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# PET Tracers for Serotonin Receptors and Their Applications

**J.S. Dileep Kumar**[1,2,*,#] and **J. John Mann**[1,3]

[1]Molecular Imaging and Neuropathology Division, New York State Psychiatric Institute, New York, USA

[2]Department of Psychiatry, Stony Brook University, New York, USA

[3]Department of Psychiatry, Columbia University Medical Center, New York, NY, USA

## Abstract

Serotonin receptors (5-HTRs) are implicated in the pathophysiology of a variety of neuropsychiatric and neurodegenerative disorders and are also targets for drug therapy. In the CNS, most of these receptors are expressed in high abundance in specific brain regions reflecting their role in brain functions. Quantifying binding to 5-HTRs *in vivo* may permit assessment of physiologic and pathologic conditions, and monitoring disease progression, evaluating treatment response, and for investigating new treatment modalities. Positron emission tomography (PET) molecular imaging has the sensitivity to quantify binding of 5-HTRs in CNS disorders and to measure drug occupancy as part of a process of new drug development. Although research on PET imaging of 5-HTRs have been performed more than two decades, the successful radiotracers so far



[*]Address correspondence to this author at the Division of Molecular Imaging and Neuropathology, New York State Psychiatric institute, 1051 Riverside Drive, Box: 42, New York, NY, 10032, USA; Tel: +1-646-774-7522; Fax: 212-543-1054; kumardi@nyspi.columbia.edu.

[#]**Author's Profile:** Dr. Kumar is a research scientist and director of PET tracer development in the Molecular Imaging and Neuropathology Division of New York State Psychiatric Institute. He is also a visiting research faculty at the department of Psychiatry, Stony Brook University, New York. His research interests are PET tracer development and applications, neuropsychopharmacology, and medicinal chemistry.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest related to the content of the review.

developed for human studies are limited to 5-$HT_{1A}$R, 5-$HT_{1B}$R, 5-$HT_{2A}$R, 5-$HT_4$R and 5-$HT_6$R. Herein we review the development and application of radioligands for PET imaging of 5-HTRs in living brain.

### Keywords

Molecular imaging; PET; 5-HTR; radioligands

## INTRODUCTION

Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter that is widely distributed in animals and plants [1–3]. Serotonin does not cross the blood brain barrier (BBB); the brain synthesizes all its serotonin from tryptophan (from diet) in two steps enzymatically with tryptophan hydroxylase (TPH) and tryptophan decarboxylase (TDC) respectively [4]. Serotonin is found in almost all brain regions and it is implicated in the pathophysiology of a variety of diseases, biological functions and biochemical pathways [1, 2, 5–8]. Serotonin neurons are confined to the brainstem and are located in the raphe nuclei. The neurons project to most of the brain including hippocampus, midbrain, prefrontal, parietal and occipital cortical regions, cingulate cortex and cerebellum, whereas, 5-HT neurons in caudal raphe nuclei project to cerebellum and spinal cord [9–11]. Serotonin effects are mediated through seven 5-HT receptor subtypes, 5-$HT_1$ to 5$HT_7$ (5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1D}$, 5-$HT_{1E}$, 5-$HT_{1F}$, 5-$HT_{2A}$, 5-$HT_{2B}$, 5-$HT_{2C}$, 5-$HT_3$, 5-$HT_4$, 5-$HT_{5A}$, 5-$HT_{5B}$, 5-$HT_6$, 5-$HT_{7A,}$ 5-$HT_{7B,}$5-$HT_{7D}$) and serotonin transporter (SERT) [1, 12]. Among these receptors, 5-$HT_3$R is a ligand gated ion channel receptor (LGICR), whereas, others are coupled with G-protein coupled receptors (GPCR) [1]. The rate of binding of 5-HT varies for 5-HTR subtypes, for example, 5$HT_{1A}$Rs ($K_i$ = 0.2–400 nM), 5-$HT_{1B}$R ($K_i$ = 1–40 nM), 5-$HT_7$R ($K_i$ = 0.2–8 nM) and 5-$HT_{2A}$R (Ki = <10 nM) have the highest affinity for 5-HT [13].

### Positron Emission Tomography (PET)

PET uses positron-radiolabeled molecules in very low mass amounts to image and measure the function of biological processes with minimum disturbance [14–17]. The primary biological mechanisms that can be studied by PET imaging are specific binding to protein structures (eg: enzymes, transporters and receptors), metabolism, and blood regulation. PET has been used for the quantification of neuroreceptor binding and release of neurotransmitters and measuring enzyme binding in disease pathology, early diagnosis, and evaluation of therapy effects. Furthermore, PET biodistribution studies are able to confirm that the drug candidate/lead ligand reaches the target site and does not accumulate in non-target sites of potential toxicity. The conventional components of PET molecular imaging include production of radioisotopes, radiotracer synthesis, PET scanning and data interpretation. Among the positron emitting isotopes, O-15 ($T_{1/2}$ = 2.037 minutes), N-13 ($T_{1/2}$ = 9.96 minutes), C-11 ($T_{1/2}$ = 20.34 minutes) and F-18 ($T_{1/2}$ = 109.77 minutes) are the common nuclei produced from cyclotron, reported for neuroimaging with PET.

There are several physiochemical criteria required for successful radioligand development for *in vivo* imaging [18]. First, the target to be imaged should have adequate density in brain

regions of interest and the degree of altered expression in the diseased state must be sufficient to be detectable. In general, PET tracers with a ratio of the target's concentration ($B_{max}$) to its affinity ($K_d$ or $K_i$) of at least 10 are expected to have a high probability of providing a reliably quantifiable specific signal *in vivo*. The radioligand should be safe to use without any adverse toxicology or pharmacology effects. The radioligand must have high affinity (in the nanomolar range) toward the imaging target and good selectivity (at least 20–100 fold, depending on the density of the non-target) over a wide spectrum of biogeneic amines, proteins, receptors, transporters and enzymes in brain. The imaging agent should have preferably low molecular weight (≤450), moderate lipophilicity (logD ~ 1–3) to facilitate BBB permeability [19, 20] and should not have binding affinity to ATP-binding cassette (ABC) transporter family efflux pump (eg: P-glycoprotein (P-gp), multi-drug resistance-associated protein (MDR1a/1b) and breast cancer resistance protein (BCRP) and soluble carrier (SLC) family influx transporters [18]. The chemical structure of the ligand should allow for a rapid radiolabeling that does not alter the pharmacological properties of the molecule and generates adequate specific activity essential to avoid self occupancy (<5%) to the target with unlabeled ligand [21, 22]. A successful radiotracer should have fast clearance from blood, rapid localization to target and fast washout from non-target tissues. The metabolite should be preferably polar, measurable or unlabeled, and should not interfere with the radioligand's binding kinetics [23]. It is proven that brain uptake of the radiotracer is in direct proportion to the free fraction ($f_p$) of the radioligand. Thus the radioligands should have measurable $f_p$ in plasma to allow marginal uptake and reliable measurements of the binding parameters. In general, radioligands with lower lipophilicity have less plasma protein binding and this allows the available $f_p$ in blood to diffuse through cell membranes and impacts binding. Furthermore, the plasma clearance rate of radioligand should not be too rapid, because this can affect the accurate determination of the input curve at later time points. The radioligand binding must equilibrate within the imaging time frame and or 3–5 half-lives of the radiotracer [24]. This will also allow reduced radiation exposure to subject. The radioligand should be amenable for quantification with tracer kinetic modeling to obtain binding parameters such as rate constants, volume of distribution ($V_T$), binding potential ($BP_F$ or $BP_P$) or specific to nonspecific equilibrium partition coefficient ($BP_{ND}$). An input function is used for kinetic modeling but some methods and outcome measures use data from a reference region instead, or estimate the input function using data from many brain regions. Estimation of binding can be achieved by compartmental, equilibrium or graphical approaches.

To permit measurement of specific binding, the radioligand should have higher target to nontarget ratio (>1.5 and preferably >3–4). The most important parameters in PET tracer development are tracer affinity, selectivity, lipophilicity and metabolism. There are several challenges associated with tracer development. These include limited synthesis time in order to incorporate radionuclei because of the short half life of some PET isotopes like $^{11}C$ or $^{18}F$, the requirement for preparation of a high specific active product in order to keep the injected mass low, metabolite analyses to generate a metabolite-corrected input function, $f_p$ measurement, rapid transit across physiological barriers such as BBB, BCRP and cellular uptake to deliver the PET probe to target. Tissue kinetics amenable to mathematical modeling to give quantitative indices are still a challenge for new tracers. The ultimate

challenge is to demonstrate whether the tracer distribution is sensitive enough to answer clinical questions relevant to diagnosis, prognosis or target occupancy by medication. In this review, we focus on the radioligands and their applications that are reported mainly in human subjects for PET imaging of the 5-HTRs, based on findings published through June 2014.

**5-HT$_1$R—**5-HT$_1$Rs have five receptor subtypes with 40–60% sequence homology and these receptors bind 5-HT resulting in activation of the Gi/o class of GPCR and inhibition of adenylate cyclase (AC) resulting in a decrease in cyclic adenosine monophosphate (cAMP) levels [2]. 5-HT$_1$R is an important class of receptors that modulate the communication and functions of 5-HT in brain and are implicated in the pathophysiology of a variety of neurogenenerative and neuropsychiatric disorders. These receptors are expressed in specific brain regions and cortical layers except for 5-HT$_{1F}$R subtype. 5-HT$_{1A}$R is the most studied receptor subtype of 5-HT$_1$R that plays a major role in transmission and regulation of 5-HT neuron firing in brain [2].

**5-HT$_{1A}$R—**Much is published about the pharmacology and pathophysiology of 5-HT$_{1A}$R receptors in brain functions [2–4, 25]. In brainstem raphe nuclei, these receptors are somatodendritic autoreceptors, while in other brain regions they are postsynaptic or heteroreceptors on nerve terminals [25, 26]. 5-HT$_{1A}$Rs have been implicated in the pathophysiology of mood and anxiety disorders, suicidal behavior, sexual functions, eating and panic disorders, epilepsy, schizophrenia, Parkinson's disease (PD) and Alzheimer's disease (AD). 5HT$_{1A}$R are involved in the mechanism of action of antidepressants and more recently they have been suggested to mediate trophic and neuroprotecting effects [2, 27–30]. 5-HT$_{1A}$R agonists and partial agonists have been evaluated as antidepressants, anxiolytics and antipsychotic drugs (APDs) with fewer side effects (31). *In vitro* and *in vivo* quantification studies of 5-HT$_{1A}$R reveal high receptor density in hippocampus (800–3,000 fmol/mg/protein) and in the entire cortical mantle where according to postmortem studies the receptors are densest in layer II (300–1900 fmol/mg/protein) [32, 34]. Lower 5-HT$_{1A}$R binding levels are found in thalamus and the lowest density is observed in adult striatum, substantia nigra and cerebellum [32, 34].

Development and evaluation of 5-HT$_{1A}$R PET tracers are documented extensively in literature [35–42]. Among these [*carbonyl*-$^{11}$C]WAY100635 ($K_i$ = 2.2 nM, Fig. 3) is the most extensively studied antagonist radiotracer in human subjects. The parent [*O-methyl*[$^{11}$C]WAY100635 has a BBB penetrating metabolite [$^{11}$C]WAY100634, hence it is unsuitable for the quantification of 5-HT$_{1A}$R [43]. Altered [*carbonyl*-$^{11}$C]WAY100635 binding are reported in a variety of psychiatric disorders in comparison to control subjects [35–42]. The advantage of [*carbonyl*-$^{11}$C]WAY100635 compared with other 5-HT$_{1A}$R PET tracers is mainly due to its higher target to nontarget ratio. However, low free fraction, rapid metabolism, complexity in radiosynthesis and low yield make this radiotracer not suitable for routine studies. Furthermore, receptor selectivity assays show that WAY100635 is not a selective 5-HT$_{1A}$R ligand and it has potential binding to α-1AR ($K_i$ = 16.4 nM, 7.45 times higher than 5-HT$_{1A}$R) and D$_4$R ($K_i$ =19.9 nM, 9 times higher than 5-HT$_{1A}$R) [44]. Literature indicates that D$_4$Rs are less abundant in brain and hence D$_4$R binding on PET

imaging with [*carbonyl*-$^{11}$C]WAY100635 is not significant with a $K_i$ of 19.9 nM [45]. However, the density of α-1AR in brain is sufficient to be detected by PET imaging, and hence there may be binding that may confuse estimation of 5-$HT_{1A}$R binding in brain regions with higher density of α-1AR using [*carbonyl*-$^{11}$C]WAY100635 [46]. The *in vivo* cross selectivity of [*carbonyl*-$^{11}$C] WAY100635 and structurally related radioligands with α-1AR is not studied or reported yet.

Several other radioligands have been reported that are analogues of WAY100635 [35]. [*carbonyl*-$^{11}$C]DWAY (desmethyl-WAY100635) (5-$HT_{1A}$R $K_i$ = 1.4 nM, Fig. 3) is a minor metabolite of WAY100635 and it provided higher signal to target ratio in various species including human in comparison to [*carbonyl*-$^{11}$C]WAY100635 [47, 48]. In contrast with WAY100635, DWAY shows less binding to α-1AR ($K_i$ = 364 nM, Kumar JSD, Mann JJ, unpublished data). [$^{11}$C](*R*)-RWAY is a reverse amide of WAY100635 (5-$HT_{1A}$R $K_i$ = 0.6 nM, Fig. 3), developed to improve the stability of WAY100635. (*R*)-RWAY possesses significant affinity to 5-$HT_{2B}$R (Ki = 7.2 nM), α-1AR ($K_i$ =10.35 nM), $D_2$R ($K_i$ = 34.5 nM), $D_3$R ($K_i$ =5.1 nM), and $D_4$R ($K_i$ =15.6 nM). In comparison to [*carbonyl*-$^{11}$C]WAY100635 or [*carbonyl*-$^{11}$C]DWAY, [$^{11}$C](*R*)-RWAY has several radiosynthesis advantages and it showed promising *in vivo* binding in rodents and non human primates [49,50]. However, the presence of radioactive metabolites in brain and its P-gp binding make this ligand problematic for studies in human subjects [51]. [$^{18}$F](*cis*)-FCWAY is a fluoro-analogue of WAY100635 (5-$HT_{1A}$R $K_i$ = 0.52 nM, Fig. 3) and it has been successfully used to study human disease with PET [52], however, *in vivo* de[$^{18}$F]fluorination is the major drawback of this tracer and makes it useful only for quantifying brain structures that are not adjacent to skull. [$^{18}$F]MPPF (5-$HT_{1A}$R $K_i$ = 3.3 nM, Fig. 3) is a fluorophenyl analogue of WAY100635, synthesized by the nucleophilic displacement of the corresponding nitro precursor with [$^{18}$F]fluoride [53]. Although in rodents and cats [$^{18}$F]MPPF was found to be sensitive enough to measure large changes of intra-synaptic 5-HT levels *in vivo* [35–37, 54], studies in awake monkeys and human subjects did not show intra synaptic changes of 5-HT with this radiotracer. [$^{18}$F]MPPF has been used to image 5-$HT_{1A}$R alteration in a variety of neuropsychatric diseases and it is the only 5-$HT_{1A}$R PET radioligand tested so far for AD imaging *in vivo* [55]. Despite the above advantages, [$^{18}$F]MPPF is a P-gp substrate and this limits the further clinical utility of this radiotracer [35]. [$^{18}$F]MeFWAY is a fluoromethyl analogue of WAY100635 and of the two isoforms, the trans isomer shows higher binding to 5-$HT_{1A}$R which is comparable to WAY100635 *in vivo*. More recently [$^{18}$F](*trans*)-MeFWAY has been successfully evaluated in human subjects with no *in vivo* de[$^{18}$F]fluorination [58]. However, kinetic analyses with arterial input functions have to be performed for the full quantification of this radiotracer. None of these radiotracers were successful for occupancy measurements of 5-$HT_{1A}$R drugs even at dose levels higher than that is used in clinics. [*carbonyl*-$^{11}$C]WAY100635 and [*carbonyl*-$^{11}$C]DWAY are synthesized by reacting [$^{11}$C]cycloalkyl magnesium chloride with the corresponding amines. [$^{18}$F](*cis*)-FCWAY, [$^{18}$F]MPPF and [$^{18}$F](*trans*)-MeFWAY are synthesized *via* the nucleophilic displacement of their corresponding nitro or tosyl precursors with [$^{18}$F]fluoride. [$^{11}$C](*R*)-RWAY is synthesized by reacting the desmethyl precursor with [$^{11}$C]$CH_3$I or [$^{11}$C]MeOTf [36, 37].

5-$HT_{1A}$R exists in high and low agonist affinity states. The antagonist ligands bind to the high affinity (HA) and low affinity (LA) conformations of 5-$HT_{1A}$R with similar affinity. Whereas, agonist ligands bind preferentially to the HA state of the receptor, which is coupled to G-protein and therefore agonist binding provides a more meaningful functional measure of the 5-$HT_{1A}$R that can reflect desensitization and supersensitivity [59, 60]. Although antagonist 5-$HT_{1A}$R PET tracers can measure the total receptor binding, they cannot detect changes in the high affinity 5-$HT_{1A}$R binding in disease states or in the context of treatment functionally larger and earlier effects such those of antidepressants that desensitize autoreceptors before they down-regulate total binding [35–40]. Antagonist binding is insensitive to the changes in the intra-synaptic 5-HT concentration and antagonist PET tracers are less sensitive for measuring agonist receptor occupancy in clinical studies to guide new drug development in dose-finding.

The development of 5-$HT_{1A}$R agonist PET tracers for the past 2 decades has met with limited success [35]. Most PET imaging studies have been carried out with amino-tetralin, apomorphine, thiochromine and arylpiperazine based 5-$HT_{1A}$R agonist ligands. Some of these ligands show promising characteristics *in vitro* but failed *in vivo* due to a lack of detectable specific binding. [$^{11}$C]MPT, an arylpiperazine derivative of 3,5-dioxo-(2*H*, 4*H*)-1,2,4-triazine is the first successful agonist PET tracer reported for 5-$HT_{1A}$R ($K_i$ = 1.35 nM, $E_{max}$ = 95%, $EC_{50}$ = 0.05 nM, Fig. 4) in non human primates [61]. The radiotracer binding in baboon brain was in excellent agreement with the known distribution of 5-$HT_{1A}$R. The $V_T$ of [$^{11}$C]MPT showed strong correlation with the antagonist tracer [*carbonyl*-$^{11}$C]WAY100635. Of note, $V_T$ of [*carbonyl*-$^{11}$C]WAY100635 was higher compared with [$^{11}$C]MPT, presumably because [*carbonyl*-$^{11}$C]WAY100635, an antagonist, binds to both HA and LA states of 5-$HT_{1A}$R. Despite the excellent binding profile of [$^{11}$C]MPT, the slow washout in baboons and unreliable measurement of free fraction, limits this radiotracer from advancing to human studies.

Several ligands based on 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine were subsequently reported as 5-$HT_{1A}$R agonist PET tracers [62, 63]. Among these [$^{11}$C]CUMI-101 or [$^{11}$C]MMP is the most successful and only radiotracer tested in nonhuman primates and human subjects [64, 65]. [$^{11}$C]CUMI-101 is a partial agonist ($K_i$ = 0.15 nM, $E_{max}$ = 80%, $EC_{50}$ = 0.1 nM, Fig. 4) to 5-$HT_{1A}$R and it is insensitive to the fluctuations in physiological intra-synaptic concentration of 5-HT and can detect robust pharmacologically induced increases in intrasynaptic 5-HT in baboons and human subjects [66, 67]. However, one study reports that [$^{11}$C]CUMI-101 did not show significant difference in endogenous 5$HT_{1A}$R changes in human [68]. The *in vivo* binding ratios of [$^{11}$C]CUMI-101 are ~55% less across brain regions in comparison to [*carbonyl*-$^{11}$C]WAY100635 and this is in agreement with the previously reported *in vitro* data of agonist and antagonist binding ratios of 5-$HT_{1A}$R [69]. Recently, it is reported that [$^{11}$C]CUMI-101 did not behave as a 5-$HT_{1A}$R agonist in brain homogenate based assays [70]. The above discrepancy can be partly attributed to the assay conditions because general GTPγS assays have a modest signal/noise ratio in tissue samples and dependent on the concentration of GDP. In another report, [$^{11}$C]CUMI-101 binding in thalamus and cerebellum in rats and monkeys is partially displaced with the α-1AR ligand prazosin indicating or supporting a cross selectivity of CUMI-101 to α-1AR [71]. However,

autoradiography experiments, a much sensitive tool than PET, with [$^{3}$H]CUMI-101 indicates no significant α1-AR binding in either baboon or human brain and the relative regional brain [$^{3}$H]CUMI-101 binding is comparable with the known distribution of 5-$HT_{1A}$Rs as defined by [$^{3}$H]WAY100635 and [$^{3}$H]8-OH-DPAT [72]. More recently [$^{18}$F]FECUMI-101, a fluoroethyl analogue of CUMI-101 is reported as a partial agonist radiotracer in nonhuman primates ($K_i$ = 0.1 nM, $E_{max}$ = 77%, $EC_{50}$ = 0.85 nM, Fig. 4) [73]. In addition to 5-$HT_{1A}$R enriched regions, [$^{18}$F]FECUMI-101 also shows binding in thalamus, which is displaceable with WAY100635. Further investigations of [$^{18}$F]FECUMI-101 are required to confirm it as a 5-$HT_{1A}$R partial agonist PET tracer [73]. [$^{18}$F]15599 and its aminomethyl analogue [$^{18}$F]13714 are recently reported as 5-$HT_{1A}$R agonist tracers with nonoptimal target to nontarget ratios [74, 75]. The high affinity ligands [$^{11}$C]S14506 and [$^{18}$F]S14506 are also not successful *in vivo* [76]. All the above radioligands are synthesized *via* the C-11 methylation of their corresponding phenolates using either [$^{11}$C]$CH_3$I or [$^{11}$C]$CH_3$OTf or nucleophilic displacement of aromatic nitro precursors with [$^{18}$F]$F^-$ or [$^{18}$F]fluoroethylation of the precursor phenolate.

**5-$HT_{1B}$R—**The existence of 5-$HT_{1B}$R was a controversy earlier and it was believed that it was a species homologue of 5-$HT_{1D}$R [2–5]. However, sequence studies and cloning of receptor confirmed that it is not an analogue of 5-$HT_{1D}$R. In CNS, 5-$HT_{1B}$R serves as a presynaptic heteroreceptor on nonserotonergic neurons and serves as an autoreceptor for serotonergic neurons in raphe nucleus. *In vitro* autoradiography studies show the presence of this receptor at higher levels in the basal ganglia (400–500 fmol/mg/tissue), especially in globus pallidus and substantia nigra followed by superior colliculus, enteropeduncular nuclei, and periaqueductal gray [77]. Lower levels of receptor density were detected in the cerebral cortex, hypothalamus, amygdala, cerebellum and dorsal horn of the spinal cord. 5-$HT_{1B}$R present in cerebral arteries mediates a major role in 5-HT-induced vasoconstriction in human cerebral arteries (HCA) [2–5]. Pharmacological studies suggest that 5-$HT_{1B}$R is involved as a regulator for aggression, memory, learning, substance abuse and premature ejaculation. 5-$HT_{1B}$R and 5-$HT_{1D}$R are autoreceptors and agonists shut off serotonin release from nerve terminals and this effect may explain their antimigraine properties in man. However, carotid vasoconstriction is an adverse side effect of 5-$HT_{1B}$R agonists during migraine therapy.

Several high affinity, selective carboxamide analogues of different core chemical structures have been radiolabeled and characterized *in vivo* with PET [36, 37, 41]. [$^{11}$C]5-methyl-8-(4-methyl-piperazin-1-yl)-4-oxo-4H-chromene-2-carboxylic acid (4-morpholin-4-yl-phenyl)-amide ([$^{11}$C]AZ10419369) is a PET ligand for 5-$HT_{1B}$Rs ($K_i$ = 0.8 nM, Fig. 5) [78]. [$^{11}$C]AZ10419369 PET obtained by the reaction of desmethyl-AZ10419369 with [$^{11}$C]$CH_3$OTf. PET studies in macaques and human subjects show high tracer uptake (3–4%) in brain. The highest uptake was found in occipital cortex and basal ganglia followed by temporal and frontal cortical regions, less in thalamus and the lowest in cerebellum [79]. Except for palliduum, all other reported brain regions reached equilibrium within the imaging time and 1-TC model with cerebellum as reference region was the optimal modeling method for [$^{11}$C]AZ10419369 in human subjects [79, 80]. [$^{11}$C]AZ10419369 shows dose-dependent binding of AZD3783, a 5-$HT_{1B}$R antagonist with potential

antidepressant properties in non-human primates and human subjects indicating its potential for receptor occupancy measurement for drug development and dose-finding in clinical studies [80]. Unlike antagonist radiotracers for 5-$HT_{1A}$Rs, [$^{11}$C]AZ10419369 was sensitive to fenfluramine-induced increase in synaptic 5-HT. PET studies in rhesus monkeys show 27% and 50% decrease in binding of [$^{11}$C]AZ10419369 binding after the administration of 1mg/kg and 5 mg/kg fenfluramine respectively [81]. [$^{11}$C]AZ10419369 PET imaging studies with citalopram shows decreased $BP_{ND}$ in the treated monkeys in comparison to controls with highest change (−25%) in raphe nucleus [82]. Human PET studies with clinically relevant citalopram doses show 10% decrease in $BP_{ND}$ in raphe nucleus and slight increase in binding (5%) in other regions [82]. Furthermore, pilot studies in human subjects found no correlation between 5-HT or its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the CSF with [$^{11}$C]AZ10419369 binding in brain. This indicates that [$^{11}$C]AZ10419369, and perhaps intrasynaptic 5-HT levels, may not correlate with serotonin and 5-HIAA levels in CSF [83]. More recently zolmitriptan, a selective 5-$HT_{1B/1D}$R agonist has been tested in human with [$^{11}$C]AZ10419369 and no significant occupancy was detected with a 5 mg dose, whereas 4–5% occupancy was found for 10 mg dose [84]. An agonist 5-$HT_{1B}$R radiotracer may be more sensitive for occupancy measurement of agonist drugs with PET. [$^{11}$C]AZ10419369 is also tested in PD and lower binding was observed in orbitofrontal cortex in comparison with control subjects [85]. [$^{11}$C](*R*)-1-[4-(2-methoxy-isopropyl)-phenyl]-3-[2-(4-methylpiperazin-1-yl)benzyl]-pyrrolidin-2-one) ([$^{11}$C]P943) ($K_i$ = 1.2 nM, Fig. 5) is also reported as a selective PET tracer for 5-$HT_{1B}$R in nonhuman primates and human subjects [86, 87]. Radiosynthesis of [$^{11}$C]P943 has been achieved by the reaction of [$^{11}$C]$CH_3$I with desmethyl-[$^{11}$C]P943. Analogues to [$^{11}$C]AZ10419369, such as [$^{11}$C]P943, are sensitive to changes of intra-synaptic 5-HT induced by fenfluramine in monkeys [88]. [$^{11}$C]P943 has been tested in depression and posttraumatic stress disorder (PTSD) subjects and lower binding was found in ventral palladium and ventral striatum in comparison with control subjects [89, 90]. Greater binding of [$^{11}$C]P943 was found in subjects with alcohol dependence and pathological gambling [90]. Whereas, lower binding of [$^{11}$C]P943 uptake was found in cocaine dependent subjects in comparison with healthy controls [91].

**5-HT1$_{D,E,F}$Rs—**The 5-$HT_{1D}$R is a heteroreceptor and a serotonin nerve terminal autoreceptor, predominantly expressed in basal ganglia and substantia nigra [2–5]. 5-$HT_{1D}$R agonists are used for migraine treatment except PNU-142633, a specific 5-$HT_{1D}$R agonist that proved ineffective for the treatment of migraine [184, 185]. The 5-$HT_{1E}$R has ~ 60% homology with 5$HT_{1B}$R. It is concentrated in caudate putamen and hippocampus with lower levels in amygdala, frontal cortex and globus pallidus. The 5-$HT_{1E}$R is species dependent and is not expressed in rats or mice [2]. There are no selective or high affinity ligands available for this receptor, which limits the elucidation of the function of 5-$HT_{E}$R. Serotonin 5-$HT_{1F}$Rs, previously known as 5-$HT_{1E}$ receptors (70% sequence homology with 5-$HT_{1E}$) and is located primarily in hippocampus, cortex and dorsal raphe nucleus. The physiological role of 5-$HT_{1F}$R is currently unknown except its antimigraine properties along with 5-$HT_{1B}$R and 5-$HT_{1D}$R affinities. Although selective ligands are available, there is no PET tracers available for 5-$HT1_{D,E,F}$ receptors.

**5-$HT_2$Rs—**5-$HT_2$Rs are coupled to Gq/11 class of G proteins that stimulate phospholipase C (PLC) producing inositol triphosphate (IP3) and intracellular $Ca^{2+}$ release [2–5]. 5-$HT_2$Rs are comprised of 3 subtypes namely 5-$HT_{2A}$R, 5-$HT_{2B}$R and 5-$HT_{2C}$R that have 70–80% sequence homology [2–5]. Among these 5-$HT_{2A}$R is the most predominant subtype and are expressed in central and peripheral tissues [2–5]. In CNS these receptors are principally located in cortex ($B_{max}$ about 500 fmol/mg/protein in neocortex), claustrum and basal ganglia [92, 93]. 5-$HT_{2A}$Rs can be in high affinity agonist state but predominantly exist in a low affinity state under normal physiological conditions [2, 94]. Alteration of 5-$HT_{2A}$R binding is reported in schizophrenia, suicide, stress, PTSD and major depression and this receptor is a target for atypical or new generation antipsychotic drugs [2, 3, 95, 96]. In addition, 5-$HT_{2A}$Rs are also implicated in learning, appetite control, glaucoma, cardiovascular functions and muscle contractions [2, 3]. Preclinical studies show that 5-$HT_{2A}$R antagonists have antipsychotic, antidepressant and antianxiety properties, whereas agonist ligands possess hallucinogenic properties. Although there are several PET tracers developed to date, their utility for imaging 5-$HT_{2A}$R *in vivo* is limited due to high nonspecific binding, tracer kinetics marked by slow washout and inadequate pharmacology [36–39, 97]. [$^{11}$C]Ketanserin ($K_i$ = 2.3 nM, Fig. 6) is the first 5-$HT_{2A}$R PET tracer studied in man, despite its affinity for histamine 1R ($H_1$R) (Ki = 2 nM) and α-1AR ($K_i$ = 40 nM) [98]. [$^{11}$C]Ketanserin is synthesized *via* the reaction of [$^{11}$C]$COCl_2$ with the corresponding aminobenzamide precursor [181]. Due to the low target to nontarget ratio and rapid metabolism [$^{11}$C]ketanserin did not advance for further development [99]. Subsequently, [$^{18}$F]FEK, a fluoroethyl analogue of ketanserin has been developed and it showed better *in vivo* characteristics in nonhuman primates [100]. [$^{18}$F]Setoperone and [$^{11}$C]NMSP are less selective ligands, whereas, [$^{18}$F]altanserin and [$^{11}$C](*R*)M100907, have high selectivity for 5-$HT_{2A}$R [36–39]. [$^{18}$F]setoperone shows significant affinity for $D_{2/3}$Rs ($K_i$ = 24 nM) in addition to 5-$HT_{2A}$R ($K_i$ = 2.3 nM, Fig. 6). Both [$^{18}$F]altanserin and [$^{18}$F]setoperone are synthesized *via* the nucleophilic displacement reaction using [$^{18}$F]$F^-$ with the corresponding nitro precursors. [$^{18}$F]setoperone *in vivo* imaging is reported in a variety of conditions such as mood disorders, schizophrenia, AD, migraine, stroke, and depression and also explored in the estimation of 5-$HT_{2A}$R occupancy by antipsychotics [36–39]. In a recent study with [$^{18}$F]setoperone, lower cortical 5-$HT_{2A}$R binding was found in adult autism spectrum disorder (ASD) [101]. However, less thalamic binding and no significant difference were found in other brain regions with [$^{18}$F]setoperone in high functioning adult autistic patients [101]. [$^{11}$C]NMSP ([$^{11}$C]MSP), a methyl analogue of spiperone, is a highly selective 5-$HT_{2A}$R ($IC_{50}$ = 1.3 nM, Fig. 6), $D_{2/3}$R ($IC_{50}$ = 0.23 nM) ligand with low affinity for α–1AR ($IC_{50}$ = 10.1 nM) [186]. It is synthesized by the [$^{11}$C]methylation of the corresponding desmethyl precursor. Similar to [$^{18}$F]setoperone, [$^{11}$C]NMSP is a dual tracer and has been studied for $D_{2/3}$R and 5-$HT_{2A}$R measurements in normal and pathological conditions and for occupancy measurements of antipsychotic drugs including inverse agonists of 5-$HT_{2A}$R [36–39]. [$^{11}$C]NMSP shows age-related decline of dopamine $D_2$R and 5-$HT_{2A}$R in human subjects, higher striatal binding in patients with PD and schizophrenic patients in comparison to controls. Lower [$^{11}$C]NMSP binding was found in putamen of Huntington patients. [$^{11}$C]NMSP is also used to evaluate the response of bromocriptine, $D_2$ agonist in pituitary tumors [101]. [$^{18}$F]Altanserin has acceptable pharmacological specificity for 5-$HT_2$Rs ($K_i$ = 0.13 nM, Fig. 6) but quantitative *in vivo* imaging of 5-$HT_2$Rs with

[$^{18}$F]altanserin has been hindered by lipophilic radiometabolites that cross the BBB and slow kinetics [36–39]. Better quantification of [$^{18}$F]altanserin binding can be achieved using a bolus plus constant infusion protocol to overcome slow equilibrium and a multicompartmental model to account for metabolites. [$^{18}$F]altanserin has been studied in several neuropsychiatric and neurodegenerative disorders. Notable findings are lower [$^{18}$F]altanserin binding in AD (neocortical regions), medication free depressed patients (right posterolateral orbitofrontal and anterior insular cortices), schizophrenia (frontal cortex), higher binding after sleep deprivation and in obsessive compulsive disorder [36–39]. The replacement of hydrogen with deuterium in [$^{18}$F]altanserin reduces the rate of undesirable radiometabolite formation. [$^{18}$F]deuteroaltanserin shows better characteristics and 26% higher brain uptake than [$^{18}$F]altanserin itself in nonhuman primates and human [102, 103]. Although higher binding in estrogen replacement and lower binding in cortical regions of AD are reported using [$^{18}$F]deuteroaltanserin, not many studies are available at present to thoroughly assess its potential [104].

[$^{11}$C](*R*)M100907 *aka* [$^{11}$C](*R*)MDL100907 is the highly selective and widely studied 5-HT$_{2A}$R ($K_i$ = 0.36 nM, Fig. 6) ligand in rodents, nonhuman primates and human [36–39]. Although [$^{11}$C]M100907 shows promising characteristics, slow off-rate kinetics makes the tracer quantification difficult unless it can be labeled with a longer half life isotope. [$^{11}$C]M100907 has been studied in few pathological conditions and greater binding reported in medication free depressed subjects and lower binding in drug naïve OCD patients [105, 106]. Receptor occupancy was found in M100907-medicated schizophrenia patients using [$^{11}$C]M100907 [107]. Several efforts have been made to develop [$^{18}$F]M100907 to permit longer duration of scanning. Muhlhausen et al reported racemic [$^{18}$F]M100907 by the coupling of (2,3-dimethoxyphenyl)(piperidin-4-yl)methanol with [$^{18}$F]fluoroethyl bromide [108]. More recently, Hooker et al reported an elegant nickel mediated radiofluoronation to synthesize [$^{18}$F]M100907 and compared its binding with [$^{11}$C]M100907 [109]. Two groups reported [$^{18}$F]fluoroethyl-M100907 ([$^{18}$F]FEM100907 aka [$^{18}$F]MH.MZ), a fluoroethyl analogue of M100907 with faster off-rate than [$^{11}$C]M100907 [110,111]. [$^{18}$F]MH.MZ has higher cortex to cerebellum ratio in nonhuman primates and pigs [112]. Further studies are required to establish the utility of [$^{18}$F]MH.MZ in human studies.

There are several reports regarding 5-HT$_{2A}$R agonist radiotracers [113–117]. An agonist ligand may be sensitive to intra-synaptic 5-HT and allow estimation of 5HT release, and measures the GPCRs and thereby provides a more meaningful functional measure of 5-HT$_{2A}$R binding. [$^{11}$C]CIMBI-5 ([$^{11}$C]IDMe aka [$^{11}$C]NBMeO) ($K_i$ = 0.15 nM, $E_{max}$ = 91%, Fig. 6) was the first 5-HT$_{2A}$R agonist tracer studied in pigs and nonhuman primates *in vivo* [113,114, 183]. In general, a markedly lower $BP_p$ is observed for [$^{11}$C]CIMBI-5 in comparison with [$^{11}$C]M100907 in baboon brain [114]. [$^{11}$C]CIMBI-5 BPp values are an average 25% of total binding of [$^{11}$C]M100907 in the brain regions examined [114]. This finding is consistent with the high affinity site binding ratio of [$^{125}$I]DOI (agonist) with [$^{3}$H]ketanserin and [$^{3}$H]M100907 (antagonists) measured by *in vitro* autoradiography and *in vitro* saturation binding studies [118, 119]. More recently [$^{11}$C]CIMBI-36, a bromo-analogue of CIMBI-5 has been reported to have higher target to non target ratio than [$^{11}$C]CIMBI-5 in pig and nonhuman primates [116]. CIMBI-36 has affinity for 5-HT$_{2B}$R ($K_i$

= 0.5 nM) and 5-$HT_{2C}$R ($K_i$ = 1.5 nM) in addition to its 5-$HT_{2A}$R affinity ($K_i$ = 0.5 nM, $E_{max}$ = 87%, Fig. 6) and it is the only agonist PET ligand tested so far in human [117]. The specificity of [$^{11}$]CIMBI-36 binding in human has been confirmed by the reduced tracer binding in challenge studies predosed with ketanserin. Radiosynthesis and *in vivo* evaluation of [$^{11}$C]AC-90179 ($K_i$ = 2.1 nM), an inverse agonist of 5-$HT_{2A}$R, is reported [120], and PET studies in baboon showed that the tracer penetrates the BBB, however no specific binding was observed.

**5-$HT_{2B}$R—**Outside the brain, 5-$HT_{2B}$Rs are involved in muscle contractions and are located in cardiovascular tissues and intestines [2–5]. These receptors are less abundant in CNS and some evidence suggests that 5-$HT_{2B}$Rs are involved in anxiety, cognition, food intake and neuroendocrine regulation in rodents. There are not many selective ligands known for 5-$HT_{2B}$R, and hence no PET ligands are currently available for this receptor subtype.

**5-$HT_{2C}$R—**5$HT_{2C}$R is expressed in the choroid plexus; $B_{max}$ 6.76 pmol/mg/protein in human, 688 fmol/mg protein in pig and 130 pmol/mg protein in rat; and spinal cord [121–123]. The major function of 5-$HT_{2C}$Rs in choroid plexus is to regulate ion exchange between the brain and the cerebrospinal fluid [2–5]. Low levels of 5-$HT_{2C}$R are expressed in basal ganglia, hippocampus, cortex and amygdala. These receptors may have clinical value in the treatment for depression, panic anxiety, OCD, bulimia, and obesity. Except for choroid plexus, the abundance of 5-$HT_{2C}$R are low in other brain regions, make this receptor less viable as an imaging target with PET. There are several attempts recently made for the development of 5-$HT_{2C}$R PET tracers with limited success. These include a low affinity azetidine analogue of pyrimidoazepine, a 5-$HT_{2C}$ agonist ($K_i$ = 75 nM) and antagonists WAY-163909 ($K_i$ = 10 nM) and vabicaserin ($K_i$ = 3 nM) [124, 125]. The radiosynthesis of [$^{11}$C]pyrimidoazepine has been achieved via [$^{11}$C]methylation using [$^{11}$C]$CH_3I$, whereas the antagonist ligands were synthesized *via* a novel C-11 Pictet-Spengler cyclization with [$^{11}$C]$CH_2O$ ([124, 125]. Although these radiotracers penetrate the BBB, no specific binding was found *in vivo* in baboon.

**5-$HT_3$R—**Unlike all the 5-HTRs, the 5-$HT_3$R belongs the class of LGICRs with a close resemblance to nicotinic acetylcholine receptor (nAChR) [2–5]. There are five subtypes of 5-$HT_3$Rs, which allow the permeability of mono ($Na^+$, $K^+$), divalent ($Ca^{2+}$), ammonium and choline cations. The major functions of these receptors are to rapidly activating and desensitizing inward currents for synaptic transmission. 5-$HT_3$Rs are expressed in central and periphery tissues [2–5]. Studies with [$^3$H]GR65630, a selective 5-$HT_3$R ligand in postmortem human brain revealed specific binding, however, the concentration of these receptors are lower in comparison with other 5-HTRs [126]. Highest expressions of 5-$HT_3$Rs were found in some areas of brain stem ($B_{max}$ = 13.1 fmol/mg/protein), striatum ($B_{max}$ = 4.8 fmol/mg/protein) and low level expression in amygdala, hippocampus, and various cortical regions [126]. 5-$HT_3$Rs have also been reported in dorsal horn, dorsal root ganglion (DRG) and vagal terminations in the digestive tract [188]. Several radioligands have been studied in various species for the quantification of 5-$HT_3$Rs, and majority of the findings show lower $B_{max}$ in brain [127–134]. The distribution and pharmacology of 5-

$HT_3R$ in brain is species dependent [134, 188]. 5-$HT_3R$ ligands have been used in antiemetic therapy for patients undergoing chemo and radiation therapy, and irritable bowel syndrome (IBS) [2–5, 188]. Preclinical data suggests that 5-$HT_3R$ could be potential drug target for mood disorder, cognitive impairment, substance abuse, addiction, bulimia, pruritis and autism [2–5, 188]. Several PET radioligands with diverse class has been reported for 5-$HT_3Rs$, however *in vivo* studies found these ligands are not successful in rodents and primates due to nonspecific binding and poor brain uptake [36,135–140]. The failure of the radioligands for *in vivo* imaging may also contribute to the low abundant of 5-$HT_3Rs$ in brain. Most of the radioligands radioligands reported for 5-$HT_3Rs$ (Fig. 8) were synthesized *via* [$^{11}$C]methylation of the corresponding precursor amine molecules. [$^{11}$C]S21007 is synthesized by the reaction of [$^{11}$C]benzyl iodide with N-nor-S21007 precursor [182].

**5-$HT_4R$—**5-$HT_4Rs$ are coupled to Gs form of GPCR and promote cAMP formation. 5-$HT_4Rs$ have 9 splice variants and are expressed in GI tract, urinary bladder, heart, adrenal gland and centrally located predominantly in striatum ($B_{max}$= 223 fmol/mg/protein), neocortex, thalamus, hippocampus and brainstem [2–5, 141, 142]. In periphery, 5-$HT_4Rs$ are involved in the GI and cardiac functions [2–5]. Centrally, these receptors are postulated in the cognitive, memory, depression, ADHD, anorexia and obesity [2–5]. The receptor subtypes of 5-$HT_4Rs$ have different degree of internalizations, hence, nonselective ligands for this receptors may cause multiple pathological effects. Several agonist and antagonist ligands are known for this receptor, however splice variant specific ligands are limited [36–38]. Regarding PET imaging, [$^{11}$C]SB207145 is the only antagonist radiotracer tested so far in human for 5-$HT_4R$ [143–146]. [$^{11}$C]SB207145 belongs to the benzodioxine class of compounds with high affinity for 5-$HT_4R$ ($K_i$ = 0.3 nM, Fig. 9) and show heterogeneous binding that corresponds to the known distribution of 5-$HT_4R$ binding in rodents, primates, pigs and human. The radioligand did not show sensitivity to endogenous competition studies with citalopram in comparison to control subjects [147]. In regards to 5-HTTLPR, [$^{11}$C]SB207145 shows 9% higher binding for *S* allele in neocortex in comparison to *LL* homozygote [148]. No significant difference of [$^{11}$C]SB207145 was found in a small group of AD patients in comparison to controls [149]. However, [$^{11}$C]SB207145 binding was positively correlated to Aβ burden and negatively correlated to MMSE score of AD patients. Several radioligands were emerged based on [$^{11}$C]SB207145 and among these, [$^{18}$F]MNI-698 and [$^{18}$F]MNI-699, the fluoroalkyl analogues of SB207145, were tested in primates [150, 151]. [$^{18}$F]MNI-698 exhibits excellent *in vivo* characteristics and it is used for the measurement of 5-$HT_4R$ in monkey [151]. Several C-11 labeled analogues of SB207145 derivatives were synthesized for 5-$HT_4R$, however, no *in vivo* data are reported for these tracers [152]. More recently [$^{11}$C]prucalopride, a potent 5-$HT_4R$ agonist has been developed and *in vivo* studies in rats show low uptake of the radioligand which is likely due to inadequate lipophilicity and possibility of being a P-gp substrate [153].

**5-$HT_5R$—**5-$HT_5R$ is a Gs-coupled GPCR and the least explored 5-HT receptor [2–5]. Of the two known subtypes, 5-$HT_{5A}R$ only been identified in human [2–5]. 5-$HT_{5A}R$ mRNA is expressed in cerebral cortex, hippocampus, hypothalamic area, amygdala and cerebellum [154, 155]. However, the physiological role of this receptor in normal brain function is still

unknown and there is a lack of selective ligands for 5-$HT_5$R. To date there is no PET ligands are reported for 5-$HT_5$R.

**5-$HT_6$R—**5-$HT_6$R is Gs-coupled GPCR having effects *via* cAMP formation. 5-$HT_6$Rs are localized almost exclusively in the CNS [2–5]. Postmortem studies demonstrated that highest 5-$HT_6$R densities are in striatum ($B_{max}$ = 215 fmol/mg/protein), nucleus accumbens and olfactory tubercle, and moderate densities in amygdala, hypothalamus, thalamus, hippocampus and cerebral cortex [156–158]. Roles for 5-$HT_6$R are postulated in cognition, seizures, feeding behavior, anxiety, epilepsy, dementia psychosis, addiction and mood disorders [3–5]. The utility of the currently developed radiotracers for *in vivo* imaging of 5-$HT_6$R are limited in scope. The [$^{18}$F]-labeled 5-$HT_6$R ligand [$^{18}$F]12ST05 ($K_i$ = 4 nM) did not reveal any specific binding to 5-$HT_6$R in rats and cats [159]. The radioligand [$^{11}$C]SB399885 ($K_i$=1 nM, Fig. 10) is unsuitable for *in vivo* studies due to low uptake and its regional binding not in agreement with the known distribution of 5-$HT_6$R [160]. [$^{11}$C]GSK224558 rapidly enters the porcine brain, but undergoes rapid metabolism with peak regional tissue concentrations reached at approximately 20 min post-injection [161]. The nonselective ligand [$^{11}$C]GSK215083 (5-$HT_6$R $K_i$ = 0.16 nM and 5-$HT_{2A}$R $K_i$ = 0.79 nM) has been tested in pigs, nonhuman primates and human [162–165]. [$^{11}$C]GSK215083 demonstrated uptake and retention in the human brain, and the highest BP was observed in caudate and putamen followed by frontal cortex. Striatal binding and cortical binding of [$^{11}$C]GSK215083 are mainly attributed to 5-$HT_6$R and 5-$HT_{2A}$R respectively. A recent patent reports radiolabeled quinolone derivatives for 5-$HT_6$R, structurally similar to GSK215083 with 5-$HT_6$R and 5-$HT_{2A}$R $K_i$s as 0.339 and 0.395 nM respectively [166, 167].

**5-$HT_7$R—**5-$HT_7$R is coupled to Gs form of GPCR to activate 5-HT [2–5]. 5-$HT_7$R is expressed in brain, gastrointestinal tract, blood vessels and heart. In brain, higher expression of 5-$HT_7$R is found in thalamus ($B_{max}$ = 68 fmol/mg/protein), hypothalamus, hippocampus and cortex [168, 169]. There are three splice variants of 5-$HT_7$R reported in human and all of them show similar pharmacology [170]. 5-$HT_7$R are involved in thermoregulation, cardiac circadian rhythm, learning, memory, mood regulation, autism, neuropathy pain and sleep [2–5]. There is a 49% sequence homology between 5-$HT_7$R and 5-$HT_{1A}$R [171, 172]. Therefore development of highly selective ligands for one or other of these two targets is a potential challenge. The anatomical distribution of the binding densities of the two receptors is significantly different from each other. For example, 5-$HT_7$R has higher density in thalamus, whereas, the density of 5-$HT_{1A}$R in thalamus is low [32, 33, 169]; higher density of 5-$HT_{1A}$R is reported in temporal cortex, whereas, 5-$HT_7$R is very low density in this region [32, 33, 169]. Among the 5-HTRs, 5-$HT_7$R has the highest affinity for 5-HT, which makes this receptor an ideal target for PET imaging [13].

There are limited reports available for the evaluation of 5-$HT_7$R with PET [36]. Although the pioneer 5-$HT_7$R PET ligand [$^{11}$C]DR4446 ($K_i$ = 9.7 nM, Fig. 11) was tested in monkeys, it did not prove successful *in vivo* despite its excellent BBB penetration and metabolic stability [173]. Several 18F- ligands based on SB-269970, a selective 5-$HT_7$R has been reported [174–178]. Among these, [$^{18}$F]2FP3 ($K_i$ = 8.4 nM, Fig. 11) and [$^{18}$F]4FP3 ($K_i$ = 14 nM, Figure 11) show specific binding *in vitro* in brain sections of rats [175]. *In vivo* studies

in cats show excellent brain uptake, regional distribution and specific binding for [$^{18}$F]2FP3 [176]. [$^{11}$C]CIMBI-806, a dimethoxy biphenyl analogue ($K_i$ = 8.6 nM, Fig. 11) shows excellent *in vitro* binding in pig brain but did not show specific binding *in vivo* in pig despite its high brain uptake [179]. More recently [$^{11}$C]CIMBI-712 ($K_i$ = 1.1 nM, Fig. 11) and [$^{11}$C]CIMBI-717 ($K_i$ = 2.6 nM, Fig. 11), the two selective phenylpiperazinyl butyloxindole derivatives have been studied as 5-HT$_7$R ligands in pigs [180] and the latter shows higher uptake and specific binding than [$^{11}$C]CIMBI-712. In summary, [$^{18}$F]2FP3 and [$^{11}$C]CIMBI-717 are the two PET ligands proven successful *in vivo* in cats and pigs, respectively.

## CONCLUSIONS

PET imaging of 5-HTRs has been progressing for almost three decades and several selective radiotracers have been developed to quantify 5-HT receptor subtypes in normal and pathological conditions in human subjects (Table 1).

The best validated PET radiotracers developed so far for serotonin receptors are for 5-HT$_{1A}$R and 5-HT$_{2A}$R. [*Carbonyl*-$^{11}$C]WAY100635, [$^{18}$F]MPPF and [$^{18}$F](*cis*)FCWAY, [$^{18}$F]altanserin and [$^{11}$C](*R*)M100907 are the best 5-HT$_{1A}$R and 5-HT$_{2A}$R ligands available but have limitations such as complex radiochemistry, fast metabolism (WAY100635), P-gp substrate (MPPF), radiodefluorination (FCWAY), interference of a radiometabolite (altanserin) and slow off-rate requiring an $^{18}$F-label to image long enough to capture equilibrium binding (M100907). The emerging tracers for 5-HTRs include [$^{11}$C]*trans*-MeFWAY (5-HT$_{1A}$R antagonist), [$^{11}$C]AZ10419369, [$^{11}$C]P943 (both 5-HT$_{1B}$R), [$^{11}$C]SB207145 (5-HT$_4$R) and [$^{11}$C]GSK215083 (5-HT$_6$R) which are successfully tested in man and reported in few brain disorders. The partial agonist ligand [$^{11}$C]CUMI-101 is promising in human and nonhuman primates to measure the HA state of 5-HT$_{1A}$R, however, no data is available so far to support its potential to image pathological conditions. The recently developed [$^{18}$F](*R*)M100907 and [$^{18}$F](*R*)MH-MZ can circumvent the disadvantages of [$^{11}$C](*R*)M100907 and are potential 5-HT$_{2A}$R PET tracers for diagnosis and drug development. The 5-HT$_{2A/2C}$R agonist ligand [$^{11}$C]CIMBI-36 is promising for quantifying high agonist affinity receptor. Despite significant efforts, no PET tracers are available for 5-HT$_3$Rs, possibly due to the low $B_{max}$ as well as intracellular localization of these receptors in brain. Similarly, due to the lack of adequate receptor density for 5-HT$_{2B}$R and lack of available ligands for 5-HT$_5$R, the development of PET tracers for these targets are hindered. [$^{11}$C]CIMBI-717 and [$^{18}$F]2FP3 have promising characteristics to image 5-HT$_7$R in human. Future development of suitable PET 5-HTRs agonist/antagonist radiotracers is needed to study more serotonin receptor subtypes and their role in pathophysiology of diseases and to study receptor occupancy of promising therapeutic medications for dose finding as a preliminary step before implementing clinical trials.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **Ab** | Amyloid beta |
| **ABC** | ATP-binding cassette |
| **AC** | Adenylate cyclase |
| **AD** | Alzheimer's disease |
| **ADHD** | Attention deficient hyperactivity syndrome |
| **APD** | Antipsychotic drug |
| **ASD** | Autism spectrum disorder |
| **a-1AR** | Adrenergic Alpha1 receptor |
| **BBB** | Blood brain barrier |
| **BCRP** | Brest cancer resistance protein |
| **$B_{max}$** | Binding maxima |
| **BP** | Binding potential |
| **C** | Carbon |
| **Ca** | Calcium |
| **cAMP** | Cyclic AMP |
| **$CH_3I$** | Methyl iodide |
| **$CH_2O$** | Formaldehyde |
| **$CH_3OTf$** | Methyl triflate |
| **$COCl_2$** | Carbonyl chloride (Phosgene) |
| **CSF** | Cerebrospinal fluid |
| **D2R** | Dopamine 2 receptor |
| **DOI** | 2,5-dimethoxy-4-iodoamphetamine |
| **DRG** | Dorsal root ganglion |
| **$E_{max}$** | Maximum efficacy |
| **$EC_{50}$** | Half maximal effective concentration |
| **F** | Fluorine |
| **$F^-$** | Fluoride |
| **$f_p$** | Free fraction |
| **fmol** | Femptomole |
| **GDP** | Guanosine diphosphate |
| **GTPgS** | Guanosine 5′-O-[gamma-thio]triphosphate |

| | |
|---|---|
| **HA** | High affinity |
| **HCA** | Human cerebral artery |
| **5-HIAA** | 5-Hydroxyindole acetic acid |
| **H1R** | Histamine 1 receptor |
| **5-HTR** | 5-Hydroxytryptamine receptor (serotonin receptor) |
| **5-HT** | 5-Hydroxytryptamine (Serotonin) |
| **5-HTTLPR** | 5-Hydroxytriptamine transporter linked polymorphic region |
| **GI** | Gastrointestinal |
| **GPCR** | G protein-coupled receptor |
| **$IC_{50}$** | Inhibitory concentration half maximal |
| **IBS** | Irritable bowel syndrome |
| **IP3** | Inositol triphosphate |
| **K** | Potassium |
| **$K_d$** | Kinetics of dissociation |
| **$K_i$** | Kinetics of inhibition |
| **LA** | Low affinity |
| **LGIC** | Ligand-gated ion channel |
| **logD** | Partition coefficient or distribution coefficient |
| **MDR** | multi-drug resistance-associated protein |
| **mg** | Milligram |
| **MMSE** | Mini mental state examination |
| **MSP** | Methyl spiperone |
| **mRNA** | Messenger ribonucleic acid |
| **nAChR** | nicotinic acetylcholine receptor |
| **N** | Nitrogen |
| **Na** | Sodium |
| **nM** | nano molar |
| **NMSP** | N-methyl spiperone |
| **O** | Oxygen |
| **OCD** | Obsessive compulsive disorder |
| **PD** | Parkinson’s disease |
| **PET** | Positron emission tomography |

| | |
|---|---|
| **P-gp** | P-glycoprotein |
| **PLC** | Phospholipase C |
| **PTSD** | Post traumatic stress disorder |
| **SERT** | Serotonin transporter |
| **SLC** | Soluble carrier |
| **$T_{1/2}$** | Half life |
| **TC** | Tissue compartment |
| **TDC** | Tryptophan decarboxylase |
| **TPH** | Tryptophan hydroxylase |
| **$V_T$** | Volume of distribution |

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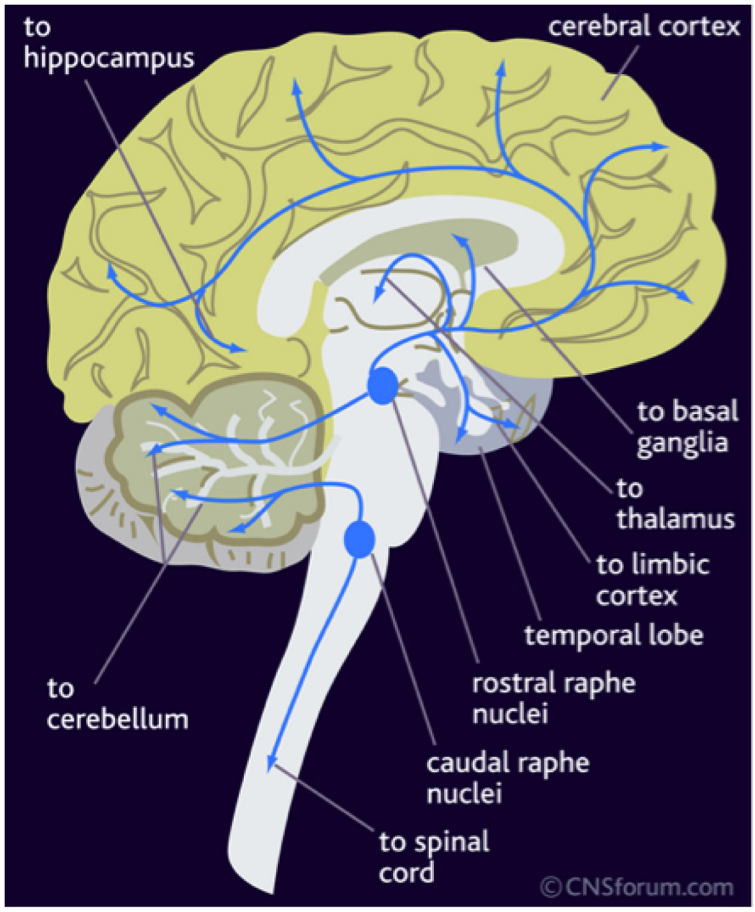


**Fig. 1.**
Serotogenic pathways in human brain (adapted from CNS forum).

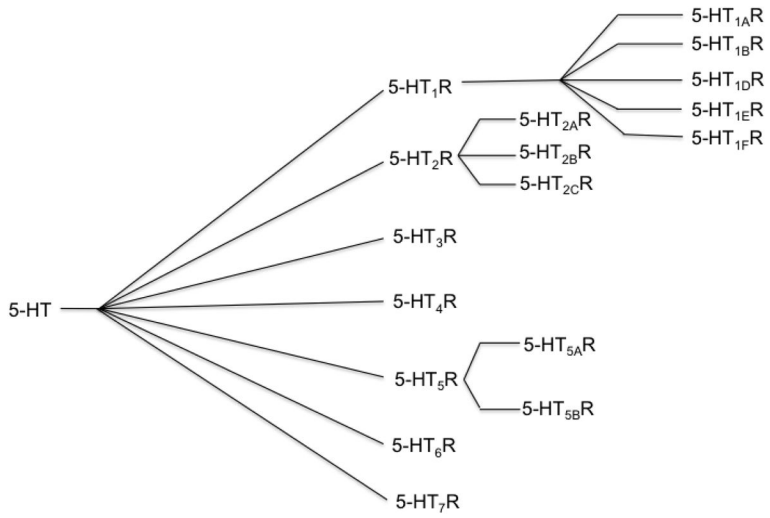


**Fig. 2.**
Serotonin receptors and subtypes.

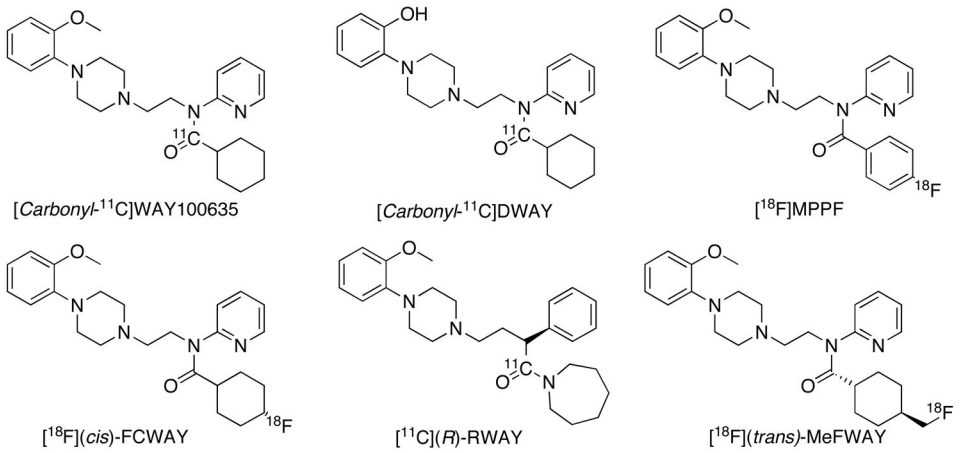


**Fig. 3.**
Examples of 5-$HT_{1A}$R antagonist PET tracers studied in human.

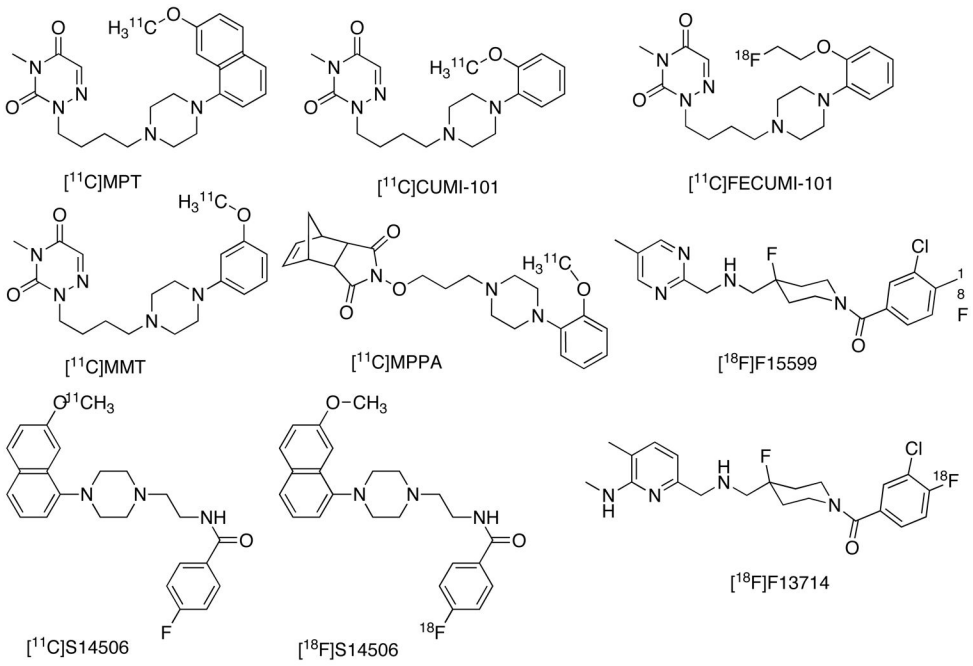


**Fig. 4.**
Examples of agonist PET tracers for 5-$HT_{1A}$R.

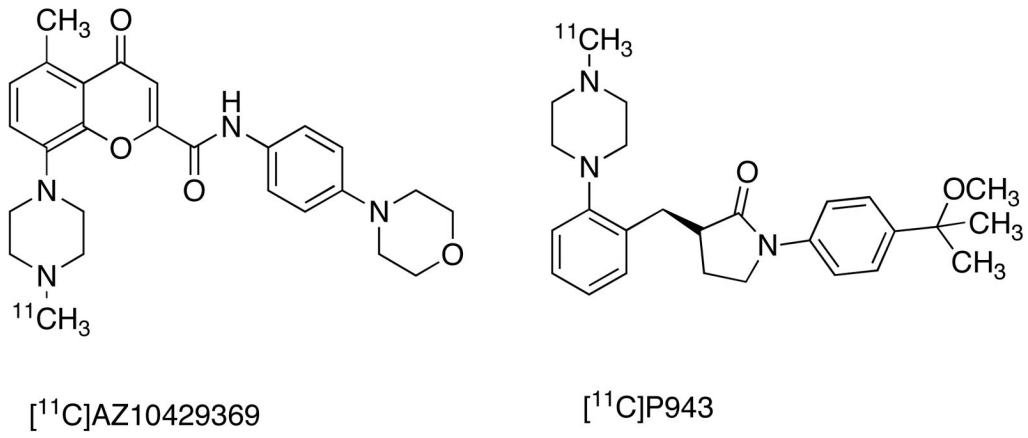


**Fig. 5.**
PET radioligands for 5-$HT_{1B}$R.

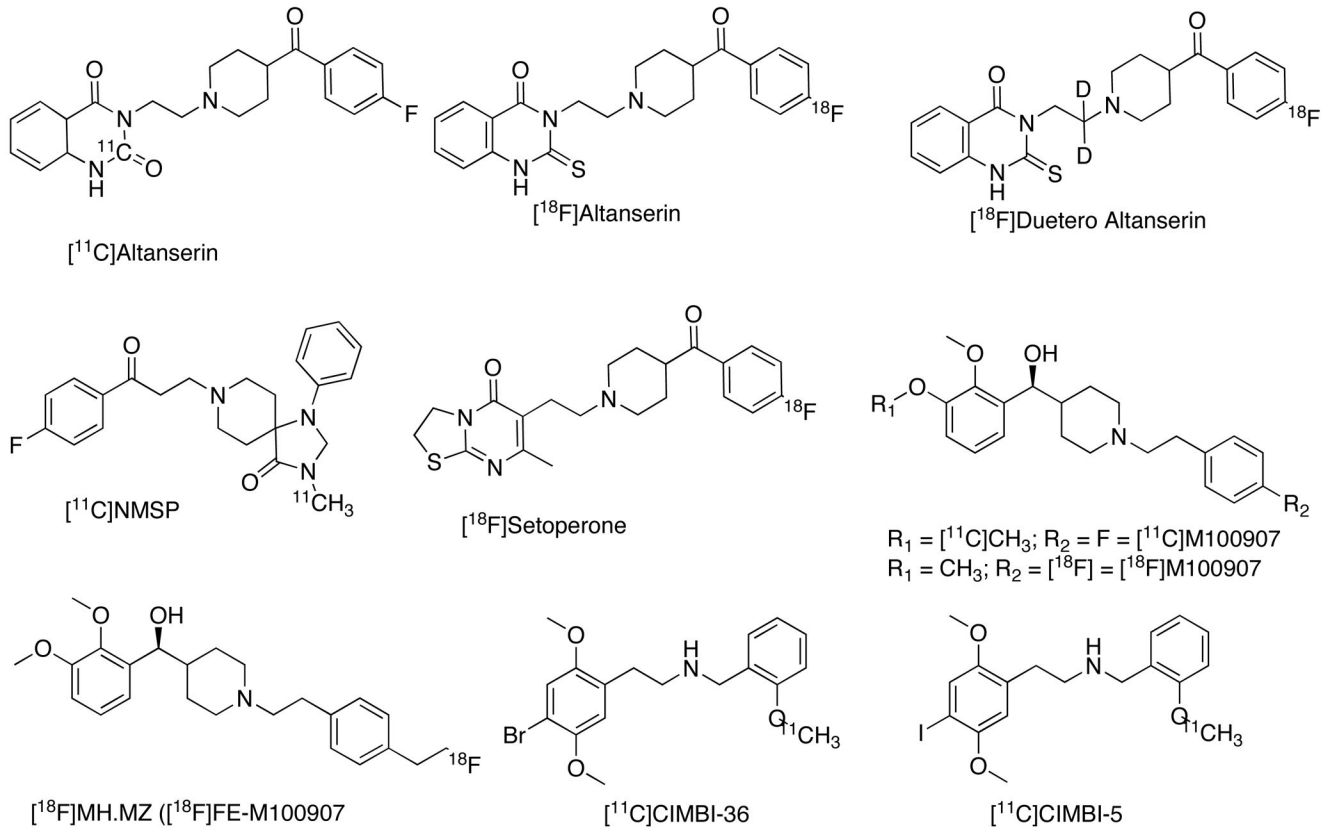


**Fig. 6.**
Examples of PET radioligands for 5-$HT_{2A}$R.

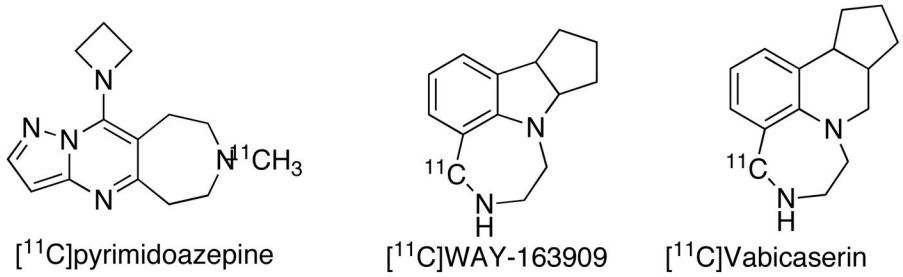


**Fig. 7.**
Examples of PET ligands for 5-$HT_{2C}$R.

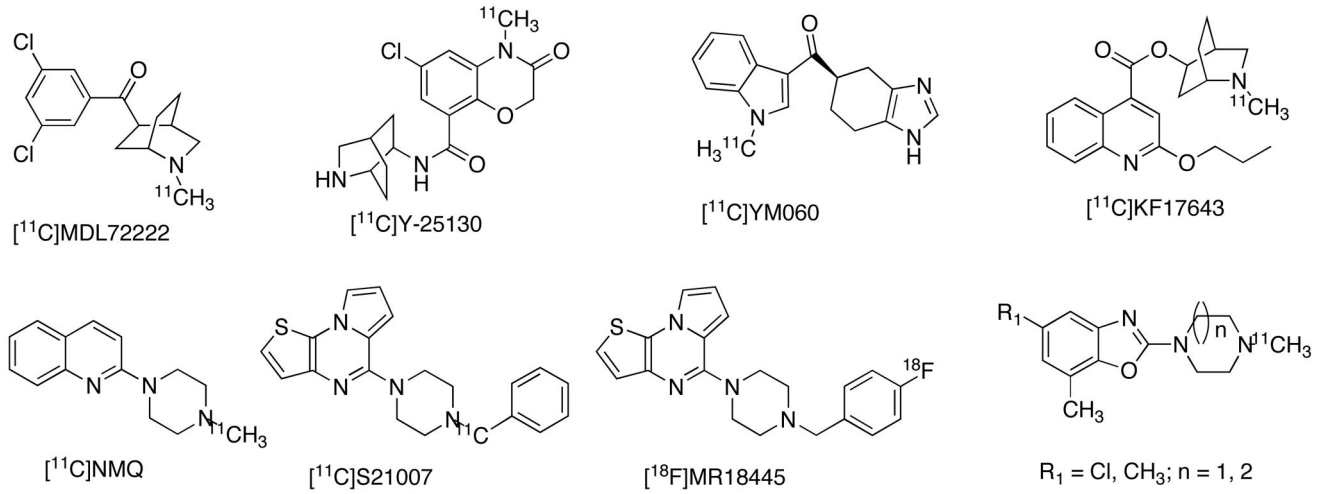


**Fig. 8.**
Examples of PET ligands for 5-$HT_{3B}$R.

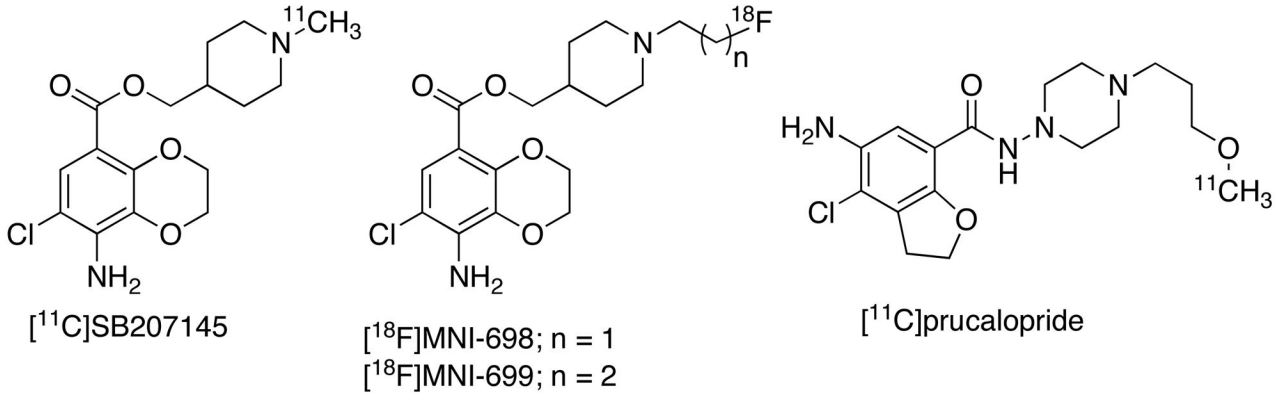


**Fig. 9.**
Examples of PET ligands for 5-$HT_4$R.

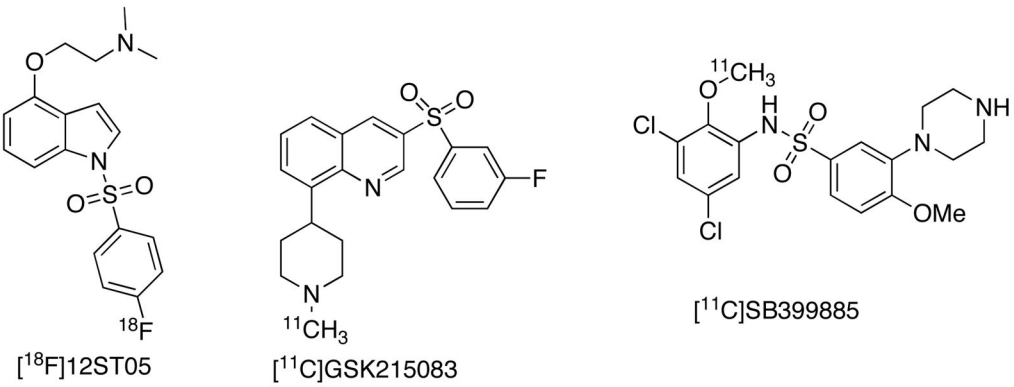


**Fig. 10.**
Examples of PET ligands for 5-$HT_6$R.

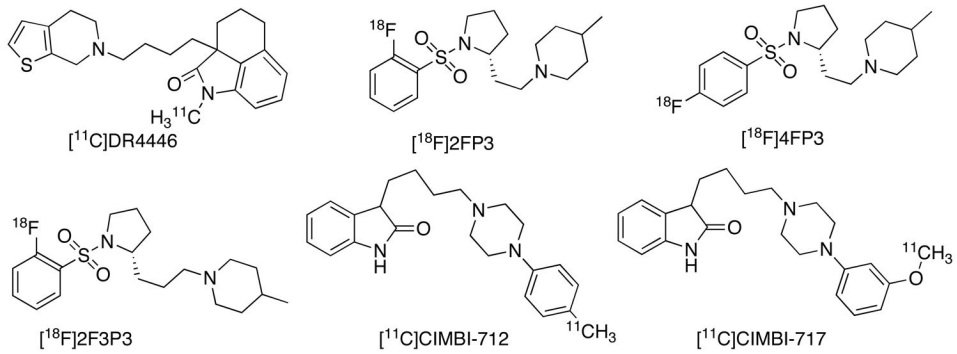


**Fig. 11.**
Examples of PET ligands for 5-$HT_7$R.

## Table 1

List of PET ligands available for 5-HTR imaging in human.

| Receptor subtype | PET Ligands | Advantages | Limitations |
|---|---|---|---|
| 5-$HT_{1A}$R antagonist | [*Carbonyl*-$^{11}$C]WAY-100635 | High target to non-target ratio | Complex radiosynthesis, fast metabolism, difficulty of quantification with arterial input functions. |
| | [$^{11}$C]*cis*-FCWAY | | Radiodefluorination |
| | [$^{11}$C]MPPF | | P-gp substrate, low target to non target ratio |
| | [$^{11}$C]*trans*-MeFWAY | High target to non-target ratio | Quantification with arterial input data is currently not available |
| 5-$HT_{1A}$R partial agonist | [$^{11}$C]CUMI-101 | Binding to high affinity site in human and NHP | Partial agonist, a-1AR cross selectivity is reported in rodents and monkey. |
| 5-$HT_{1B}$R antagonist | [$^{11}$C]AZ10419369 | | |
| | [$^{11}$C]P943 | Reported altered binding in a variety of pathological conditions | |
| 5-$HT_{2A}$R antagonist | [$^{18}$F]Altanserin | | Interference of radiometabolite in quantification, need bolus infusion paradigm for injection. |
| | [$^{18}$F]Setoperone | | Nonselective for $D_2$R |
| | [$^{11}$C]NMSP | | Cross affinity for $D_2$, a-1R, 5-$HT_{1A}$R, and 5-$HT_{2C}$R |
| | [$^{11}$C](*R*)-M100907 | | Slow kinetics |
| 5-$HT_{2A}$R agonist | [$^{11}$C]CIMBI-36 | | |
| 5-$HT_4$R antagonist | [$^{11}$C]SB207145 | | Not sensitive to endogenous 5-HT changes, Altered binding in 5-HTTLPR |
| 5-$HT_6$R antagonist | [$^{11}$C]GSK215083 | | Cross selectivity to 5-$HT_{2A}$R |