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Synthesis, fluorine-18 radiolabeling, and in vitro characterization of 1-iodophenyl-*N*-methyl-*N*-fluoroalkyl-3-isoquinoline carboxamide derivatives as potential PET radioligands for imaging peripheral benzodiazepine receptor

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Abstract—The isoquinoline carboxamide derivative 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide (PK11195) has been shown to bind strongly and selectively to the peripheral benzodiazepine receptor (PBR) binding sites. A series of PK11195 analogues have been synthesized and biologically characterized. The affinities of the analogues for the PBR were determined using in vitro competitive binding assays with [^{3}H]PK11195 in rat kidney mitochondrial membranes. The results showed that the 1-(2-iodophenyl)-*N*-methyl-*N*-(3-fluoropropyl)-3-isoquinoline carboxamide (**9a**) was the most potent compound (K_i = 0.26 nM) of this series and is an excellent lead ligand for additional studies for labeling with fluorine-18 to determine whether it possesses the desired in vivo performance in non-human primates by PET imaging. Thus, radiolabeling of **9a** with fluorine-18 was developed.

1. Introduction

The development and biological evaluation of radioligands for positron emission tomography (PET) studies of central benzodiazepine receptors (CBRs) and peripheral benzodiazepine receptors (PBRs) are of great value as they have been associated with a variety of disease processes. Although CBR and PBR are pharmacologically and structurally distinct from each other,[1,2] despite their historical connection through the benzodiazepines, they both hold interest for present and future PET studies on, for example, epilepsy, Alzheimer's disease, alcoholism, and anxiety with respect to CBR, and for stroke, neuro-protection, inflammation, and tumors with respect to PBR.

Advances in our understanding of the molecular biology of cancer and other diseases have identified molecules and signaling pathways that we can visualize, in vivo, for diagnosis and staging, for identifying optimal therapies, and for monitoring patient response to therapy. These advances have also helped to identify targets for molecular radiotherapy, making it possible to target radiation at the cellular and molecular level. The understanding of the structure and the function of PBR is not as advanced as that of CBR. Nonetheless, its concentration at brain lesions, tumors, and inflammatory sites now engenders much clinical interest.

PBR has been implicated in the regulation of various cellular processes, including regulation of steroidogenesis, immunomodulation, porphyrin transport, heme synthesis, cell apoptosis and proliferation.[1,3–5] PBR expression levels were shown to be increased in a variety of cancers indicating its important role in cancer development.[6–12] PBR has been reported to be a sensitive marker for various pathologies of glial activation in brain injuries.[13–22] Increased and decreased PBR levels have been demonstrated as biological indicators of stressful conditions[23] in several psychiatric disorders as it is closely associated with personality traits for anxiety tolerance.[24]

PBR is a ubiquitous high affinity drug ligand and cholesterol binding protein. Functional PBR is described as a

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multimeric complex composed of evolutionarily conserved, highly hydrophobic 18 kDa receptor protein,[25] the 32 kDa voltage-dependent anion channel (VDAC) protein required for benzodiazepine binding and the 30 kDa adenine nucleotide carrier (ANC) of an as yet unclear function in the complex.[26–29] PBR is mainly located and spanned on the outer mitochondrial membrane, but its localization has also been detected on plasma membranes,[30,31] the Golgi apparatus, lysosomes, rough endoplasmic reticular microsomes, peroxisomes,[30] and nuclear membranes.[9,32]

Specific PBR ligands are divided into two classes, endogenous and synthetic ligands. Endogenous PBR ligands include the human protein sequences of diazepam binding inhibitor (DBI)[33] and its derived fragments (TTN, triakontatetraneuropeptide, DBI 17–50; EPN, eicosaneuropeptide, DBI 26–50; and ODN, octadecaneuropeptide, DBI 33–50),[34] porphyrines[35] and cholesterol.[36,37] The representative synthetic PBR ligands include 4′-chlorodiazepam Ro54864 [7-chloro-5-(4-chlorophenyl)-1-methyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one],[38,39] isoquinoline carboxamide PK11195 [1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide],[40,41] indolacetamide derivative FGIN-1-27 [*N*,*N*-di-*n*-hexyl-2-(4-fluorophenyl) indole-3-acetamide][40,42–44], and pyridazinoindole derivative SSR180575 (7-chloro-*N*,*N*,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4H-pyridazino[4,5-*b*]indole-1-acetamide)[45–47] (Fig. 1). These compounds' affinity (K_d) for human PBR is selective in the nanomolar range: Ro54864, 6 nM; PK11195, 2 nM; FGIN-1-27, 4 nM and SSR180575, 3.5 nM.[37]

The development of specific potent PBR ligands may be of great value for the management of a broad range of different pathological applications such as cancer, autoimmune diseases, viral infection, neurodegeneration, stress, and brain injury. PK11195 was the first non-benzodiazepine high affinity PBR ligand and is the most widely used pharmacological tool for the study of the expression and the function of PBR. The isoquinolines that bind specifically to PBR interact with the 18 kDa subunit of the receptor complex. PK11195 does not show the variations in affinity that the majority of benzodiazepines do, and it has a consistent high affinity (K_d < 20 nM) in all species[40,41,43,48,49] whereas the affinity for Ro54864 is highly species-dependent[50,51] and temperature sensitive.[40]

Based on but not limited to the above mentioned facts, we decided to develop new isoquinoline carboxamide analogues labeled with fluorine-18 to image PBR with PET. In this paper, we describe the synthesis and the preliminary in vitro evaluation of a series of 1-iodophenyl-*N*-methyl-*N*-fluoroalkyl-3-isoquinoline carboxamides (**9a–d**). The best PBR affinity ligand **9a** was chosen to explore the fluorine-18 labeling methodology. Although [^{11}C]PK11195 has been used as a valuable PET reagent to image PBR, the development of 1-iodophenyl-*N*-methyl-*N*-[^{18}F]fluoroalkyl-3-isoquinoline carboxamides as new PBR raioligands has at least three advantages over [^{11}C]PK11195. Firstly, [^{18}F] has a half-life of 110 min while [^{11}C] has a 20 min half-life. The longer half-life of [^{18}F] allows for longer imaging time which gives rise to effective clearance of non-specific binding and thus allows for imaging of more subtle specific PBR binding. Secondly, the new radioligands [^{18}F]**9a–d** do not possess a chiral center whereas [^{11}C]-PK11195 does, including its analogues [^{125}I]-PK11195,[52] [^{11}C]PK11211,[53,54] and [^{18}F]PK14105.[55,56] It is well known that in general individual enantiomers may behave differently than racemic mixtures in vivo with respect to specific receptor binding, protein binding,[57] pharmacokinetics,[57,58] and metabolism.[59] Where such differences exist the mathematical interpretation of kinetic data from the use of the racemic radioligands may lead to more complicated kinetic modeling.[60] It has also been reported that the binding of PK11195 to PBR binding sites may be inhibited stereoselectively.[61] These factors have not been evaluated for PK11195.[2] By using non-chiral radiotracers for imaging PBR with PET, it not only eliminates all the above mentioned complications caused by racemic ligands, but also significantly simplifies the synthesis procedures, since synthesis of >99% enantiomerically enriched compounds poses significant challenges for chemists. An additional advantage of [^{18}F] ligands is that it can be produced off-site whereas [^{11}C] requires production via an on-site cyclotron. Results of these new tracers' in vivo biodistribution and displacement experiments, in vivo metabolic stability, and comparative microPET studies with PK11195 in non-human primates are currently in progress and will be reported in a separate publication.

2. Results and discussion

2.1. Synthesis of reference compounds

The syntheses of 1-iodophenyl-3-isoquinoline carboxylic acids **7a–c** were shown in Scheme 1. Iodobenzoyl chlorides **2a–c**, which were prepared from their correspond-

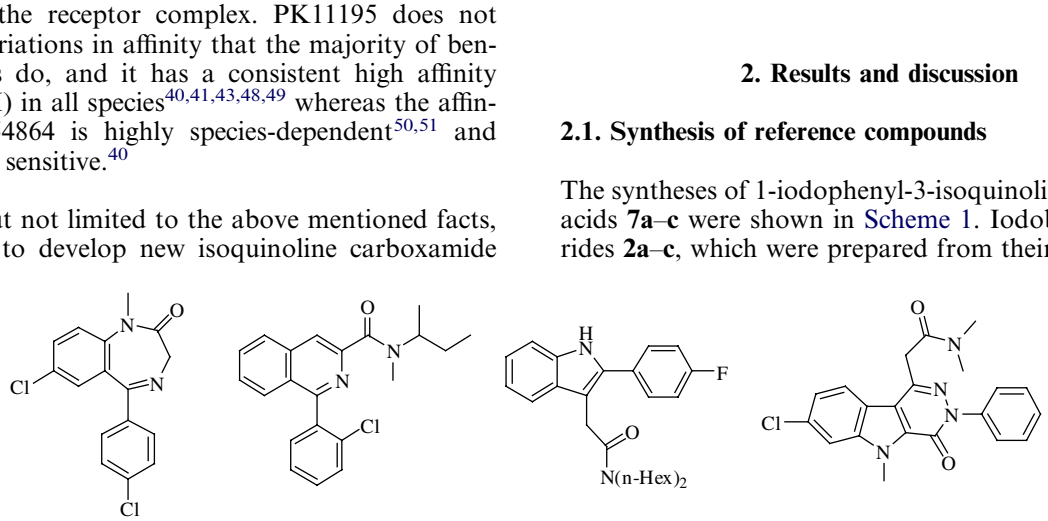
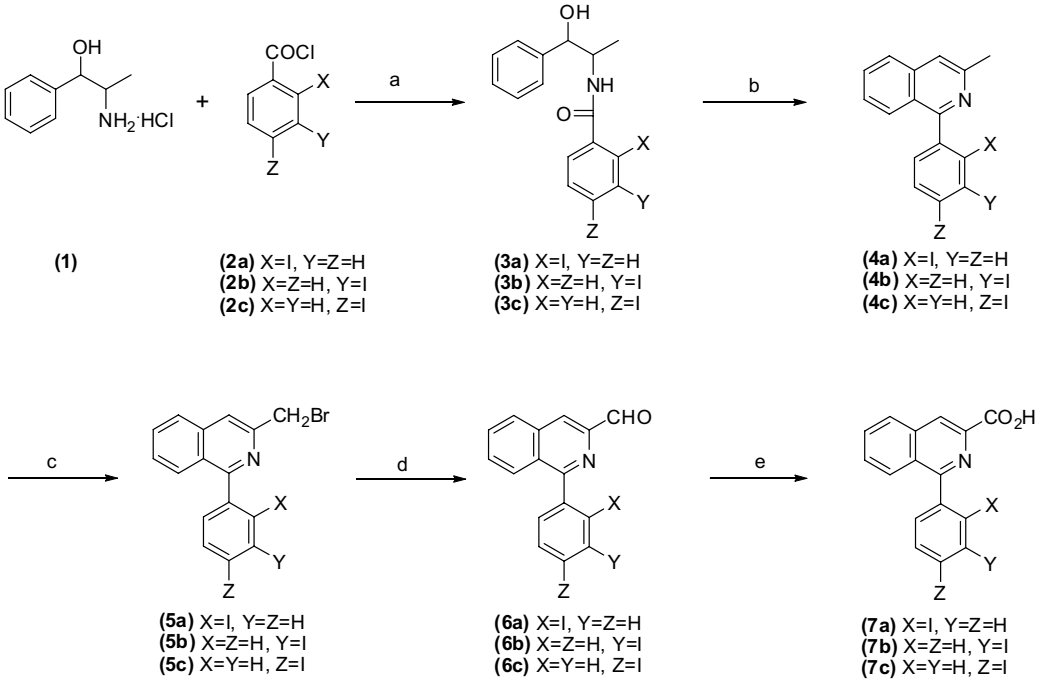


Figure 1. Representative synthetic PBR ligands.



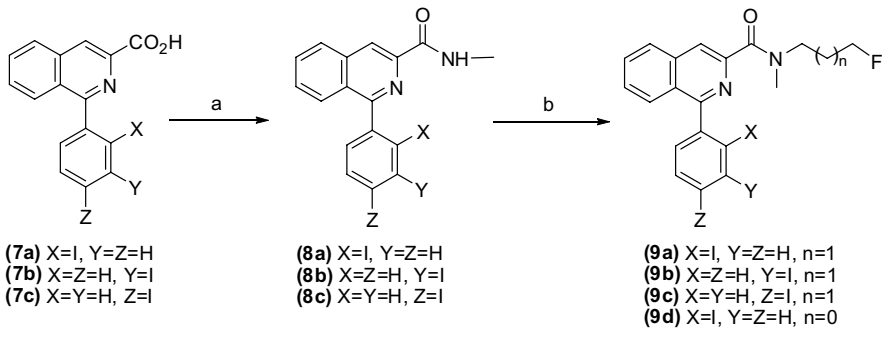
Scheme 1. Syntheses of 1-iodophenyl-3-isoquinoline carboxylic acids **7a–c**. Reagents and conditions: (a) 5% NaOH, CH_2Cl_2, 0 °C to rt, overnight; (b) P_2O_5, *o*-dichlorobenzene, reflux, overnight; (c) NBS, AIBN, CCl_4, reflux, overnight; (d) $NaIO_4$, DMF, reflux, overnight; (e) $AgNO_3$, NaOH, $EtOH/H_2O$, rt, 2 h.

ing acids by treatment with phosphorous (V) chloride, were reacted with norephedrine hydrochloride (**1**), respectively, to afford benzamides **3a–c**. The benzamides were cyclized by a modified Pictet–Gams reaction[62] to provide isoquinoline derivatives **4a–c**. The bromides **5a–c** were obtained by free radical-promoted bromination of **4a–c**, respectively, using *N*-bromosuccinimide (NBS)/2,2-azobisisobutyro-nitrile (AIBN). Oxidative hydrolysis of the bromides by treatment of **5a–c** with sodium periodate gave the aldehydes **6a–c**, respectively. Compounds **5b–c** and **6b–c** were used for the next reaction without further purification. For characterization, compounds **5a** and **6a** were isolated and analyzed to confirm their identities. 1-Iodophenyl-3-isoquinoline carboxylic acids **7a–c** were synthesized by further oxidation of aldehydes **6a–c** with silver nitrate, respectively.

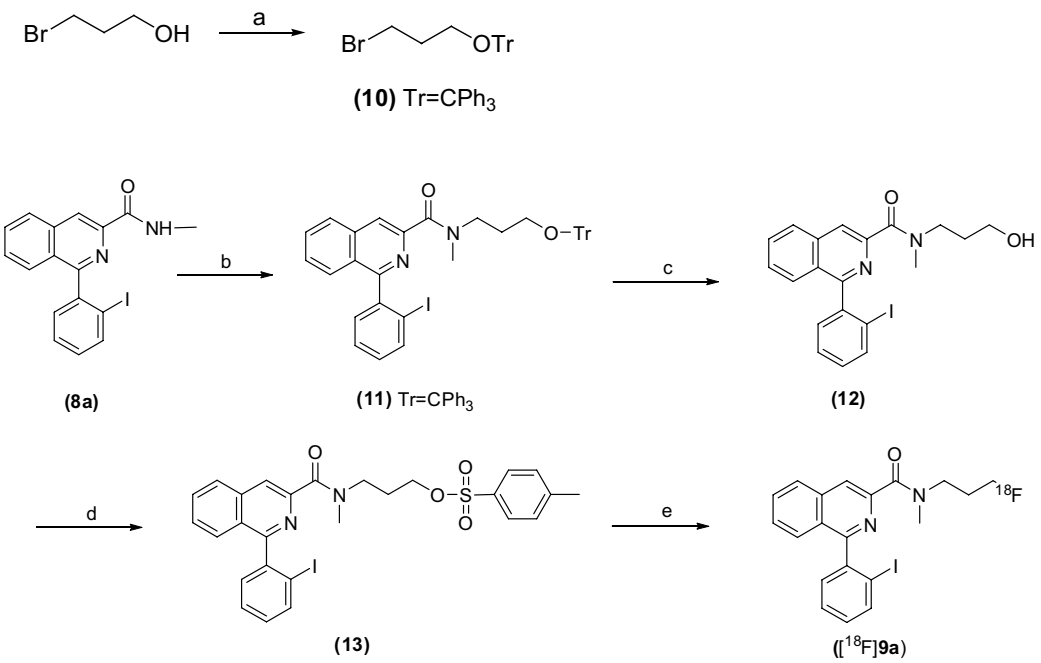
Preparations of fluorine-19 reference compounds **9a–d** are displayed in Scheme 2. 1-Iodophenyl-3-isoquinoline carboxylic acids **7a–c** were first coupled with methylamine to give secondary amides **8a–c**, respectively, which were then N-alkylated with 3-fluoropropyl bromide to provide the N-fluoropropylated PK11195 analogues **9a–c**, respectively. Amide **8a** was reacted with 2-fluoroethyl bromide and afforded 1-(2-iodophenyl)-*N*-methyl-*N*-(2-fluoroethyl)-3-isoquinoline carboxamide **9d**.

2.2. Synthesis of labeling precursor and radiosynthesis

The precursor for fluorine-18 radiolabeling of the most potent isoquinoline carboxamide **9a** (see Section 2.3) was prepared as shown in Scheme 3. First amide **8a** was reacted with 3-triphenylmethoxypropylbromide **10**, which was prepared by tritylation of 3-bromopropyl alcohol, in the presence of sodium hydride in DMF to give amide **11**. The trityl group of **11** was removed by treatment with trifluoroacetic acid (TFA) to afford the alcohol **12**, followed by using *p*-toluenesulfonyl chloride to produce the tosylate **13** as F-18 labeling precursor.



Scheme 2. Syntheses of F-19 references of isoquinoline carboxamides **9a–d**. Reagents and conditions: (a) 1—$SOCl_2$, CH_2Cl_2, 50 °C, 1 h; 2—CH_3NH_2, THF, −70 °C to rt, overnight; (b) 1—NaH, DMF, rt, 40 min; 2—$BrCH_2CH_2CH_2F$ (for **9a–c**) or $BrCH_2CH_2F$ (for **9d**), rt, 24 h.



Scheme 3. Synthesis of [^{18}F] labeling precursor **13** and radiosynthesis of iosquinoline carboxamide [^{18}F]**9a**. Reagents and conditions: (a) TrCl, Py, CH_2Cl_2, 60 °C, 5 h then rt, overnight; (b) (1) NaH, DMF, rt, 30 min. (2) **10**, rt, overnight; (c) TFA, CH_2Cl_2, rt, 40 min; (d) *p*-TsCl, Et_3N, CH_2Cl_2, 0 °C, 4 h; (e) [^{18}F]KF, K_2CO_3, K_{222}, DMSO, 120 °C, 10 min.

Radiofluorination of [^{18}F]**9a** was achieved through no-carrier-added (NCA) nucleophilic substitution with dried [^{18}F]KF, potassium carbonate, and Kryptofix in DMSO from the tosylate **13** (Scheme 3). The exchange between [^{18}F]fluoride and the leaving group occurred in 10 min at 120 °C. Unreacted [^{18}F]fluoride and any radiolabel-charged by-products were eliminated by passing the reaction mixture through a silica SepPak. Purification by semipreparative, reverse-phase HPLC afforded [^{18}F]**9a** in an average (n = 4) radiochemical yield of 8%, decay corrected from the end of bombardment (EOB). The identity of the final [^{18}F] radioligand was confirmed by co-injection with the authentic sample of non-radioactive **9a** on analytical HPLC. Analytical HPLC demonstrated that the radiochemical purity of [^{18}F]**9a** was over 99%. The total synthesis time including HPLC purification and radiopharmaceutical formulation for intravenous administration was approximately 90 min from EOB.

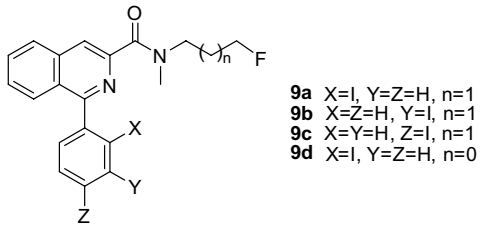
The lipophilicity of radioligand [^{18}F]**9a** was measured according to Wilson et al.'s procedure,[63] by extracting the test compound between octanol and pH 7.4, phosphate buffer. The log $P_{7.4}$ value of [^{18}F]**9a** is 3.0 ± 0.05 (n = 3). It has been reported that the log $P_{7.4}$ value of radiotracers in the range of 1.0–3.0 was considered optimal for brain penetrance thus, the compound [^{18}F]**9a** should be expected to enter the brain readily.[64]

2.3. Binding affinity assays

New isoquinoline carboxamide derivatives **9a**–**d** were screened for binding affinities to PBR using in vitro competition binding assays with a known PBR antagonist [^{3}H]PK11195 in rat kidney mitochondrial membranes according to previously reported procedures.[65–67] We chose rat renal mitochondria as a tissue source since it has been demonstrated that in kidney[68] and adrenal,[69] these receptors are primarily associated with the mitochondrial compartment stably expressing PBR. In this study, the binding affinity experiments were performed by NovaScreen (Hanover, MD) under the NIMH Psychotherapeutic Drug Discovery & Development Program. The compounds **9a**–**d** were tested for their ability to inhibit the binding of [^{3}H]PK11195, and their affinities are given as K_i and IC_{50} in Table 1. The K_i values of PK11195, Ro54864, and diazepam (CBR ligand)[2] were also screened under the same assay system for references.

The data in Table 1 indicate that compounds **9a** and **9b** displayed high affinities for PBR, with K_i values of 0.26 nM and 1.30 nM, respectively, approximately 110 and 22 times more potent compared to the K_i value of PK11195, respectively, and 130 and 26 times to Ro54864, respectively, in this in vitro assay model. Changing the iodo group on the benzene ring of the isoquinoline carboxamide from *ortho* (**9a**) to *meta* (**9b**) position resulted in 5-fold decreased affinity for PBR. Further decreased affinity occurred when the iodo group is on *para* (**9c**) position, over 240-fold decrease compared to compound **9a**. Replacing the *N*-fluoropropyl group of **9a** to *N*-fluoroethyl group afforded **9d**, which led to even more dramatic decrease in PBR affinity, exhibited over 1600-fold less K_i value relative to **9a**. Thus the order of PBR affinities in this series (K_i in nM) was **9a** > **9b** > **9c** > **9d**. This structure–affinity relationship may be of value to help predicting new PBR ligands as it may apply on other halogens (fluoro, chloro and bromo), or other groups (nitro, alkoxy, alkyl, etc.) instead of iodo on the benzene ring in the similar molecules. Also the best PBR ligand **9a** in this study provided a suitable candidate to label this compound with [^{11}C] (*N*-methyl), [^{18}F] (*N*-fluoropropyl), and [^{123}I] (iodo-

Table 1. In vitro competition assays of 1-iodophenyl-*N*-methyl-*N*-fluoroalkyl-3-isoquinoline carboxamides **9a–d** with [^{3}H]PK11195 in rat kidney mitochondrial membranes



9a X=I, Y=Z=H, n=1
9b X=Z=H, Y=I, n=1
9c X=Y=H, Z=I, n=1
9d X=I, Y=Z=H, n=0

Compound	K_i (nM)	IC_{50} (nM)	K_i(PK11195)	K_i(Ro54864)
9a	0.26	0.28	111	131
9b	1.3	1.4	22.2	26.2
9c	63.1	66.5	0.46	0.54
9d	421	445	0.07	0.08
PK11195	28.8			
Ro54864	34			
Diazepam	574			

phenyl) to imaging PBR with PET and SPECT. Further work is in progress in this laboratory.

3. Conclusions

A series of 1-iodophenyl-*N*-methyl-*N*-fluoroalkyl-3-isoquinoline carboxamide derivatives **9a–d** have been prepared and the in vitro affinities of these compounds to PBR in rat kidney mitochondrial membranes have been conducted. Competition binding assays showed that the ability of these compounds to inhibit the standard PBR ligand PK11195 decreased in the order of **9a**, **9b**, **9c**, and **9d**. The most potent compound **9a** was radiolabeled with [^{18}F] in moderate radiochemical yield with high radiochemical purity from its tosylated precursor **13**. The lipophilicity of [^{18}F]**9a** was measured as log$P_{7.4}$, which indicates that this tracer is suitable to image PBR in organs in the body, as well as in the brain. This tracer model labeled with [^{11}C], [^{18}F], or [^{123}I] may be used as a tool to evaluate the potential of isoquinoline carboxamide derivatives as PET and SPECT radioligands for imaging PBR.

4. Experimental

4.1. Materials and instrumentation

All chemicals used were obtained from commercially available sources (Aldrich Chemicals Co., Milwaulkee, WI, USA; Sigma Chemical Co. St. Louis, MO, USA), were of analytical or higher grade, and were used without further purification. Solvents used in reactions and chromatographic purification were purchased from Aldrich Chemicals Co. and from VWR Scientific Products (West Chester, PA, USA). Thin-layer chromatography (TLC) analyses were performed with 250 μm thick layers of fluorescence UV254 silica gel backing on aluminum plates purchased from Whatman Ltd (Maidstone, Kent, England). Flash chromatography was carried out using Merck Kieselgel silica gel 60 (230–400 mesh). Silica and C-18 SepPaks were purchased from Waters, Inc. (Milford, MA, USA). The Gelman teflon filters were purchased from VWR.

Melting points were measured in capillary tubes using a Mel-Temp II apparatus (Laboratory Devices, Inc., Holliston, MA, USA) and are uncorrected. ^{1}H NMR and ^{13}C spectra were recorded on Varian 400 MHz, 300 MHz or GE 300 MHz spectrometers at NMR Center in Emory University, and chemical shifts (δ values) were reported as parts per million (ppm) downfield from tetramethylsilane (TMS). Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA, USA) and were within ±0.4% of the theoretical values. Mass Spectra were performed on JEOL JMS-SX102/SX102A/E or VG 70-S double focusing mass spectrometers at Mass Spectroscopy Center at Emory University using high-resolution electrospray ionization (ESI), or electron impact ionization (EI). Semipreparative HPLC column (Waters XTerra Prep RP_{18} 5 μm, 19× 100 mm) and analytical HPLC column (Waters Nova-Pak C_{18}, 3.9× 150 mm) were purchased from Waters Inc.

The [^{18}F]fluoride was produced at Emory University with a 11 MeV Siemens RDS 112 negative-ion cyclotron (Knoxville, TN, USA) by the ^{18}O (p,n) ^{18}F reaction using [^{18}O]H_2O (95%). The isolated radiochemical yields were determined using a dose-calibrator (Capintec CRC-712M). Thin-layer chromatograms of the radiolabeled compounds were analyzed with a Raytest System (model: Rita Star, Germany) using the same type of silica TLC plates from Whatman.

4.2. 2-Iodo-*N*-(2-hydroxy-1-methyl-2-phenylethyl)benzamide (3a)

To an ice-cooled mixture of L,D-norephedrine hydrochloride (**1**, 5.28 g, 28.1 mmol) in CH_2Cl_2 (100 mL) and 5% sodium hydroxide aqueous solution (40 mL) was added dropwise a solution of 2-iodobenzoyl chloride (**2a**, 7.5 g, 28.1 mmol) in CH_2Cl_2 (15 mL). The resulting mixture was stirred at 0 °C for 4 h and at room temperature overnight. The solvent was removed in vacuo. The residue was washed with water, dried, and recrystallized from ethanol to afford a white solid (9.07 g, 84.7%). Mp 129–130 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12, 1.13 (3H, d, J = 6.8 Hz, -CH_3), 4.51 (1H, m, -$CHCH_3$), 5.05 (1H, d, J = 2.8 Hz, -CHOH), 5.96 (1H, d, J = 8 Hz, -NH), 7.08–7.12 (1H, m, aromatic), 7.26–7.42 (m, 7H, aromatic), 7.84, 7.86 (1H, d, J = 8.0 Hz, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.74, 51.55, 75.64, 92.44, 126.02, 139.70, 140.66, 141.85, 169.57. Anal. Calcd for $C_{16}H_{16}INO_2$: C, 50.41; H, 4.23; N, 3.67. Found: C 50.25; H, 4.18; N, 3.58.

4.3. 3-Iodo-*N*-(2-hydroxy-1-methyl-2-phenylethyl)benzamide (3b)

The title compound **3b** was obtained from L,D-norephedrine hydrochloride (**1**, 7.02 g, 37.5 mmol) and 3-iodobenzoyl chloride (**2b**, 10 g, 37.5 mmol) following the procedure described for preparation of compound **3a**. Compound **3b**, white solid (12.84 g, 90%). Mp 139–

141 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10 (3H, d, J = 6.9 Hz, CH_3), 3.51 (1H, d, J = 3.3 Hz, –$CHCH_3$), 4.42–4.52 (1H, m, –OH), 4.97 (1H, bs, CHOH), 6.42 (1H, d, J = 7.8 Hz, NH), 7.14 (1H, t, J = 7.8 Hz, aromatic), 7.25–7.42(5H, m, aromatic), 7.69 (1H, d, J = 7.8 Hz, aromatic), 7.82 (1H, d, J = 8.1 Hz, aromatic), 8.08 (1H, s, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.20, 51.45, 76.18, 94.24, 126.14, 127.63, 128.26, 130.19, 136.00, 136.25, 140.41, 140.55, 166.26. Anal. Calcd for $C_{16}H_{16}INO_2$: C, 50.41; H, 4.23; N, 3.67. Found: C, 50.48; H, 4.28; N, 3.62.

4.4. 4-Iodo-*N*-(2-hydroxy-1-methyl-2-phenylethyl)benzamide (3c)

The title compound **3c** was obtained from L,D-norephedrine hydrochloride (**1**, 7.02 g, 37.5 mmol) and 4-iodobenzoyl chloride (**2c**, 10.1 g, 37.9 mmol) following the procedure described for preparation of compound **3a**. Compound **3c**, white solid (12.8 g, 90%). Mp 209–210 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (3H, d, J = 6.6 Hz, CH_3), 4.05–4.12 (1H, m, –$CHCH_3$), 4.65 (1H, s, –CHOH), 5.46 (1H, d, J = 3.6 Hz, NH), 7.14 (1H, t, J = 7.2 Hz, aromatic), 7.23 (2H, t, J = 735 Hz, aromatic), 7.32 (2H, d, J = 7.2 Hz, aromatic), 7.53 (2H, d, J = 8.4 Hz, aromatic), 7.75 (2H, d, J = 8.1 Hz, aromatic), 8.30 (1H, d, J = 8.4 Hz, aromatic). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.70, 51.30, 74.41, 98.48, 126.23, 126.73, 127.75, 129.29, 134.19, 137.03, 143.59, 164.70. Anal. Calcd for $C_{16}H_{16}INO_2$: C, 50341; H, 4.23; N, 3.67. Found: C, 50.35; H, 4.24; N, 3.65.

4.5. 1-(2-Iodophenyl)-3-methyl-isoquinoline (4a)

A mixture of amide (**3a**, 820 mg, 2.15 mmol) and P_2O_5 (3.8 g) in *o*-dichlorobenzene (15 mL) was refluxed overnight. After it was cooled to room temperature, it was further cooled to 0 °C by application of an external ice-water bath. To this cooled mixture was cautiously added 30 mL of water. After the vigorous reaction had subsided, the dark solution was washed with toluene (2× 10 mL). The aqueous layer was cooled to 0 °C and was adjusted to pH > 11 with 50% aqueous sodium hydroxide. The resulting mixture was extracted with toluene (4× 10 mL). The organic layers were dried over sodium sulfate and filtered. The filtrate was concentrated in vacuo. The residue was recrystallized from benzene, thereby affording a white solid (366 mg, 49.4%). Mp 111–112 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.77 (3H, s, -CH_3), 7.16–7.19 (1H, m, aromatic), 7.39–7.65 (6H, m, aromatic), 7.80 (1H, d, J = 8.0 Hz, aromatic), 7.98 (1H, d, J = 8.0 Hz, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.05, 97.82, 118.26, 124.45, 125.98, 126.77, 127.70, 129.43, 129.79, 129.92, 136.73, 138.78, 143.74, 150.24, 161.34. HRMS (ESI), calcd for $C_{16}H_{13}{}^{127}IN$ $[M+H]^+$, 346.00873; found 346.00949 (100%). Anal. Calcd for $C_{16}H_{12}IN$: C, 55.67; H, 3.50; N, 4.06. Found: C, 55.77; H, 3.51; N, 3.96.

4.6. 1-(3-Iodophenyl)-3-methyl-isoquinoline (4b)

The title compound **4b** was obtained from amide (**3b**, 11.54 g, 30.3 mmol) following the procedure described for preparation of compound **4a**. Compound **4b**, white solid (6.5 g, 62%). Mp 77–78 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.72 (3H, s, CH_3), 7.23 (1H, t, J = 7.8 Hz, aromatic), 7.40–7.52 (2H, m, aromatic), 7.57–7.66 (2H, m, aromatic), 7.71–7.85 (2H, m, aromatic), 7.92 (1H, d, J = 8.7 Hz, aromatic), 8.02 (1H, s, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.31, 94.31, 118.36, 126.39, 126.92, 129.08, 129.83, 129.99, 137.34, 137.47, 138.53, 141.70, 150.80, 158.36. Anal. Calcd for $C_{16}H_{12}IN$: C, 55.67; H, 3.50; N, 4.06. Found: C, 55.53; H, 3.56; N, 4.05.

4.7. 1-(4-Iodophenyl)-3-methyl-isoquinoline (4c)

The title compound **4c** was obtained from amide (**3c**, 8.54 g, 22.4 mmol) following the procedure described for preparation of compound **4a**. Compound **4c**, white solid (6.1 g, 79%). Mp 147–148 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.72 (3H, s, CH_3), 7.38–7.51 (4H, m, aromatic), 7.61 (1H, t, J = 6.9 Hz, aromatic), 7.75 (1H, d, J = 8.1 Hz, aromatic), 7.84 (1H, d, J = 8.1 Hz, aromatic), 7.94 (1H, d, J = 8.7 Hz, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.34, 94.58, 118.20, 124.56, 126.31, 126.42, 126.94, 129.96, 131.68, 137.41, 137.51, 139.12, 150.88, 158.97. Anal. Calcd for $C_{16}H_{12}IN$: C, 55.67; H, 3.50; N, 4.06. Found: C, 55.63; H, 3.55; N, 4.07.

4.8. 1-(2-Iodophenyl)-3-bromomethyl-isoquinoline (5a)

A mixture of 1-(2-iodophenyl)-3-methyl-isoquinoline (**4a**, 112 mg, 0.33 mmol), *N*-bromosuccinimide (NBS) (104 mg, 0.59 mmol), and 2,2-azobisisobutyro-nitrile (AIBN) (12 mg, 0.07 mmol) in CCl_4 (6 mL) was heated to reflux for 20 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was washed with saturated $NaHCO_3$ aqueous solution (1× 5 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo, thereby affording crude 1-(2-iodophenyl)-3-bromomethyl-isoquinoline **5a** as yellow oil, which was purified by flash chromatography (20% ethyl acetate/hexane) to give a clear oil (90.2 mg, 64.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.80 (2H, s, –CH_2Br), 7.17–8.01 (9H, m, aromatic). HRMS (ESI), calcd for $C_{16}H_{12}{}^{79}Br^{127}IN$ $[M+H]^+$, 423.91924; found 423.91898 (85.7%).

4.9. 1-(2-Iodophenyl)-3-formyl-isoquinoline (6a)

The mixture of bromide (**5a**, 216 mg, 0.51 mmol) and sodium periodate (110 mg, 0.51 mmol) in DMF (10 mL) was refluxed for 19 h. Water (20 mL) was added and the product was extracted into ether (2× 10 mL). The combined organic layers were washed with water (1× 10 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was subjected to flash chromatography (20% ethyl acetate/hexane) to give **6a** as white semisolid (116 mg, 63.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21–8.11 (8H, m, aromatic), 8.47 (1H, s, aromatic), 10.31 (1H, s, CHO). HRMS (ESI), calcd for $C_{16}H_{11}{}^{127}INO$ $[M+H]^+$, 359.98854; found 359.98798 (100%).

4.10. 1-(2-Iodophenyl)-3-isoquinoline carboxylic acid (7a)

To a solution of aldehyde (**6a**, 274 mg, 0.76 mmol) in ethanol (10 mL) was slowly added a solution of silver ni-

trate (320 mg, 1.9 mmol) in water (5 mL). To this stirred solution was added dropwise a solution of sodium hydroxide (240 mg, 6.1 mmol) in water (5 mL). The resulting black slurry was stirred at room temperature for 2 h. Then the mixture was filtered through a small Celite column. The filter cake was washed with ether. The ether was evaporated and the aqueous solution was adjusted to pH 5 with concentrated hydrochloric acid. The precipitate was collected by filtration, and washed with water. It was recrystallized from CH_3CN, thereby affording a pale yellow crystalline (246 mg, 86.3%). Mp 174–176 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–8.12 (8H, m, aromatic), 8.73 (1H, s, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.05, 97.82, 118.26, 124.45, 125.98, 126.77, 127.70, 129.43, 129.79, 129.92, 136.73, 138.78, 143.74, 150.24, 161.34. HRMS (ESI), calcd for $C_{16}H_{11}{}^{127}INO_2$ $[M+H]^+$, 375.98291; found, 375.98203 (100%).

4.11. 1-(3-Iodophenyl)-3-isoquinoline carboxylic acid (7b)

The crude compound **5b** (530 mg, yellow oil) was obtained from1-(3-iodophenyl)-3-methyl-isoquinoline (**4b**, 573 mg, 1.66 mmol) following the procedure described for preparation of compound **5a**, which was used directly in next step without further purification. The crude product 1-(3-iodophenyl)-3-formyl-isoquinoline **6b** was obtained as dark yellow oil (315 mg) following the procedure described for preparation of compound **6a**. This oil was used without further purification. The title compound **7b** was obtained following the procedure described for preparation of compound **7a**. Compound **7b**, yellow crystalline (222 mg, 36%). Mp 145–146 °C. ^{1}H NMR (300 MHz, DMAO-d_6) δ 7.37 (1H, t, J = 6.0 Hz, aromatic), 7.65–7.71 (2H, m, aromatic), 7.80 (1H, t, J = 4.8 Hz, aromatic), 7.91 (1H, d, J = 6.6 Hz, aromatic), 7.95 (1H, d, J = 6.3 Hz, aromatic), 8.01 (1H, s, aromatic), 8.14 (1H, d, J = 6.0 Hz, aromatic), and 8.44 (1H, s, aromatic). HRMS (EI) calcd for $C1_6H_{10}INO_2$, 374.9756; found: 374.9756.

4.12. 1-(4-Iodophenyl)-3-isoquinoline carboxylic acid (7c)

The crude compound **5c** (240 mg, yellow oil) was obtained from1-(4-iodophenyl)-3-methyl-isoquinoline (**4c**, 259 mg, 0.75 mmol) following the procedure described for preparation of compound **5a**, which was used directly in next step without further purification. The crude product 1-(4-iodophenyl)-3-formyl-isoquinoline **6c** was obtained as dark yellow oil (140 mg) following the procedure described for preparation of compound **6a**. This oil was used without further purification. The title compound **7c** was obtained following the procedure described for preparation of compound **7a**. Compound **7c**, a pale yellow solid (101 mg, 37%). Mp 243–245 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.44 (1H, d, J = 8.1 Hz, aromatic), 7.59 (1H, d, J = 8.1 Hz, aromatic), 7.68–7.78 (2H, m, aromatic), 7.84 (1H, t, J = 7.8 Hz, aromatic), 7.90 (1H, d, J = 8.4 Hz, aromatic), 7.97 (1H, d, J = 8.4 Hz, aromatic), 8.20 (1H, d, J = 8.1 Hz, aromatic), and 8.56 (1H, s, aromatic). HRMS (EI) calcd for $C_{16}H_{10}INO_2$, 374.9756; found: 374.9761.

4.13. 1-(2-Iodophenyl)-*N*-methyl-3-isoquinoline carboxamide (8a)

To a solution of 1-(2-iodophenyl)-3-isoquinoline carboxylic acid (**7a**, 189 mg, 0.5 mmol) in 5 mL of dichloromethane was added dropwise thionyl chloride (180 mg, 1.5 mmol). The mixture was heated to 50 °C for 1 h and the solvent was removed under reduced pressure. The resulting residue was re-dissolved in 5 mL of THF and the mixture was cooled to −70 °C followed by the addition of methylamine (78 mg, 2.5 mmol) in THF (2 mL). The reaction was allowed to warm up to room temperature overnight. Water (5 mL) was added and the compound **8a** was extracted with dichloromethane (3× 5 mL). The combined organic layers were washed with water (1× 5 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo. The residue was purified on column (SiO_2, 50% ethyl acetate/hexane, 0.1% Et_3N), thereby giving a white foam (189.2 mg, 97.5%). Mp 167–168 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05, 3.06 (3H, two s, N-CH_3), 7.21–8.07 (8H, m, aromatic), 8.21 (1H, br s, NH), 8.67 (1H, s, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.13, 97.79, 120.02, 127.29, 127.75, 127.98, 128.53, 128.76, 130.13, 130.31, 130.81, 136.81, 139.42, 142.59, 143.30, 160.79, 165.32. HRMS (ESI), calcd for $C_{17}H_{14}{}^{127}IN_2O$ $[M+H]^+$, 389.01454; found 389.01309 (100%).

4.14. 1-(3-Iodophenyl)-*N*-methyl-3-isoquinoline carboxamide (8b)

The title compound **8b** was obtained from 1-(3-iodophenyl)-3-isoquinoline carboxylic acid (**7b**, 119 mg, 0.32 mmol) following the procedure described for preparation of compound **8a**. Compound **8b**, white solid (59.2 mg, 48%). Mp 172–174 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.03, 3.04 (3H, two s, N-CH_3), 7.24 (1H, t, J = 8.7 Hz, aromatic), 7.55–7.65 (2H, m, aromatic), 7.71 (1H, t, J = 8.1 Hz, aromatic), 7.82 (1H, d, J = 8.7 Hz, aromatic), 7.95–8.05 (3H, m, aromatic), and 8.19 (1H, broad s, CONH), 8.57 (1H, s, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.16, 94.24, 119.86, 127.15, 128.70, 128.96, 129.13, 129.91, 130.77, 137.14, 137.85, 138.57, 140.99, 142.67, 157.62, 165.25. HRMS (EI) calcd for $C_{17}H1_3IN_2O$, 388.0073; found: 388.0071.

4.15. 1-(4-Iodophenyl)-*N*-methyl-3-isoquinoline carboxamide (8c)

The title compound **8c** was obtained from 1-(4-iodophenyl)-3-isoquinoline carboxylic acid (**7c**, 207 mg, 0.55 mmol) following the procedure described for preparation of compound **8a**. Compound **8c**, white solid (172 mg, 81%). Mp 163–164 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.07, 3.09 (3H, two s, N-CH_3), 7.46 (2H, d, J = 8.4 Hz, aromatic), 7.64 (1H, t, J = 7.2 Hz, aromatic), 7.77 (1H, t, J = 6.9 Hz, aromatic), 7.92 (2H, d, J = 8.4 Hz, aromatic), 8.07 (2H, t, J = 7.8 Hz, aromatic), 8.24 (1H, br s, NH), 8.02 (1H, s, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.05, 95.14, 119.54, 127.02, 128.58, 128.75, 130.60, 131.43, 131.56, 137.02, 137.40, 138.28, 142.58, 158.09, 165.12. HRMS (EI) calcd for $C_{17}H_{13}IN_2O$, 388.0073; found: 388.0077.

4.16. 1-(2-Iodophenyl)-*N*-methyl-*N*-(3-fluoropropyl)-3-isoquinoline carboxamide (9a)

To a solution of 1-(2-iodophenyl)-*N*-methyl-3-isoquinoline carboxamide (**8a**, 20 mg, 0.05 mmol) in DMF (2 mL) was added sodium hydride (12 mg, 0.5 mmol) in one portion. The mixture was stirred under argon at room temperature for 40 min and 3-fluropropyl bromide (36 mg, 0.25 mmol) was added. After stirring under argon at room temperature for 24 h, the mixture was quenched by adding water (5 mL). The mixture was extracted with dichloromethane (3× 15 mL). The combined organic layers were dried (Na_2SO_4) and filtered. The filtrate was concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 30% ethyl acetate/hexane, 0.1% Et_3N), thereby affording clear oil (7.9 mg, 35.3%) and the starting material (**8a**, 12.2 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96–2.18 (2H, m, CH_2), 3.16, 3.19 (3H, two s, *N*-CH_3), 3.58–3.82 (2H, m, *N*-CH_2), 4.27, 4.39, 4.54, 4.66 (2H, qt, J = 79.8, 47.1, 6 Hz, CH_2F), 7.20–7.98 (8H, m, aromatic), 8.10, 8.17 (1H, two s, aromatic). ^{19}F NMR (376 MHz, $CDCl_3$) δ −221.17 to −220.31 (1F, m, FCH_2). HRMS (ESI), calcd for $C_{20}H_{19}F^{127}IN_2O$ $[M+H]^+$, 449.05207; found 449.05186 (100%).

4.17. 1-(3-Iodophenyl)-*N*-methyl-*N*-(3-fluoropropyl)-3-isoquinoline carboxamide (9b)

The title compound **9b** was obtained from 1-(3-iodophenyl)-*N*-methyl-3-isoquinoline carboxamide (**8b**, 22 mg, 0.06 mmol) following the procedure described for preparation of compound **9a**. Compound **9b**, semisolid (4.4 mg, 37%). ^{1}H NMR (300 MHz, $CDCl_3$) δ *2.04–2.24 (2H, m, CH_2), 3.16,3.18(3H, two s,* *N*-CH_3), 3.60–3.75 (2H, m, *N*-CH_2), 4.25–4.70(2H, qt, J = 67.8, 47.1, 5.7 Hz, CH_2F), 7.20–7.30 (1H, m, aromatic), 7.55–7.65 (2H, m, aromatic), 7.73 (1H, t, J = 7.2 Hz, aromatic), 7.83 (1H, d, J = 7.8 Hz, aromatic), 7.94 (1H, d, J = 8.4 Hz, aromatic), 7.98–8.09 (2H, m, aromatic), and 8.12 (1H, s, aromatic). HRMS (EI) calcd for $C_{20}H_{18}FIN_2O$, 448.0448; found: 448.0461.

4.18. 1-(4-Iodophenyl)-*N*-methyl-*N*-(3-fluoropropyl)-3-isoquinoline carboxamide (9c)

The title compound **9c** was obtained from 1-(4-iodophenyl)-*N*-methyl-3-isoquinoline carboxamide (**8c**, 25 mg, 0.06 mmol) following the procedure described for preparation of compound **9a**. Compound **9c**, semisolid (11 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ *2.01–2.22 (2H, m, CH_2), 3.15, 3.17 (3H, two s,* *N*-CH_3), 3.60–3.76 (2H, m, *N*-CH_2), 4.22–4.76 (2H, qt, J = 77.7, 47.1, 5.7 Hz, CH_2F), 7.36–7.47 (1H, m, aromatic), 7.5–7.81 (4H, m, aromatic), 7.82–7.99 (2H, m, aromatic), 7.83–8.16 (2H, m, aromatic). HRMS (EI), calcd for $C_{20}H_{18}FIN_2O$, 448.0448; found: 448.0435.

4.19. 1-(2-Iodophenyl)-*N*-methyl-*N*-(2-fluoroethyl)-3-isoquinoline carboxamide (9d)

The title compound **9d** was obtained from 1-(2-iodophenyl)-*N*-methyl-3-isoquinoline carboxamide (**8a**, 104 mg, 0.27 mmol) and 2-fluoroethylbromide (274 mg, 2.16 mmol) following the procedure described for preparation of compound **9a**. Compound **9d**, white solid (48 mg, 41%) was obtained. Mp 142–143 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.22, 3.27 (3H, two s, *N*-CH_3), 3.67–4.15 (2H, m, *N*-CH_2), 4.42–4.92 (2H, m, CH_2F), 7.15–7.25 (1H, m, aromatic), 7.27–7.37(1H, m, aromatic), 7.45–7.64 (3H, m, aromatic), 7.67–7.77 (1H, m, aromatic), 7.97 (2H, t, J = 7.5 Hz, aromatic), 8.11, 8.21 (1H, two s, aromatic). Anal. Calcd for $C_{19}H_{16}FIN_2O$: C, 52.55; H, 3.71; N, 6.45. Found: C, 52.64; H, 3.78; N, 6.38.

4.20. 3-Triphenylmethoxypropyl bromide (10)

To a mixture of 3-bromo-1-propanol (1.39 g, 10 mmol), pyridine (4 mL, 50 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added dropwise a solution of triphenylmethyl chloride (3.13 g, 11 mmol) in CH_2Cl_2 (5 mL). The resulting mixture was heated at 60 °C for 5 h and then kept at room temperature overnight. It was poured into ice-water and extracted with dichloromethane (3× 20 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 10% ethyl acetate/hexane), thereby affording a colorless oil (1.44 g, 37.8%). ^{1}H NMR ($CDCl_3$) δ 2.09–2.13 (2H, m, CH_2), 3.20 (2H, t, J = 6 Hz, CH_2O), 3.56 (2H, t, J = 6.8 Hz, CH_2Br), and 7.21–7.31 (15H, m, aromatic).

4.21. 1-(2-Iodophenyl)-*N*-methyl-*N*-(3-triphenylmethoxypropyl)-3-isoquinoline carboxamide (11)

A mixture 1-(2-iodophenyl)-*N*-methyl-3-isoquinoline carboxamide (**8a**, 65 mg, 0.17 mmol) and sodium hydride (20.4 mg, 0.84 mmol) in DMF (4 mL) was stirred at room temperature for 30 min. To this mixture was added dropwise a solution of 3-triphenylmethoxypropyl bromide (**10**, 128 mg, 0.34 mmol) in DMF (2 mL). The resulting mixture was stirred under argon at room temperature overnight and then quenched by addition of 5 mL water. The mixture was extracted with dichloromethane (3× 15 mL). The combined organic layers were dried (Na_2SO_4) and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography (silical gel, 50% ethyl acetate/hexane, 0.1% Et_3N), thereby affording clear oil (56 mg, 48.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82–1.98 (2H, m, CH_2), 2.95–3.88 (4H, m, CH_2O, CH_2N), 3.05, 3.06 (3H, two s, *N*-CH_3), 7.14–8.07 (23H, m, aromatic), 8.67 (1H, s, aromatic). HRMS (ESI), calcd for $C_{39}H_{34}F^{127}IN_2O_2$ $[M+H]^+$, 689.16596; found 689.16620 (47.9%).

4.22. 1-(2-Iodophenyl)-*N*-methyl-*N*-(3-hydroxypropyl)-3-isoquinoline carboxamide (12)

To a solution of 1-(2-iodophenyl)-*N*-methyl-*N*-(3-triphenylmethoxypropyl)-3-isoquinoline carboxamide (**11**, 187 mg, 0.27 mmol) in dichloromethane (5 mL) was added dropwise trifluoroacetic acid (TFA) (1 mL). The mixture was stirred at room temperature for 40 min. Then it was transferred into a separatory funnel and

washed with saturated sodium bicarbonate solution (2× 10 mL), water (1× 10 mL), and brine (1× 10 mL), then dried (Na_2SO_4), and filtered. After removing the solvent in vacuo, the residue was purified on column (silica gel, 2% MeOH/CH_2Cl_2, 0.1% Et_3N), thereby affording starting material (32 mg) and the title compound as clear oil (50 mg, 41%). ^{1}H NMR ($CDCl_3$) δ 1.85–1.87 (2H, m, CH_2), 3.11, 3.18 (3H, two s, N-CH_3), 3.52–3.95 (4H, m, CH_2N, CH_2O), 7.20–8.02 (8H, m, aromatic), 8.18, 8.26 (1H, two s, aromatic). HRMS (ESI), calcd for $C_{20}H_{20}{}^{127}IN_2O_2$ $[M+H]^+$, 447.05641; found: 447.05692 (100%).

4.23. 1-(2-Iodophenyl)-*N*-methyl-*N*-(3-*p*-toluenesulfonyl-oxypropyl)-3-isoquinoline carboxamide (13)

To a solution of 1-(2-iodophenyl)-*N*-methyl-*N*-(3-hydroxypropyl)-3-isoquinoline carboxamide (**12**, 42 mg, 0.09 mmol) and Et_3N (0.02 mL, 0.14 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added dropwise *p*-toluenesulfonyl chloride (69 mg, 0.36 mmol) in CH_2Cl_2 (1 mL). The resulting mixture was stirred at 0 °C and monitored by TLC. After stirring at 0 °C for 4 h, TLC showed that no starting material was left. Then it was transferred into a separatory funnel and washed with water (1× 10 mL). The organic layer was dried (Na_2SO_4) and filtered. The filtrate was concentrated in vacuo. The residue was purified on column (silica gel, 2% MeOH/CH_2Cl_2, 0.1% Et_3N), thereby affording an oil (19 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97–2.08 (2H, m, CH_2) 2.39, 2.41 (3H, two s, –PhCH_3), 3.05, 3.11 (3H, two s, N-CH_3), 3.45–3.72 (2H, m, CH_2N), 3.89, 4.15 (2H, two m, CH_2O), 7.17–7.99 (12H, m, aromatic), 8.06, 8.15 (1H, two s, aromatic). HRMS (ESI), calcd for $C_{27}H_{26}{}^{127}IN_2O_4{}^{32}S$ $[M+H]^+$, 601.06526; found: 601.06537 (100%).

4.24. Radiosynthesis of [^{18}F]9a

[^{18}F]HF was produced with a Siemens 11 MeV RDS 112 cyclotron by the ^{18}O (p,n) ^{18}F reaction using [^{18}O]H_2O. A fully automated synthesis program developed for the Siemens computer programmable chemistry process control unit (CPCU) was used for the preparation of [^{18}F]**9a**. The CPCU is a valve-and-tubing system which is designed to accommodate one or two glass reaction vessels and a number of reagent and solvent reservoir containers. Briefly, the automated production of [^{18}F]**9a** was performed by reacting 3–5 mg (5–8 μmol) of tosylate precursor **13** in 0.3 mL of DMSO with 3.7–5.6×10^7 MBq NCA [^{18}F]fluoride in the presence of Kryptofix 222 (5 mg, 13.3 μmol) and potassium carbonate (3 mg, 21.7 μmol) at 120 °C for 10 min. After passing a silica SepPak, the product [^{18}F]**9a** was purified by semipreparative reverse-phase HPLC, using mobile phase consisting of 60:40 MeOH/H_2O (v/v) at a flow rate of 6 mL per minute. The desired fractions were combined, diluted 1:2 (v/v) with sterile water, and loaded onto a C-18 SepPak. After washing with 40 mL of saline (0.9% NaCl aq), the radioactive product was eluted from C-18 SepPak with 1 mL of ethanol into a sealed sterile vial containing 9 mL of saline. The resulting solution was passed successively through a 1 μm and then a 0.2 μm Gelman teflon filters into a dose vial, which was ready for use in in vitro and in vivo studies.

4.25. Quality control of purified radioligand [^{18}F]9a

The identity of the radiolabeled product [^{18}F]**9a** was confirmed by co-injection with the authentic sample of non-radioactive **9a** on an analytical HPLC, equipped with UV and radio detectors, eluted with 70:30 MeOH/H_2O (v/v) at a flow rate of 1 mL per minute. The retention time of [^{18}F]**9a** was 4.48 min. Its radiochemical purity was also determined by analytical HPLC.

4.26. Lipophilicity measurement

Measurement of distribution coefficient of radiolabeled [^{18}F]**9a** was performed according to a previously reported procedure.[63] Briefly, the test tubes containing 2 mL of 1-octanol, 2 mL of 0.02 M, pH 7.4, sodium phosphate buffer, and approximately 185–370 MBq portion of the radiotracer were mixed for 10 min at room temperature and then centrifuged at 75g for 5 min. A 0.5 mL portion of samples from the 1-octanol and the buffer layers, respectively, were counted for radioactivity on a Packard Cobra II automated gamma-counter. Each assay condition was performed in triplicate. The $\log P_{7.4}$ values were calculated with decay corrected for each replicate, which were averaged to give the $\log P_{7.4}$ value for the radiotracer.

4.27. Binding assays

PBR binding affinity assays were performed by NovaScreen using a previously reported procedure which was modified.[65–67] Briefly, tissue preparations of rat kidney homogenate were incubated with [^{3}H]PK11195 and competitors (i.e. **9a**–**d**, at 12 concentrations ranging from 10^{-11} to 10^{-6} nM) in an assay buffer. The assay mixture was incubated for 60 min at room temperature with stirring and the resulting samples were rapidly filtered through Whatman GF/B glass-fiber filters pretreated with 0.2% protamine base and washed with cold buffer, pH 7.4. The filters were counted in a liquid scintillation counter at an efficiency of 65% for tritium saturation binding, scatchard and competition experiments were analyzed with the iterative nonlinear least square curve-fitting program.

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