Journal of Medicinal Chemistry

Brief Article

pubs.acs.org/jmc

Evaluation of 3-Ethyl-3-(phenylpiperazinylbutyl)oxindoles as PET Ligands for the Serotonin 5-HT$_7$ Receptor: Synthesis, Pharmacology, Radiolabeling, and in Vivo Brain Imaging in Pigs

Matthias M. Herth,[†,‡] Valdemar L. Andersen,[†,‡] Hanne D. Hansen,[†] Nikolas Stroth,[§] Balázs Volk,[∥] Szabolcs Lehel,[⊥] Agnete Dyssegaard,[†] Anders Ettrup,[†] Per Svenningsson,[§] Gitte M. Knudsen,[†] and Jesper L. Kristensen*[,†,‡]

[†]Center for Integrated Molecular Brain Imaging, Rigshospitalet and University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

[‡]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

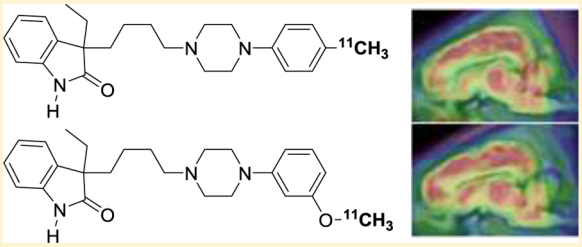
[§]Center for Molecular Medicine, Department of Neurology and Clinical Neuroscience, Karolinska Institute and Karolinska University Hospital, 17176 Stockholm, Sweden

[∥]Egis Pharmaceuticals Plc, P.O. Box 100, H-1475 Budapest, Hungary

[⊥]PET and Cyclotron Unit, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Supporting Information

ABSTRACT: We have investigated several oxindole derivatives in the pursuit of a 5-HT$_7$ receptor PET ligand. Herein the synthesis, chiral separation, and pharmacological profiling of two possible PET candidates toward a wide selection of CNS-targets are detailed. Subsequent ^{11}C-labeling and in vivo evaluation in Danish landrace pigs showed that both ligands displayed high brain uptake. However, neither of the radioligands could be displaced by the 5-HT$_7$ receptor selective inverse agonist SB-269970.



INTRODUCTION

The 5-HT$_7$ G protein-coupled receptor is the latest addition to the serotonin receptor subfamily.[1,2] Although the functional significance of this receptor is largely unknown, several reports have associated the human 5-HT$_7$ receptor with a variety of central nervous system (CNS) functions and disorders.[3] For example, the antidepressant effect of the atypical antipsychotic drugs amisulpride, vortioxetine, and lurasidone have been explained by 5-HT$_7$ receptor antagonism.[4−8] The availability of an appropriate 5-HT$_7$ receptor positron emission tomography (PET) radioligand would be an important tool that could significantly advance our understanding of the neurobiology and eventual dysfunctions of the 5-HT$_7$ receptor, as PET molecular imaging enables quantification of neuroreceptor binding in vivo.

We and others have investigated a number of ligands from different compound classes for that purpose.[9−17] Of those ligands, Cimbi-712 (**1**) and Cimbi-717 (**2**) displayed the most promising profiles for selective 5-HT$_7$ receptor PET imaging in thalamic regions (Figure 1).[12] Subsequently, ^{11}C-labeling of both compounds was carried out and the in vivo PET behavior of both radioligands were evaluated in pigs.[13,18] High brain uptake and specific labeling of 5-HT$_7$ receptors was observed.[13] These results encouraged us to further evaluate the potential of this compound class as PET ligands for the 5-HT$_7$ receptor. Initially, **1** and **2** were evaluated in their racemic forms.

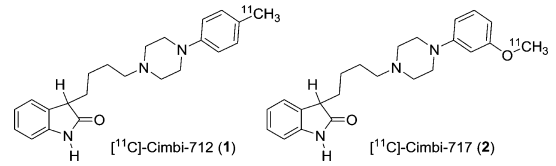


Figure 1. Oxindole ligands previously investigated as PET ligands for the 5-HT$_7$ receptor.[13]

The use of racemic ligands in PET can complicate data interpretation as the two enantiomers may very well have different pharmacological profiles and kinetics (on/off-rates etc.).

Thus, we set out to evaluate the pharmacology of the individual enantiomers of **1** and **2**. However, rapid racemization was observed after separation on chiral HPLC at room temperature for both compounds (see Supporting Information for further details). The observed racemization is due to the acidic nature of the chiral center at the 3-position of the oxindole moiety. Consequently, we decided to investigate the

Received: January 19, 2015
Published: March 31, 2015

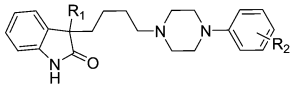
DOI: 10.1021/acs.jmedchem.5b00095
J. Med. Chem. 2015, 58, 3631−3636

influence of blocking the 3-position as this should enable the separation of the compounds into their corresponding enantiomers.

RESULTS AND DISCUSSION

Volk et al. have previously reported that 3-ethyloxindoles have a similar pharmacological profile to nonsubstituted oxindoles.[19,20] We could also show that other alkyl chains at the 3-position (−Me, −Et, −EtF, and −Pr) did not alter the affinity toward the 5-HT$_7$ receptor significantly (Supporting Information).

Thus, the two compounds in which the H is replaced by an ethyl group (**3** and **4** compared to **1** and **2**) were selected for further studies (Figure 2). The compounds were synthesized using procedures similar to those previously described (see Supporting Information for details).[12,19−21]



	K_i (nM)	
	R_1 =	
R_2 =	H	Et
4-Me	1.1 (**1**)	2.5 (**3**)
3-OMe	2.6 (**2**)	2.0 (**4**)

Figure 2. Affinities of **1**, **2**, **3**, and **4** for the 5-HT$_7$ receptor. Data represent n = 6 from three independent experiments, each carried out in duplicate.

As seen in Figure 2, the affinity for 5-HT$_7$ receptor is not influenced to any great extent after blocking the labile 3-position. The affinity of the racemic 4′-Me derivative goes from 1.1 to 2.5 nM, whereas the racemic 3′-OMe is essentially the same, going from 2.6 to 2.0 nM. All four racemic compounds (**1**−**4**) were subsequently tested for efficacy in a heterologous cell line overexpressing human 5-HT$_{7(a)}$ receptor.

Initial experiments indicated inverse agonism, in that cellular cAMP concentrations were reduced below the level found in untreated control cells (Figure 3A). Subsequent concentration−response studies showed complete antagonism of 1 μM 5-HT-induced cAMP accumulation (Figure 3B), and curve fitting of the data (Figure 3C) yielded inhibitory potencies in the low nanomolar range (IC_{50} values: 1.9, 5.6, 15.3, and 6.8 nM for compounds **1**−**4**).

Encouraged by the preliminary pharmacological evaluation, we proceeded with the chiral resolution of **3** and **4** on HPLC, which provided both enantiomers, and as expected, epimerization of the chiral center is no longer an issue (see Experimental Section for details). Subsequently, the racemic mixtures as well as the pure enantiomers were screened against a broad selection of targets at the PDSP screening facilities to fully evaluate the potential of these compounds as PET ligands (Table 1). For several of the investigated targets, the affinities of the racemic mixtures are on par with or even higher than the pure enantiomers of **3** and **4**. This is of course not to be expected, but because the values are within a factor of 5, we speculate that this can be attributed to the uncertainty in the assays. The data was generated by the PDSP, and we present the data as received.

(+)-**3** and (+)-**4** showed more than 5-fold higher affinity toward the 5-HT$_7$ receptor compared to (−)-**3** and (−)-**4**. The racemic compounds **3** and **4** had affinities comparable to (+)-**3** and (+)-**4**, respectively and also comparable to the 3-unsubstituted oxindole derivatives **1** and **2**. However, α_{1A}

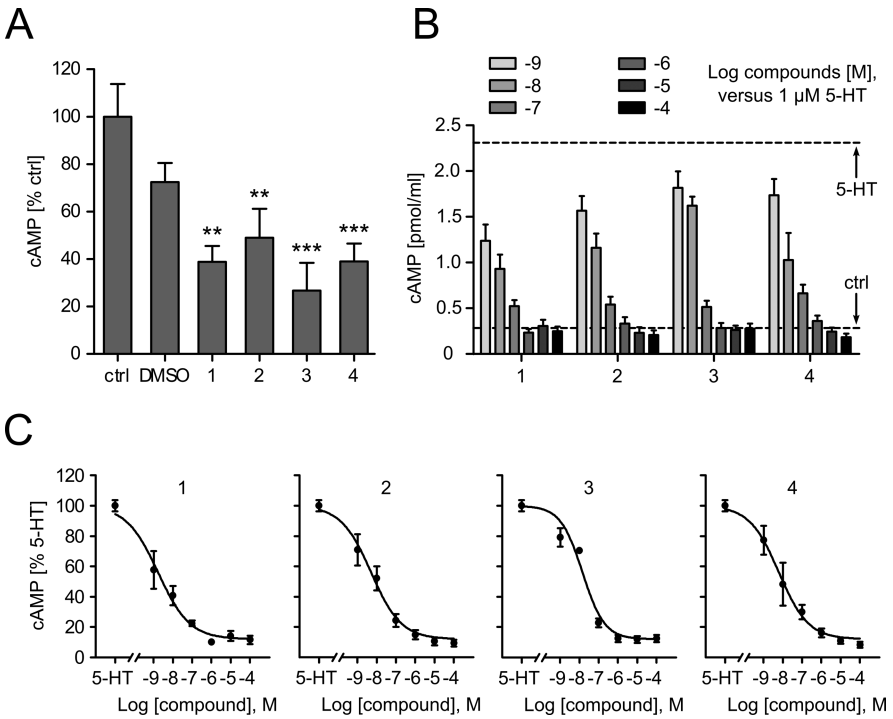


Figure 3. Pharmacological characterization of compound efficacy in HeLa cells stably expressing human 5-HT$_{7(a)}$. Cells were exposed to test compounds in the absence or presence of 1 μM 5-HT, and cellular cAMP was measured after 15 min of treatment. In the absence of 5-HT, all four compounds (at 10 μM) exhibit inverse agonist activity (A). In the presence of 5-HT, all compounds are potent and efficacious antagonists (B,C). All data are displayed as means ± SEM. Data in (A) were analyzed by one-way ANOVA with Dunnett's multiple comparison test, and asterisks indicate significant differences from untreated controls (**p < 0.01, ***p < 0.001; n = 6−11 from four independent experiments). Concentration−response curves in (C) were derived from data in (B) via nonlinear regression (n = 6 from three independent experiments, each carried out in duplicate).

DOI: 10.1021/acs.jmedchem.5b00095
J. Med. Chem. 2015, 58, 3631−3636

Table 1. Selectivity Profile of Racemic Mixtures and Enantiomers of 3 and 4

	K_i [nM][a]							
	1	**3**	(+)-**3**	(−)-**3**	**2**	**4**	(+)-**4**	(−)-**4**
5-HT$_7$	4.1	6.5	5.6	82	7.5	7.8	11	56
5-HT$_{1A}$	491	469	787	633	130	83	192	151
5-HT$_{2A}$	35	39	94	110	239	98	352	66
5-HTT	112	295	271	827	253	250	376	1439
H$_1$	323	529	135	61	164	502	42	2405
α_{1A}	103	431	951	487	167	434	>10000	794
σ_1	45	37	33	390	59	42	46	249
σ_2	24	40	347	61	49	22	152	16

[a]Affinities [K_i values] were determined by PDSP (n = 3) and are reported in nM.

affinities were decreased in comparison with **1** and **2**. Table 1 displays selected affinities toward targets where cross-affinity could become an issue.

Other tested targets showed affinity >3 μM (5-HT$_6$, α_{1B}, α_{1D}). Compounds **1**, **2**, **3**, (+)-**3**, **4**, and (+)-**4** were at least 2.5-fold selective over σ-receptors and at least 5-fold selective over 5-HT$_{2A}$ receptors. All other targets tested showed a selectivity of more than 1 order of magnitude, except for (+)-**4**, which displayed ∼4-fold lower selectivity for H$_1$ receptors. In general, it appears that C-3 alkylation does not improve the affinity toward the 5-HT$_7$ receptor but improves the selectivity especially toward the α_1 receptor.

A PET radioligand has to fulfill several requirements to selectively image only one target. For example, high affinity toward the target-in-question must be accompanied by an acceptable level of selectivity toward other targets in the regions of interest. The receptor density (B_{max}) is a measure of how many receptors are present in a given region of interest. A seeming lack of selectivity of a compound toward a certain receptor may be compensated by a high B_{max} value in a particular region.[22] Thus, the observed PET images are a function of the relative affinity of the ligand toward a target and the B_{max} value of that target in a specific region. In general, a 10−100-fold higher binding to the target compared to other targets in a particular region is acceptable.[22] On the basis of these guidelines, **3**, (+)-**3**, **4**, and (+)-**4** displayed very promising profiles for selective 5-HT$_7$ receptor imaging in the thalamus (Table 2; see Supporting Information for further details).

In general, introduction of an alkyl group to the 3-position of the oxindole moiety increased the selectivity/B_{max} ratio toward the critical α_1 receptor. This could be further improved by enantioselective resolution of the 3-ethyl derivatives. Unfortunately, selectivity/B_{max} ratios for the σ-receptors could not be calculated due to a lack of human B_{max} literature values. Future studies have to determine if these receptors have to be considered off-targets.

Table 2. Selectivity/B_{max} Ratios for the 5-HT$_7$ Receptor Relative to Other Cross-Affinity Targets in the Thalamus

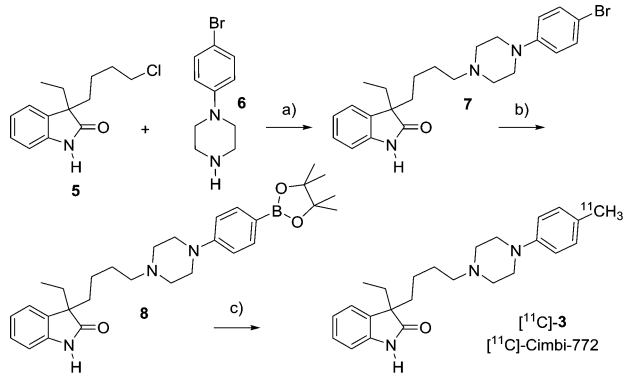
	selectivity over B_{max} ratio[a]							
	1	**3**	(+)-**3**	(−)-**3**	**2**	**4**	(+)-**4**	(−)-**4**
5-HT$_{1A}$	359	216	421	23	52	31	52	8
5-HT$_{2A}$	17	12	34	3	63	25	64	2
5-HTT	32	54	58	12	40	38	41	30
H$_1$	236	244	72	2	65	193	11	128
α_1	4	9	23	1	3	8	123	2

[a]Data are based on values reported in Table 1 and Supporting Information Table S2. PDSP determined K_i values and human B_{max} values.

We decided to start with ^{11}C-labeling of both racemic compounds to allow us to optimize the labeling procedure. In addition, use of the racemic compounds (**3**, **4**) would enable a direct in vivo PET imaging comparison between these and the unsubstituted oxindoles (**1**, **2**).

The required precursor was accessed in two steps from readily available **5** as outlined in Scheme 1. The radiosynthesis of [^{11}C]-**3** ([^{11}C]-Cimbi-772) was carried out similarly to a recently published procedure for ^{11}C-Suzuki cross couplings.[18] It is noteworthy that the formation of the [^{11}C]Me-Pd-I complex prior to the addition of the precursor is essential for a successful radiolabeling. The average specific activity was 343 GBq/μmol (range 183−542 GBq/μmol), with radiochemical purity being above 97%. Thus, typically an amount of 122−230 MBq could be isolated from a 40 min beam. Scheme 1 summarizes the synthesis of precursor **8** and radiosynthesis of [^{11}C]-**3**.

Scheme 1. Radiosynthesis of [^{11}C]-3[a]



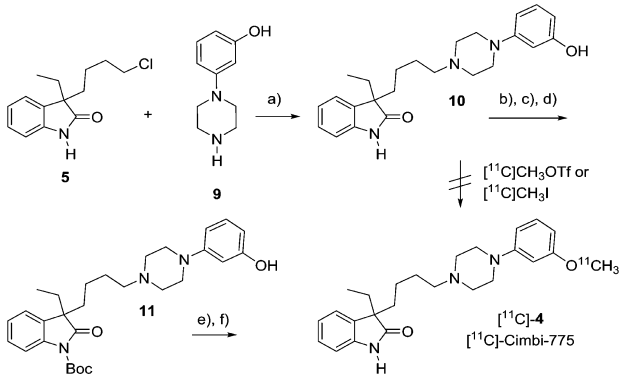
[a]Reagents and conditions: (a) Na_2CO_3, 180 °C, 1 h; (b) KOAc, bis(pinacolato)diboron, Pd(dppf)Cl$_2$, 1,4-dioxane, 100 °C, 12 h; (c) ^{11}C-labeling: Pd$_2$(dba)$_3$, P(*o*-tolyl)$_3$, K_2CO_3, [^{11}C]CH_3I, DMF:water 9:1, 60 °C, 5 min.

Initially, ^{11}C-labeling of compound **4** ([^{11}C]-Cimbi-775) was attempted using the same conditions as reported for the synthesis of the structurally related compound **2** via direct ^{11}C-methylation at the phenolic position of the corresponding precursor. However, all attempts to produce the desired PET ligand using this approach were unsuccessful and a single undesired byproduct was observed.

DOI: 10.1021/acs.jmedchem.5b00095
J. Med. Chem. 2015, 58, 3631−3636

We speculate that C-3 alkylation of the oxindole influences the reactivity of the oxindole core leading to competing *N*-labeling. Therefore, a new labeling strategy was developed in which the phenolic hydroxyl group was transiently TBDPS-protected, facilitating selective Boc-protection of the oxindole (Scheme 2). Subsequent desilylation provided a suitable

Scheme 2. Radiosynthesis of [^{11}C]-4[a]



[a]Reagents and conditions: (a) Na_2CO_3, 180 °C, 1 h; (b) TBDPSCl, NaH, DMF, 110 °C, 12 h; (c) Boc_2O, NaHDMS, THF, −5 °C, 30 min; (d) NH_4F, MeOH, 70 °C, 30 min; (e) ^{11}C-labeling: [^{11}C]CH_3I, 2 M NaOH, DMF, 140 °C, 5 min; (f) TFA/CH_2Cl_2 (1:1), 80 °C, 5 min.

precursor, which was O-alkylated with [^{11}C]CH_3I followed by Boc-deprotection, yielding the desired tracer [^{11}C]-**4**. Average specific activities were around 234 GBq/μmol (range 78−331 GBq/μmol) with a radiochemical purity above 97%. Typically, an amount of 152−211 MBq could be isolated using a 40 min beam (Scheme 2).

A preliminary ex vivo investigation into the in vivo binding behavior of [^{11}C]-**4** was performed on autoradiography on pig brain slices in a competition experiment with SB-269970,[23,24] a selective 5-HT_7 receptor inverse agonist (see Supporting Information for details). Those studies indicated that [^{11}C]-**4** could indeed be displaced in this setup, and we proceeded with the full in vivo evaluation of the ligands.

[^{11}C]-**3** and [^{11}C]-**4** were evaluated in Danish Landrace pigs using a high resolution research tomography (HRRT) PET scanner. Summed PET images (Figure 4A,B) show that both radioligands readily enter the pig brain and distribute according to the known 5-HT_7 receptor distribution. Following iv injection of either [^{11}C]-**3** or [^{11}C]-**4**, the peak uptake reached 2.5 SUV in the thalamus. Peak uptake was reached at around ∼30 min for [^{11}C]-**3** and ∼10 min for [^{11}C]-**4**.

To investigate the specificity of binding of the radioligands, we administered 1.0 mg/kg/h of SB-269970 30 min prior to the second injection of the radioligand and continued the infusion throughout the scan time. However, no significant blocking effects could be detected for either of the radioligands (Figure 4D,E). This lack of displacement has been observed with other potent [^{11}C]-5HT_7-ligands from other compound classes.[11,17]

This lack of blocking could simply occur because [^{11}C]-**3** and [^{11}C]-**4** are not selective for the 5-HT_7 receptor in vivo and that the observed brain uptake is nonspecific binding (NSB). However, assuming that NSB is uniform throughout the brain, the observed binding cannot be solely attributed to this because the radioligands distribute according to the 5-HT_7 receptor distribution determined in vitro. Higher NSB of [^{11}C]-**3** and

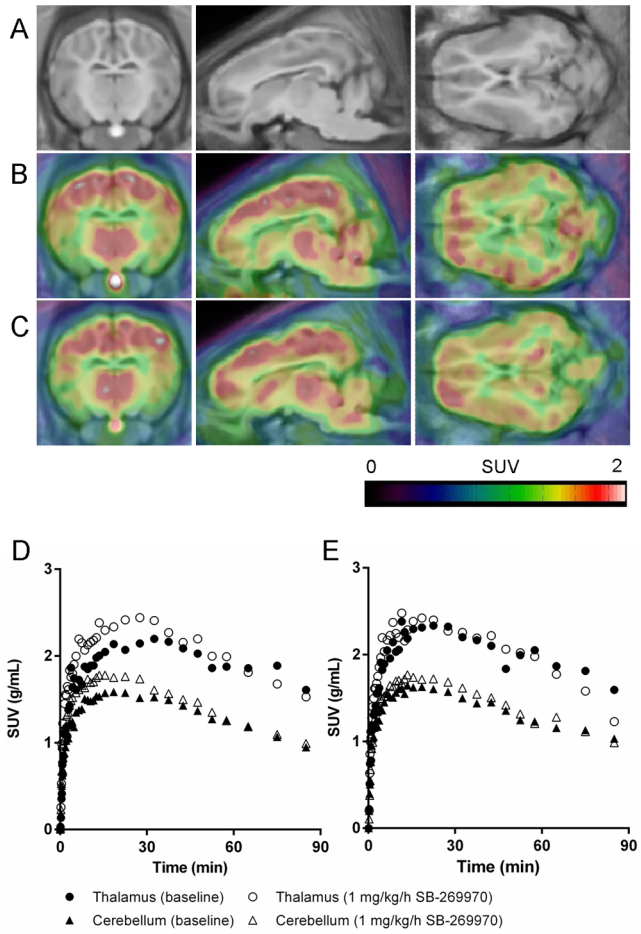


Figure 4. Summed PET images (0−90 min) of [^{11}C]-**3** (A) and [^{11}C]-**4** (B). MRI-based atlas of the pig brain (C). Time activity curves at baseline (solid symbols) and after pretreatment with SB-269970 (open symbols) for [^{11}C]-**3** (n = 2) (D) and [^{11}C]-**4** (n = 1) (E).

[^{11}C]-**4** compared to [^{11}C]-**1** and [^{11}C]-**2** is not unlikely given that **3** and **4** have higher lipophilicity (see Supporting Information for details, Table S1). Second, despite the improved selectivity profile of [^{11}C]-**3** and [^{11}C]-**4**, we cannot explicitly rule out binding to unknown targets. A third explanation could be that the dose of the blocking agent was too low. However, the dose of SB-269970 used in this study has previously resulted in 60% blocking of [^{11}C]-**2** binding.[13]

Another concern could be binding to the σ-receptors, for which the ligands have relatively high affinity, see Table 1. However, the relative affinities of **3** and **4** for 5-HT_7/σ are higher than that for **1** and **2** and SB-269970 is able to displace the binding of those ligands in vivo.[13] Thus, it is unlikely that binding to σ-receptors can explain the inability of SB-269970 to block the binding of [^{11}C]-**3** and [^{11}C]-**4**.

Finally, we speculate whether the two radioligands evaluated here could bind to different receptor binding sites because of their different functional profiles or because they cause different degrees of receptor internalization, both of which may influence the possibility to perform blocking or competition studies. Volk reported all oxindole structures as antagonists but also observed a difference in the antagonistic efficacy between the C-3 alkylated and unsubstituted oxindole derivatives.[19] We report herein that **1**−**4** are inverse agonists (Figure 3). The functional properties (inverse agonist, antagonist, partial, or full agonist) of the radioligand may influence the ability to block or displace

DOI: 10.1021/acs.jmedchem.5b00095
J. Med. Chem. 2015, 58, 3631−3636

the radioligand in vivo, which has been shown in other studies where other receptor systems were investigated.[25−27] However, more studies are warranted in order to investigate the importance of functional differences of compounds for PET radioligand binding.

CONCLUSION

A set of potential 5-HT$_7$ receptor PET radioligands of the 3-ethyl-3-(phenylpiperazinyl-butyl)oxindole family was successfully synthesized and evaluated. Compounds **3**, (+)-**3**, **4**, and (+)-**4** displayed a promising in vitro profile for PET imaging of the 5-HT$_7$ receptor in thalamus. In comparison to the nonalkylated derivatives, **1** and **2**, it appears that alkylation of the oxindole 3-position improves the overall selectivity profile but does not improve the affinity toward the 5-HT$_7$ receptor. Radiolabeling of **3** and **4** allowed us to evaluate both racemates in vivo, where they entered the brain and accumulated in brain regions reflecting the expected distribution of 5-HT$_7$ receptors. However, no significant blocking of radioligand binding was observed after administration of the 5-HT$_7$ receptor specific inverse agonist, SB-269970, which calls into question the selectivity of both radioligands in vivo. Further studies have to be conducted to clarify if a functional difference between alkylated and nonalkylated oxindole PET ligands could lead to the observed effect in vivo.

EXPERIMENTAL SECTION

General. The labeling procedure of selected compounds and the PET scanning protocol 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%.

Labeling Procedures. *[^{11}C]-3-Ethyl-3-(4-(4-(4-methylphenyl)-piperazin-1-yl)butyl)indolin-2-one ([^{11}C]-**3**).* [^{11}C]Methyl iodide ([^{11}C]MeI) produced using a fully automated system was transferred in a stream of helium to a 1.1 mL vial containing DMF (300 μL). To this vial was added $Pd_2(dba)_3$ (0.3 mg), P(*o*-tolyl)$_3$ (0.2 mg), and a 0.5 M K_2CO_3 (2.6 μL) dissolved in DMF:H_2O 9:1 (150 μL). The resulting mixture was heated at 60 °C for 2 min. Afterward, the precursor (**8**) dissolved in DMF:H_2O 9:1 (150 μL) was added and the mixture was heated at 60 °C for another 5 min. Purification of the crude product was accomplished using HPLC (Luna 5 μ C_{18}(2) 100 Å, 250 mm × 10.00 mm 5 μm; 0.01 M borax buffer:MeCN (30:70; flow rate, 9 mL/min; RT, 475 s ([^{11}C]-**3**). The fraction corresponding to the labeled product was collected in sterile water (150 mL), and the resulting solution was passed through a solid-phase C18 Sep-Pak extraction column (Waters Co.), which had been preconditioned with ethanol (10 mL), followed by an isotonic sodium chloride solution (20 mL). The column was flushed with sterile water (3 mL). Then, the trapped radioactivity was eluted with ethanol (3 mL) into a 20 mL vial containing phosphate buffer (9 mL, 100 mM, pH 7), giving a 12 mL solution of [^{11}C]-**3**. In a total synthesis time of 45−50 min, 0.1−0.2 GBq of [^{11}C]-**3** was produced.

*[^{11}C]-3-Ethyl-3-(4-(4-(3-(methoxy)phenyl)piperazin-1-yl)butyl)-indolin-2-one ([^{11}C]-**4**).* [^{11}C]Methyl iodide ([^{11}C]MeI) produced using a fully automated system was transferred in a stream of helium to a 1.1 mL vial containing precursor **11** (0.3−0.4 mg), DMF (300 μL), and a 2 M NaOH solution (4 μL). The resulting mixture was heated at 140 °C for 5 min. Afterward, the solution was subsequently cooled to 80 °C by nitrogen cooling before 500 μL of a TFA:CH_2Cl_2 solution (1:1) was added. The solution was stirred for further 5 min at this temperature and then quenched with 3.5 mL of the HPLC eluent (see below). Purification of the crude product was accomplished using HPLC (Luna 5μ C_{18}(2) 100 Å, 250 mm × 10.00 mm 5 μm; EtOH/0.1% H_3PO_4 20:80; flow rate, 6 mL/min; RT, 650 s ([^{11}C]-**4**)). The fraction corresponding to the labeled product was collected in sterile water (150 mL), and the resulting solution was passed through a solid-phase C18 Sep-Pak extraction column (Waters Co.), which had been preconditioned with ethanol (10 mL), followed by isotonic sodium chloride solution (20 mL). The column was flushed with sterile water (3 mL). Then, the trapped radioactivity was eluted with ethanol (3 mL), followed by anisotonic sodium chloride solution (3 mL) into a 20 mL vial containing phosphate buffer (9 mL, 100 mM, pH 7), giving a 15 mL solution of [^{11}C]-**4** with a pH of approximately 7. In a total synthesis time of 45−50 min, 0.4−0.5 GBq of [^{11}C]-**4** was produced.

PET Scanning Protocol. [^{11}C]-**3** was given as an intravenous (iv) bolus injection, and the injected dose was 61 and 153 MBq for baseline scans (n = 2) and 153 and 194 MBq for scans where SB-269970 was preadministered (n = 2). [^{11}C]-**4** was also given as an iv bolus injection, and the injected dose was 286 and 129 MBq for baseline scans (n = 2) and 155 MBq for the scan where SB-269970 was administered (n = 1). The pigs were subsequently scanned for 90 min in list-mode with a high resolution research tomography (HRRT) scanner (Siemens AG, Munich, Germany), where scanning started at the time of injection (0 min). Immediately after the baseline scan (90 min), SB-269970 (Tocris Bioscience, Bristol, United Kingdom) was given iv as bolus infusion (1.0 mg/kg/h) and rescanning started after 30 min of pretreatment with SB-269970.

ASSOCIATED CONTENT

Supporting Information

Full experimental details on the synthesis of compounds, the conditions for the chiral resolution, in vitro characterization, lipophilicity measurements, animal procedures, and quantification of PET data. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: (+45) 35336487. Fax: +45 35336040. E-mail: jesper.kristensen@sund.ku.dk.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Intra European Fellowship (MC-IEF-275329). The Faculty of Health and Medical Sciences, University of Copenhagen, and the Lundbeck Foundation (Cimbi) is gratefully acknowledged. We thank the staff at the PET and Cyclotron unit for expert technical assistance and Mette Værum Olesen for animal assistance. K_i determinations at selected neuroreceptors were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, contract no. HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth, MD Ph.D., at the University of North Carolina at Chapel Hill, and Project Officer Jamie Driscol at NIMH, Bethesda MD, USA. The John & Birthe Meyer Foundation and the Toyota Foundation are acknowledged for granting the HRRT scanner and the HPLC system, respectively.

ABBREVIATIONS USED

5-HT, serotonin; NSB, nonspecific binding; HRRT, high resolution research tomography

REFERENCES

(1) Glennon, R. A. Higher-end serotonin receptors: 5-HT(5), 5-HT(6), and 5-HT(7). *J. Med. Chem.* **2003**, *46*, 2795−2812.

DOI: 10.1021/acs.jmedchem.5b00095
J. Med. Chem. 2015, 58, 3631−3636

(2) Hoyer, D.; Hannon, J. P.; Martin, G. R. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol., Biochem. Behav.* **2002**, *71*, 533−554.
(3) Matthys, A.; Haegeman, G.; Van Craenenbroeck, K.; Vanhoenacker, P. Role of the 5-HT7 receptor in the central nervous system: from current status to future perspectives. *Mol. Neurobiol.* **2011**, *43*, 228−253.
(4) Cassano, G. B.; Jori, M. C. Efficacy and safety of amisulpride 50 mg versus paroxetine 20 mg in major depression: a randomized, double-blind, parallel group study. *Int. Clin. Psychopharmacol.* **2002**, *17*, 27−32.
(5) Lecrubier, Y.; Boyer, P.; Turjanski, S.; Rein, W. Amisulpride versus imipramine and placebo in dysthymia and major depression. Amisulpride Study Group. *J. Affect. Disord.* **1997**, *43*, 95−103.
(6) Abbas, A. I.; Hedlund, P. B.; Huang, X. P.; Tran, T. B.; Meltzer, H. Y.; Roth, B. L. Amisulpride is a potent 5-HT7 antagonist: relevance for antidepressant actions in vivo. *Psychopharmacology (Berlin)* **2009**, *205*, 119−128.
(7) Cates, L. N.; Roberts, A. J.; Huitron-Resendiz, S.; Hedlund, P. B. Effects of lurasidone in behavioral models of depression. Role of the 5-HT(7) receptor subtype. *Neuropharmacology* **2013**, *70*, 211−217.
(8) Andersen, V. L.; Hansen, H. D.; Herth, M. M.; Knudsen, G. M.; Kristensen, J. L. (11)C-Labeling and preliminary evaluation of vortioxetine as a PET radioligand. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2408−2411.
(9) Zhang, M. R.; Haradahira, T.; Maeda, J.; Okauchi, T.; Kida, T.; Obayashi, S.; Suzuki, K.; Suhara, T. Synthesis and preliminary PET study of the 5-HT7 receptor antagonist [C-11]DR4446. *J. Labelled Compd. Radiopharm.* **2002**, *45*, 857−866.
(10) Andries, J.; Lemoine, L.; Mouchel-Blaisot, A.; Tang, S.; Verdurand, M.; Le Bars, D.; Zimmer, L.; Billard, T. Looking for a 5-HT7 radiotracer for positron emission tomography. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3730−3733.
(11) Herth, M. M.; Hansen, H. D.; Ettrup, A.; Dyssegaard, A.; Lehel, S.; Kristensen, J.; Knudsen, G. M. Synthesis and evaluation of [C-11]Cimbi-806 as a potential PET ligand for 5-HT7 receptor imaging. *Bioorg. Med. Chem.* **2012**, *20*, 4574−4581.
(12) Herth, M. M.; Volk, B.; Pallagi, K.; Bech, L. K.; Antoni, F. A.; Knudsen, G. M.; Kristensen, J. L. Synthesis and in vitro evaluation of oxindole derivatives as potential radioligands for 5-HT7 receptor imaging with PET. *ACS Chem. Neurosci.* **2012**, *3*, 1002−1007.
(13) Hansen, H. D.; Herth, M. M.; Ettrup, A.; Andersen, V. L.; Lehel, S.; Dyssegaard, A.; Kristensen, J. L.; Knudsen, G. M. Radiosynthesis and in vivo evaluation of novel radioligands for PET imaging of cerebral 5-HT7 receptors. *J. Nucl. Med.* **2014**, *55*, 640−646.
(14) Lacivita, E.; Niso, M.; Hansen, H. D.; Di Pilato, P.; Herth, M. M.; Lehel, S.; Ettrup, A.; Montenegro, L.; Perrone, R.; Berardi, F.; Colabufo, N. A.; Leopoldo, M.; Knudsen, G. M. Design, synthesis, radiolabeling and in vivo evaluation of potential positron emission tomography (PET) radioligands for brain imaging of the 5-HT7 receptor. *Bioorg. Med. Chem.* **2014**, *22*, 1736−1750.
(15) Zimmer, L.; Billard, T. Molecular imaging of the serotonin 5-HT7 receptors: from autoradiography to positron emission tomography. *Rev. Neurosci.* **2014**, *25*, 357−365.
(16) Lemoine, L.; Andries, J.; Le Bars, D.; Billard, T.; Zimmer, L. Comparison of 4 radiolabeled antagonists for serotonin 5-HT7 receptor neuroimaging: toward the first PET radiotracer. *J. Nucl. Med.* **2011**, *52*, 1811−1818.
(17) Hansen, H. C.; Andersen, V. L.; Lehelc, S.; Magnussen, J. H.; Dyssegaard, A.; Stroth, N.; Kristensen, J. L.; Knudsen, G. M.; Herth, M. M. Labeling and preliminary in vivo evaluation of the 5-HT7 receptor selective agonist [11C]E-55888. *Bioorg. Med. Chem. Lett.* **2015**, DOI: 10.1016/j.bmcl.2015.03.039.
(18) Andersen, V. L.; Herth, M. M.; Lehel, S.; Knudsen, G. M.; Kristensen, J. L. Palladium-mediated conversion of *para*-aminoarylboronic esters into *para*-aminoaryl-C-11-methanes. *Tetrahedron Lett.* **2013**, *54*, 213−216.
(19) Volk, B.; Barkoczy, J.; Hegedus, E.; Udvari, S.; Gacsalyi, I.; Mezei, T.; Pallagi, K.; Kompagne, H.; Levay, G.; Egyed, A.; Harsing, L. G.; Spedding, M.; Simig, G. Phenylpiperazinyl-butyl)oxindoles as selective 5-HT(7) receptor antagonists. *J. Med. Chem.* **2008**, *51*, 2522−2532.
(20) Volk, B.; Gacsalyi, I.; Pallagi, K.; Poszavacz, L.; Gyonos, I.; Szabo, E.; Bako, T.; Spedding, M.; Simig, G.; Szenasi, G. Optimization of (arylpiperazinylbutyl)oxindoles exhibiting selective 5-HT7 receptor antagonist activity. *J. Med. Chem.* **2011**, *54*, 6657−6669.
(21) Volk, B.; Simig, G. New one-pot synthesis of 3-alkyl- and 3-(ω-hydroxyalkyl)oxindoles from isatins. *Eur. J. Org. Chem.* **2003**, 3991−3996.
(22) Paterson, L. M.; Kornum, B. R.; Nutt, D. J.; Pike, V. W.; Knudsen, G. M. 5-HT radioligands for human brain imaging with PET and SPECT. *Med. Res. Rev.* **2013**, *33*, 54−111.
(23) Lovell, P. J.; Bromidge, S. M.; Dabbs, S.; Duckworth, D. M.; Forbes, I. T.; Jennings, A. J.; King, F. D.; Middlemiss, D. N.; Rahman, S. K.; Saunders, D. V.; Collin, L. L.; Hagan, J. J.; Riley, G. J.; Thomas, D. R. A novel, potent, and selective 5-HT7 antagonist: (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl)phenol (SB-269970). *J. Med. Chem.* **2000**, *43*, 342−345.
(24) Romero, G.; Pujol, M.; Pauwels, P. J. Reanalysis of constitutively active rat and human 5-HT7(a) receptors in HEK-293F cells demonstrates lack of silent properties for reported neutral antagonists. *Naunym-Schmiedeberg's Arch. Pharmacol.* **2006**, *374*, 31−39.
(25) Suter, T. M.; Chesterfield, A. K.; Bao, C.; Schaus, J. M.; Krushinski, J. H.; Statnick, M. A.; Felder, C. C. Pharmacological characterization of the cannabinoid CB1 receptor PET ligand ortholog, [H-3]MePPEP. *Eur. J. Pharmacol.* **2010**, *649*, 44−50.
(26) Terry, G.; Liow, J. S.; Chernet, E.; Zoghbi, S. S.; Phebus, L.; Felder, C. C.; Tauscher, J.; Schaus, J. M.; Pike, V. W.; Halldin, C.; Innis, R. B. Positron emission tomography imaging using an inverse agonist radioligand to assess cannabinoid CB(1) receptors in rodents. *Neuroimage* **2008**, *41*, 690−698.
(27) Terry, G. E.; Liow, J. S.; Chernet, E.; Zoghbi, S. S.; Phebus, L.; Lerner, A.; Felder, C. C.; Tauscher, J.; Schaus, J. M.; Pike, V. W.; Halldin, C.; Innis, R. B. Evaluation of an inverse agonist with high affinity for the CB1 receptor, [C-11]MePPEP, in rodents and humans. *Neuroimage* **2008**, *41*, T197−T197.

DOI: 10.1021/acs.jmedchem.5b00095
J. Med. Chem. 2015, 58, 3631−3636