activation in rodents. The 25CN-NBOH-induced HTR was lower in magnitude than that induced by DOI, and the fact that the HTR was blocked by administration of a 5-HT2A-selective antagonist but not by a 5-HT2C antagonist supports the notion that HTR primarily arises from 5-HT2A activation.[61,63] Repeated once-per-day injections of 25CN-NBOH led to reduced occurrence of the induced HTRs, indicating the build-up of drug-tolerance over time.[65] Recent studies have indicated that there is a correlation between the subjective effects of 5-HT2A agonists in humans and HTR in rodents, making this a valuable translational model to anticipate effects in humans.[63]

Cognitive effects.

25CN-NBOH has also been used to evaluate the role of 5-HT2A activation on behavioral flexibility in rodents, where it impaired probabilistic spatial discrimination and reversal learning tasks in male mice.[64] 25CN-NBOH-mediated 5-HT2A activation was shown not to affect the reward-driven decision-making tasks in mice, in contrast to the effects seen by LSD.[65] Studies have indicated that 5-HT2A activation may induce neuroplasticity and the elevated neuronal growth.[66] Investigations to evaluate this hypothesis measured the effects of sustained 5-HT2A stimulation using 25CN-NBOH on brain-derived neurotrophic factor (BDNF) system in mice, compared to other known 5-HT2A agonists. The investigations indicated that this system is indeed effected by the prolonged activation of this receptor although failing to affect memory and spatial learning.[66] 25CN-NBOH has also been used to induce cortical changes in non-human primates in order to perform functional neuroimaging using dynamic radial 3D UTE pulse sequences thus demonstrating its wide applicability as a tool compound in various species and models.[55]

Conclusion

We have presented an updated summary of the current literature data on 25CN-NBOH in the hope that this will further its application as a tool for future investigations into the pharmacology of the 5-HT2A and the serotoninergic system in general.

The selective 5-HT2A agonism mediated by 25CN-NBOH makes it a valuable addition to the toolbox of compounds that can be used to probe the function of the 5-HT2A. Due to its pronounced selectivity for this receptor over other targets, its benign toxicology and favorable physiochemical profile, we propose that 25CN-NBOH be considered a viable alternative to DOI.
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References


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MINIREVIEW


The highly subtype selective 5-HT2A agonist 25CN-NBOH, has become widely used as a tool compound for various investigations, specifically as a comparator or token selective agonist in an array of studies investigating the implications of 5-HT2A R activation and related effects. We here present a comprehensive overview of the synthesis, properties and reported applications of this interesting pharmacological tool.