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Synthesis and In Vitro Evaluation of Oxindole Derivatives as Potential Radioligands for 5-HT$_7$ Receptor Imaging with PET

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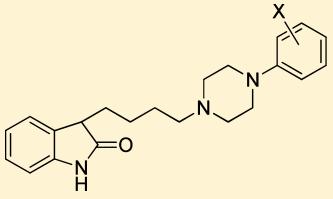
S *Supporting Information*

ABSTRACT: The most recently discovered serotonin (5-HT) receptor subtype, 5-HT$_7$, is considered to be associated with several CNS disorders. Noninvasive in vivo positron emission tomography (PET) studies of cerebral 5-HT$_7$ receptors could provide a significant advance in the understanding of the neurobiology and eventual dysfunctions of the 5-HT$_7$ receptor. To date, no appropriate 5-HT$_7$ receptor PET ligand has been developed. Here, we modified known 5-HT$_7$ selective phenylpiperazinyl-butyloxindole derivatives so that they may be labeled either with carbon-11 or fluorine-18. A set of potential 5-HT$_7$ ligands for PET molecular imaging was successfully synthesized. Two compounds (**10** and **14**) were tested against a range of targets. Both compounds display a promising in vitro profile with respect to PET imaging of the 5-HT$_7$ receptor in thalamic regions.



KEYWORDS: *Oxindole, 5-HT$_7$ receptor distribution, PET*

The relatively recently discovered G-protein coupled 5-HT$_7$ receptor has been implicated in various central nervous system (CNS) disorders such as schizophrenia, depression, epilepsy, migraine, and in the control of circadian rhythm.[1] For example, the atypical antipsychotic drug amisulpride has antidepressant effects,[2,3] and a study in 5-HT$_7$ receptor knockout mice supports that the 5-HT$_7$ receptor antagonism of amisulpride alleviates depression symptoms.[4] Other atypical antipsychotics also have relatively high affinity for the 5-HT$_7$ receptor, but their involvement in alleviating depressive symptoms through blocking the 5-HT$_7$ receptor remains to be investigated.[5,6]

In vivo studies of cerebral 5-HT$_7$ receptor binding in humans would thus provide a significant advance in the understanding of the above-mentioned physiology and pathophysiology. Positron emission tomography (PET) is used to quantify neuroreceptor binding in vivo, and the availability of an appropriate PET radiotracer for the 5-HT$_7$ receptor would be of particular interest.

Previous attempts of other groups to develop a 5-HT$_7$ receptor selective PET tracer have not been convincingly successful.[7,8] Most recently, ^{18}F-labeled SB-269970 derivatives were synthesized and evaluated in vivo in cats,[9,10] but in the absence of a validated reference region or an arterial input function it was not possible to fully evaluate the validity of those radiolabeled compounds.[11]

Several lead structures of 5-HT$_7$ receptor ligands have been identified within various structural classes.[12] Among these structures, phenylpiperazinyl-butyloxindoles display an interesting selectivity profile (Figure 1).[13,14] Some oxindoles showed

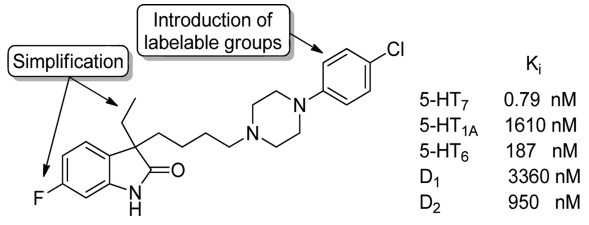


Figure 1. Proposed structural modifications of a known 5-HT$_7$ receptor selective phenylpiperazinyl-butyloxindole derivative and its binding affinities.

inhibition constants (K_i) 2000-fold lower for the 5-HT$_7$ receptor than for the 5-HT$_{1A}$ receptor. This large difference in K_i is necessary because of low brain tissue 5-HT$_7$ receptor density (B_{max}) compared to 5-HT$_{1A}$ receptor values in, e.g., hippocampus and cortical areas.[15,16]

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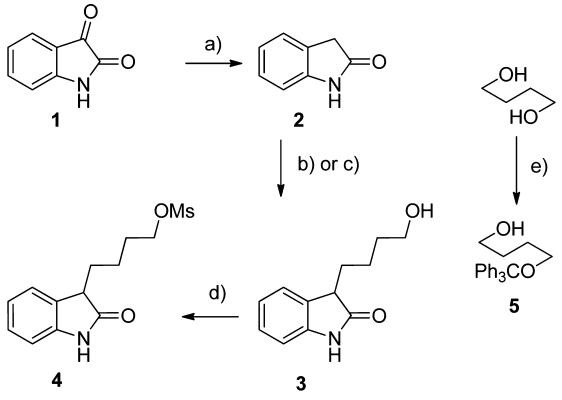
Therefore, the aim of this study was to synthesize, simplify, and fine-tune phenylpiperazinyl-butyloxindole derivatives in the search of radioligands for 5-HT_7 receptor PET imaging (Figure 1). Thereby, we aimed to develop selective reference compounds suitable either for ^{11}C- or for ^{18}F-labeling. Whereas ^{11}C with its half-life (20.4 min) is limited to short experimental time frames, it concurrently allows for test−retest experiments within the same day. In contrast, the longer half-life of ^{18}F (110 min) provides the possibility of a longer experimental setup or even the shipment of the tracer to other facilities. Further, its lower β^+-traveling energy should in principle lead to a higher resolution in PET experiments.

RESULTS AND DISCUSSION

A convenient synthetic route to phenylpiperazinyl-butyloxindole derivatives has been described by Volk and coworkers.[13,17] The method is based on a reductive alkylation of isatins,[17] which circumvents the usual problem of *N*-alkylation and *C*(3)-dialkylation of oxindoles.[18−20] However, the described one-pot reductive alkylation to the corresponding 3-alkyloxindoles made use of high hydrogen pressure (15 bar) at 180 °C. Recently, two different approaches using oxindoles as starting material were published to synthesize *C*(3)-monoalkylated oxindole derivatives by reductive alkylation of alcohols using either $[Cp{\cdot}IrCl_2]_2$[21] or Raney nickel[22] as the reductive agent without high hydrogen pressure.

Scheme 1 illustrates the applied approach to synthesize *C*(3)-monoalkylated oxindole derivatives starting from isatin (**1**),

Scheme 1. Synthesis of *C*(3)-Monoalkylated Oxindole Derivatives via Reductive Alkylation[a]



[a](a) $N_2H_4{\cdot}H_2O$, 130 °C, 30 min, 70%; (b) 1,4-butanediol, Ra−Ni, MW, 200 °C, 4 h, 95%; (c) (1) Compound **5**, $[Cp{\cdot}IrCl_2]_2$, KOH, toluene, MW, 110 °C, 20 min, 37%; (2) 1 M HCl, THF, RT, 20 min, 60%; (d) MsCl, Et_3N, THF, −78 °C, 1 h, 83%; (e) TrCl, pyridine, CH_2Cl_2, RT, 90 min, 83%.

which is first reduced under Wolff−Kishner conditions to oxindole (**2**). In contrast to reported procedures, hydroxyalkylation of **2** was performed under microwave (MW) conditions either resulting quantitatively in **3** in the presence of Raney nickel or yielding 60% using $[Cp{\cdot}IrCl_2]_2$. In both cases, the reaction time could be minimized from 12 h to 20 min−4 h using MW irradiation.

However, the iridium catalyzed *C*(3)-alkylation is hampered not only by lower yields but also by increased synthetic efforts as butane-1,4-diol could not directly be applied to the reductive alkylation. Many side-products were detected using this strategy. Finally, the last synthetic step, that is, mesylation of **3** proceeded uneventfully.

The synthesis of the desired phenylpiperazinyl-butyloxindole derivatives proceeded in reasonable yields when the corresponding arylpiperazine and **4** were heated under neat reaction conditions (Table 1). Interestingly, treatment of **4** with various arylpiperazines using DMF and Na_2CO_3 did not lead to the desired products (for further data, see the Supporting Information). The arylpiperazine derivatives applied were either commercially available or synthesized using previously published methods.[23,24]

The development of a successful in vivo PET probe for neuroreceptor imaging requires a range of properties such as high selectivity for the target, the ability to cross the blood-brain barrier (BBB), and low relative nonspecific binding. To guide the selection of suitable candidates for in vivo PET, the lipophilicity and in vitro affinity for the 5-HT_7 receptor was determined for all compounds.

Lipophilicities were determined using the HPLC method, according to Krass et al.[25] Extrapolated log $D_{7.4}$ values are displayed in Tables 1 and 2. As expected, hydroxyl derivatives showed lower log $D_{7.4}$ values (between 3 and 4) compared to the less polar compounds (log $D_{7.4}$ between 4 and 6). In general, all log $D_{7.4}$ values appear relatively high considering that Rowley et al. suggested the ideal interval for small molecules to penetrate the BBB to be 2−3.[26] However, with our setup, other known CNS PET ligands (e.g., MDL 100907, altanserin, or WAY 100635) show similarly high values.[11] This suggests that our ligands may also have good properties for molecular imaging.

Consequently, we investigated the influence of various substitution patterns on the affinity toward the 5-HT_7 receptor. Thereby, we concentrated on structure−activity relationships at the arylpiperazine moiety (Table 1).

Interestingly, substitution of the 4-position of the phenylpiperazine by a fluorine atom and a methyl group (**8** and **10**) showed a slight improvement in binding profile toward the 5-HT_7 receptor compared to its 4-Cl and 4-unsubstituted analogues. Both compounds (**8**, **10**) represent possible reference structures for PET tracers with nanomolar affinity which can in principle be labeled either by ^{11}C-cross-coupling reactions[27,28] or by a ^{18}F-nucleophilic substitution followed by decarbonylation.[29] Surprisingly, no general trend of the substitution pattern in 2- and 3-position was observed. Whereas the 2-OMe moiety (**13**) resulted in lower affinity compared to its 3-OMe analogue (**14**), 2-(2-fluoroethoxy) and 3-(2-fluoroethoxy) derivatives (**11** and **12**, respectively) showed contrary findings. However, all ether compounds (**11**−**14**) demonstrated nanomolar affinity and could in theory be labeled with either $[^{11}C]CH_3I$ or $[^{18}F]$FETos.[30] In addition, their corresponding precursors showed at least 3 times lower affinity.

Furthermore, the influence of three different possible labeling moieties at the 2-position were explored. ^{11}C-Labeling of thioethers, amines or cyano compounds is well described.[31] All three compounds (**18**−**20**) showed reasonable affinities, and thus, they proved to be promising PET ligands. Finally, a possibility to introduce ^{18}F via a direct one-step fluorination would be desirable. Compound **21** could be labeled with this approach using a trimethylammonium leaving group, which is the preferred site of attack of $[^{18}F]$fluoride compared to the nitro-group.[32] Unfortunately, this compound displayed lower affinity.

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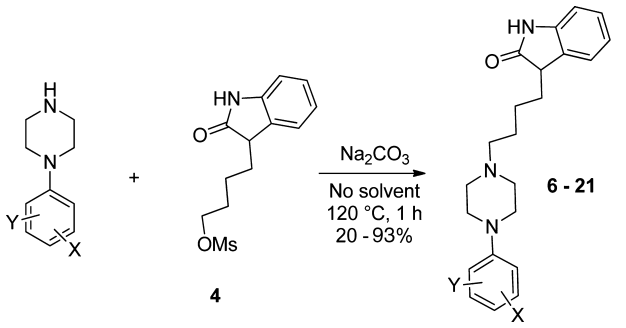
Table 1. Chemical yield of the condensation reaction between the mesylate (4) and the phenylpiperazine derivatives, human recombinant 5-HT$_7$ receptor affinity and lipophilicity of compounds 6−21



compd	X	Y	yield (%)	5-HT$_7$ K_i (nM)[a]	log $D_{7.4}$
6	H	H	58	2.1[16,b]	nd[c]
7	4-Cl	H	71	7.0[7,b]	5.56
8	4-F	H	72	1.1[5,b]	4.65
9	2-Me	H	72	6.3	5.97
10	4-Me	H	76	1.1	5.19
11	2-OCH$_2$CH$_2$F	H	20	3.5	4.15
12	3-OCH$_2$CH$_2$F	H	27	8.5	4.66
13	2-OMe	H	93	7.6	4.15
14	3-OMe	H	32	2.6	4.73
15	2-OH	H	52	7.4	3.91
16	3-OH	H	60	27.5	3.40
17	4-OH	H	34	6.8	3.33
18	2-SMe	H	64	6.7	5.89
19	2-NMe$_2$	H	83	2.0	3.58
20	2-CN	H	73	2.0	4.67
21	4-F	3-NO$_2$	78	25.0	4.90

[a]Receptor and radioligand used in binding assays and data analysis; see Methods. [b]Published in Volk et al.[13,14] [c]Not determined.

Encouraged by the promising results regarding lipophilicity and affinity, **10** and **14** were submitted to a commercial screening package for their selectivity profile on 37 receptors. Compound **14** was found to be over 100-fold selective against a total of 33, whereas **10** against 34 receptors, enzymes, or ion channels (see the Supporting Information). However, both compounds showed some affinity toward a small set of receptors (Table 2).

To avoid significant contributions from targets other than 5-HT$_7$ receptors to the PET imaging signal, selectivity versus the B_{max} value of the target has to be taken into account. The selectivity toward the 5-HT$_{1A}$ and α_1 receptor, because of the high density (B_{max}) of these two receptors in certain brain regions, is of particular concern. Since the displaceable PET-signal consists of density multiplied by affinity, a high B_{max} and high K_d will give a large PET signal compared to the corresponding signal from a relatively low abundance of the 5-HT$_7$ receptor. For example, a ∼350-fold K_d difference over the 5-HT$_{1A}$ receptor is necessary in order to avoid more than 10% PET signal interference from the 5-HT$_{1A}$ receptors in cortical regions, whereas in the thalamus only a 35-fold selectivity must be achieved. For the α_1 receptor a ∼2000-fold selectivity is necessary in cortical regions, but only a 100−200-fold selectivity in thalamic regions.

Table 2. K_i (nM) of Selected Receptors for 10 and 14[ab]

	compd			
	10		**14**	
receptors	K_i	selectivity (receptor/5-HT$_7$)	K_i	selectivity (receptor/5-HT$_7$)
5-HT$_7$	1.1	1	2.6	1
5-HT$_{1A}$	2410	2191	261	100
5-HT$_{2B}$	121	110	192	74
5-HT$_{2A}$	113	103	132	51
5-HT$_{2C}$	676	515	3297	1268
α_1	53	48	47	18

[a]Receptors and radioligands used in binding assays and data analysis; see Methods. [b]K_i values are based on at least two independent experiments.

In contrast to **14**, compound **10** fulfils the requirement for selectively imaging the 5-HT$_7$ receptor against the 5-HT$_{1A}$ receptor in thalamic and cortical regions, whereas **14** is predicted to represent 5-HT$_7$ receptors binding only in the thalamus. Unfortunately, both compounds show a limited specificity against the α_1 receptor. They should image the α_1 receptor rather than the 5-HT$_7$ receptor in cortical regions. But in thalamic regions a major PET signal belonging to the 5-HT$_7$ receptor is predicted for both compounds, and with a slightly higher selectivity for **10**.

However, **10** also displays affinity toward the 5-HT$_{2A}$ receptor. Therefore, the PET images would be anticipated to be composed of a 5-HT$_{2A}$ signal in cortex regions, but requirements for imaging thalamic regions should be fulfilled. In addition, both compounds show a 70−80% inhibition of the H$_1$ receptor at a concentration of 10^{-7} M and **10** displays some affinity toward the SERT. But in both cases (for the H$_1$ and also for the SERT), an interference of the PET signal is not expected due to the low B_{max} number in the relevant brain regions. Finally, both compounds showed inhibition on σ-receptors. σ-Receptors are abundant in cortex regions and could cause decomposition during PET imaging.

The discussion is based on human binding data determined by autoradiography and human brain membranes (Table 3).[33−40]

CONCLUSION

A set of potential 5-HT$_7$ receptor reference ligands for PET molecular imaging were successfully synthesized. Compounds **10** and **14** were tested toward a broader range of receptors. Both compounds display a promising in vitro profile for PET imaging of the 5-HT$_7$ receptor in thalamic regions. Based on in vitro data, it is possible that they may not provide target-specific imaging as signals from the α_1 receptor may contaminate the signal detected from the 5-HT$_7$ receptor. However, we believe that those compounds display an interesting starting point for developing 5-HT$_7$ selective PET ligands with even higher selectivity and affinity.

METHODS

General. The syntheses of selected compounds are described below. The general chemistry, experimental information, spectral data of all new compounds, and determination of lipophilicities and K_i values are supplied in the Supporting Information. Purity of all final compounds was determined by HPLC or GC analysis and is >96%.

General Procedure to Couple 3-[4-(Methanesulfonyloxy)-butyl]oxindole 4 with Substituted 4-Phenylpiperazines. The melt of the secondary amine (12 mmol) was heated to 120 °C under slow stirring. The appropriate 3-[4-(methanesulfonyloxy)butyl]-oxindole (**4**, 12 mmol) and sodium carbonate (1.36 g, 12 mmol)

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Table 3. Receptor Distribution of Colocalized Targets

	5-HT$_7$ (fmol/mg tissue)	5-HT$_{1A}$ (fmol/mg tissue)	5-HT$_{2A}$ (fmol/mg tissue)	α_1 (fmol/mg protein)	H$_1$ (fmol/mg tissue)	σ fmol/mg protein	SERT (fmol/mg tissue)
frontal cortex	2.3	73	80	283	19.1	101	4.3
temporal cortex	2.3	78.6		467	23.5		3.5
parietal cortex		78	75	434	16.6		
occipital cortex	1.2	30	75		13.2	106	3.8
cingulate cortex			75	381	22.3		
hippocampus	5.7	76	25	568		73	2.6
thalamus	12	4.16		299	4.3	58	6.7
caudate			23	277	5.3	84	17
putamen			10	215	4.4		9.8
amygdala	4.2	18	25	393			3
ratios (receptor/5-HT$_7$)		5-HT$_{1A}$	5-HT$_{2A}$	α_1	H$_1$	σ	SERT
frontal cortex		32	35	123	8.3	44	1.86
temporal cortex		34		203	10		1.5
occipital cortex		25	33			88	3.2
hippocampus		13	4.4	99		13	0.46
thalamus		0.35		25	0.36	5	0.56
amygdala		4.3	6.0	94			0.71

were added. After 1 h reaction time, the brown melt was cooled to ambient temperature. EtOAc and water were added, and the layers were separated. The organic layer was dried over $MgSO_4$ and evaporated. The residual oil or solid was purified by column chromatography using EtOAc as eluent.

3-{4-[4-(4-Methylphenyl)piperazine-1-yl]butyl}-1,3-dihydro-2*H*-indol-2-one (10). Compound **10** was prepared using the aboved mentioned procedure. 1-(4-Methylphenyl)piperazine (0.704 g, 3.98 mmol), sodium carbonate (0.452 g, 4.0 mmol), and **4** (1.14 g, 4.0 mmol) yield in **10** (1.09 g, 3.0 mmol, 76%) as a white solid. Mp 109−110 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.98 (1H, s), 7.23−7.17 (2H, m), 7.07−6.99 (3H, m), 6.89−6.80 (3H, m), 3.48 (1H, t, J = 6.0 Hz), 3.14 (4H, t, J = 6.0 Hz), 2.58 (4H, t, J = 6.0 Hz), 2.37 (2H, t, J = 7.5 Hz), 2.28 (3H, s), 2.04−1.96 (2H, m), 1.61−1.51 (2H, m), 1.49−1.37 (2H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 181.0, 149.76, 141.87, 130.07, 130.0, 129.61, 128.51, 122.63, 116.75, 109.99, 58.72, 53.67, 50.03, 46.00, 30.81, 27.18, 24.19, 20.87. MS (FD) m/z (% rel. int.): 363.41 (100.0 $[M]^+$); 364.41 (27.74 $[M+1]^+$); 365.44 (3.14 $[M+2]^+$). LC-MS (ESI): RT: 4.83 min, m/z: 364.2 $[M+H]^+$ at 210 and 254 nm. R_f: 0.2 (EtOAc). HRMS (ESI) $[MH^+]$ calcd for $C_{23}H_{30}N_3O$, 364.2383; found, 364.2386.

3-{4-[4-(3-Methoxyphenyl)piperazine-1-yl]butyl}-1,3-dihydro-2*H*-indol-2-one (14). Compound **14** was prepared using the above-mentioned procedure. 1-(3-Methoxyphenyl)piperazine (0.674 g, 3.51 mmol), sodium carbonate (0.40 g, 3.51 mmol) and **4** (1.0 g, 3.51 mmol) yield in **14** (0.42 g, 1.10 mmol, 32%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (1H, s), 7.17−7.04 (3H, m), 6.93 (1H, t, J = 6.0 Hz), 6.79 (1H, d, J = 1.5 Hz), 6.46−6.42 (1H, dt, J_1 = 3.0 Hz, J_2 = 6.0 Hz), 6.46−6.31 (2H, m), 3.70 (3H, s), 3.40 (1H, t, J = 6.0 Hz), 3.10 (4H, t, J = 4.5 Hz), 2.49 (4H, t, J = 4.5 Hz), 2.30 (2H, t, J = 7.5 Hz), 1.92−1.88 (2H, m), 1.54−1.41 (2H, m), 1.40−1.29 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.60, 160.68, 152.81, 141.77, 129.90, 129.83, 128.02, 124.28, 122.42, 109.86, 108.99, 104.56, 102.60, 102.60, 58.45, 55.35, 53.30, 49.12, 46.16, 30.54, 26.90, 23.93. MS (FD) m/z (% rel. int.): 379.41 (100.0 $[M]^+$); 380.41 (18.26 $[M+1]^+$). LC-MS (ESI): RT: 4.99 min, m/z: 380.4 $[M+H]^+$ at 210 and 254 nm. R_f: 0.1 (EtOAc). HRMS (ESI) $[MH^+]$ calcd for $C_{23}H_{30}N_3O_2$, 380.2338; found, 380.2340.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, spectroscopic data for selected compounds, receptor distribution, and detailed in vitro profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

M.M.H., G.M.K., and J.L.K. conceived the project. M.M.H., J.L.K., B.V., and F.A. designed the experiments. M.M.H. performed all chemical syntheses. B.V. provided M.H.H. with intermediates. K.P. and F.A. carried out all affinity measurements. Compounds were analyzed by M.M.H. HRMS data were provided by B.V. The project was coordinated by M.H.H., G.M.K., and J.L.K. M.H.H. wrote the manuscript with the help of B.V., G.M.K., and J.L.K. L.K.B. did the log *D* measurements.

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Notes

The authors declare no competing financial interest.

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