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3-Substituted 1,5-Diaryl-1*H*-1,2,4-triazoles as Prospective PET Radioligands for Imaging Brain COX-1 in Monkey. Part 1: Synthesis and Pharmacology

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Abstract

Cyclooxygenase-1 (COX-1) is a key enzyme in the biosynthesis of proinflammatory thromboxanes and prostaglandins and is found in glial and neuronal cells within brain. COX-1 expression is implicated in numerous neuroinflammatory states. We aim to find a direct-acting positron emission tomography (PET) radioligand for imaging COX-1 in human brain as a potential biomarker of neuroinflammation and for serving as a tool in drug development. Seventeen 3-substituted 1,5-diaryl-1*H*-1,2,4-triazoles were prepared as prospective COX-1 PET radioligands. From this set, three 1,5-(4-methoxyphenyl)-1*H*-1,2,4-triazoles, carrying a 3-methoxy (**5**), 3-(1,1,1-trifluoroethoxy) (**20**), or 3-fluoromethoxy substituent (**6**), were selected for radioligand development, based mainly on their high affinities and selectivities for inhibiting human COX-1, absence of carboxyl group, moderate computed lipophilicities, and scope for radiolabeling with carbon-11 ($t_{1/2}$ = 20.4 min) or fluorine-18 ($t_{1/2}$ = 109.8 min). Methods were developed for producing [^{11}C]**5**, [^{11}C]**20**, and [d_2 - ^{18}F]**6** from hydroxy precursors in a form ready for intravenous injection for prospective evaluation in monkey with PET.

Graphical Abstract

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Author Contributions

P.S. contributed to design of the study and performed chemistry and radiochemistry. S.S., K.J.J., and S.S.Z. established and performed inhibitor assays. M.Y.C.-S. performed chemistry and radiochemistry. C.L.M. performed radiochemistry. R.B.I. provided oversight on assays. V.W.P. conceived and directed the project and drafted the paper. All authors contributed to data analysis, writing, and correcting the manuscript.

Supporting Information

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NMR spectra and HPLC purity data on new compounds, radiochromatograms for radioligand separations and analyses, and examples of inhibitor assay binding curves (PDF)

The authors declare no competing financial interest.

Keywords

COX-1; PET; radioligand; brain; carbon-11; fluorine-18

INTRODUCTION

Cyclooxygenases (COXs), also known as prostaglandin endoperoxide synthases, occur as two isoforms, COX-1 and COX-2, that play a rate-limiting step in the syntheses of proinflammatory thromboxanes and prostaglandins from arachidonic acid.¹ Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen, inhibit both COX-1 and COX-2. Inhibition of the COXs by NSAIDs attenuates both central and peripheral inflammation.² Each COX isoform is considered to play a distinct role^{3,4} in these inflammatory processes.

Although the role of COXs in peripheral inflammation has been investigated extensively, very little is known about the roles of the COXs in central inflammation. Neuroinflammatory processes are now associated with several neurodegenerative and psychiatric illnesses, such as Alzheimer's disease, Parkinson's disease, depression, schizophrenia, autism, and addiction.⁵ Although COX-2 plays a much larger role than COX-1 in peripheral inflammation, COX-1 is considered to be a key contributor to neuroinflammation.³ COX-1 is constitutively expressed in most tissues and organs, including brain.⁶ In healthy brain, COX-1 is almost exclusively localized to resting microglia. In response to various types of inflammatory stimuli, microglia become activated and COX-1 expression increases. Consequently, proinflammatory mediators are increased, generating oxidative stress and processes that ultimately lead to cytotoxicity and neuronal loss.

Genetic modulations in rodents⁷⁻⁹ and neuroinflammation models in animals^{8,10} have unveiled some details of the role of COX-1 in the neuroinflammatory process. For instance, genetic knockout of COX-1 in mice reduced oxidative stress and neuronal damage compared to those in wild-type mice.⁹ In a monkey neuroinflammation model, COX-1 expression increased globally in activated microglia and macrophages.¹⁰ Taken together, these findings suggest that COX-1 could be a promising biomarker for studying neuroinflammation.

An effective radioligand for the selective imaging of COX-1 in the brains of living animal and human subjects with positron emission tomography (PET) would be a useful tool for increasing our understanding of the roles of COX-1 in neuroinflammation and the progression of neuropsychiatric disorders. An effective radioligand could also assist in the development of drugs targeting COX-1.

Only two candidates have been evaluated as potential direct-acting PET radioligands for imaging COX-1 within brain, but neither has been found successful. These radioligands, [¹⁸F]1 ([¹⁸F]SC63217)¹¹ and [¹¹C]2,¹² are both 1,5-diarylpyrazoles (Chart 1), and they failed because of low enzyme affinity, low brain uptake, and high nonspecific binding. *S*-Ketoprofen (4) is a selective inhibitor of human COX-1,¹³ but has poor ability to cross the blood-brain barrier because of almost complete ionization of its carboxyl group at physiological pH. To circumvent this issue with 4, [¹¹C](*S*)-ketoprofen methyl ester ([¹¹C]3;

Scheme 1)¹⁴ has been studied as a “prodrug” type PET radioligand. After intravenous administration, [¹¹C]**3** readily enters brain where it rapidly undergoes hydrolysis to [¹¹C] (*S*)-ketoprofen ([¹¹C]**4**; Scheme 1). [¹¹C]**3** has been successful for imaging COX-1 in brains of mice¹⁴ and rats¹⁵ that have been treated with lipopolysaccharide to induce neuroinflammation with accompanying activated microglia. Although [¹¹C]**3** also enters human brain,¹⁶ it does not detect neuroinflammation in Alzheimer’s disease.¹⁷ [¹¹C]**4** likely has too low an affinity for human COX-1 (*IC*₅₀ of 47 nM)¹³ to be effective for detection of COX-1 in human brain. Such a low affinity would require very high concentrations of COX-1 (>250 nM) to exist in one or more brain regions to provide sufficient binding potential ($B_{\text{max}}/K_D > 5$)¹⁸ for measurement with PET using [¹¹C]**4**. In general, successful radioligands for imaging enzymes in brain have low nanomolar or subnanomolar affinities.¹⁹ COX-1 is known to be widely expressed in human brain, primarily in frontal, temporal and cerebellar cortices, hippocampus (CA2, CA3, and CA4), and caudate, but the absolute concentrations (B_{max} values) are unknown.²⁰ Another drawback of using a prodrug-type radioligand, such as [¹¹C]**3**, is that satisfactory biomathematical modeling of the PET data may be difficult to achieve because of the extra requirement to consider the kinetics of radioligand hydrolysis. Hitherto, there have been no direct-acting radioligands to image COX-1 in human brain with PET.

Our eventual aim is to find a direct-acting PET radioligand to image COX-1 in human brain. For this purpose, we searched the literature to find compounds that showed properties^{19,21,22} in vitro that are desirable for PET radioligand development, such as high potency and selectivity for inhibiting human COX-1, absence of a carboxyl group, moderate computed lipophilicity (clog *D*), and scope for labeling with either carbon-11 (*t*_{1/2} = 20 min) or fluorine-18 (*t*_{1/2} = 110 min). We found that the 1,5-diaryl-1,2,4-triazole-based COX-1 inhibitor, **5** (**FK881**) (*IC*₅₀ = 4.9 nM; clog *D* = 3.72)²³ (Chart 2), met these prima facie requirements.

Several patented analogues of **5**, having various substituents on the 1*H*-1,2,4-triazole ring, also have high inhibitory potencies for human COX-1 (*IC*₅₀ < 10 nM) with appreciable selectivities (> 10-fold) over inhibitory potencies for human COX-2.^{24,25} On this basis, we decided to prepare **5** and several analogues that might present even better properties for PET radioligand development. Altogether, 17 compounds were prepared and tested for COX-1 and COX-2 inhibitory potencies in enzymatic immunoassays on rat, monkey, and human blood. cLog *D* values were also calculated. Three COX-1 inhibitors emerged as meriting labeling with a positron-emitter for subsequent evaluation of radioligand behavior with PET in monkey.

RESULTS AND DISCUSSION

Chemistry.

All the prepared inhibitors feature a common 3-substituted 1,5-bis(aryl)-1*H*-1,2,4-triazole scaffold. Compounds **5–14** (Table 1) were chosen for synthesis based on reported²⁴ high potency for inhibiting human COX-1 (*IC*₅₀ < 10 nM) and much lower potency for inhibiting human COX-2 (*IC*₅₀ > 100 nM), plus our observations of (i) a clog *D* value of less than 4,

(ii) fewer than 8 heteroatoms, (iii) a predicted pK_a of less than 9, (iv) a calculated tPSA of less than 80 Å, (v) a molecular weight of less than 500 Da, and (vi) the presence of at least one group amenable to labeling with carbon-11 or fluorine-18 by radioalkylation with either [^{11}C]iodomethane or [d_2 - ^{18}F]-fluorobromomethane (Table 1). Such properties are generally considered desirable in candidate PET radioligands.^{19,19} Analogues (**24**, **26–28**, **31**, and **32**) of these inhibitors, with unknown inhibitory potencies for COX-1 or COX-2 but otherwise desirable radioligand properties, were also chosen for synthesis. These included a 2-fluoropyridyl compound (**24**) and four fluoromethyl compounds (**26–28**, **32**) with potential to be labeled with fluorine-18 or with carbon-11. All the inhibitors, except for **14**, carried a relatively small substituent in the 3-position. In some inhibitors (**10**, **12**, **13**, **31**, and **32**), the pendant 1-aryl or 5-aryl substituent, or both, differed from a more usual 4-methoxyphenyl substituent.

Inhibitors **5–14** were prepared as described.²⁴ The known inhibitor **20** was prepared by a different route to that reported.²⁴ Thus, 2-(4-methoxyphenyl)hydrazine carboxamide (**15**)²⁴ was treated with 4-(benzyloxy)benzoyl chloride to give 2-(4-(benzyloxy)benzoyl)-2-(4-methoxyphenyl)hydrazine carboxamide (**16**). Cyclization of **16** under basic conditions gave 5-(4-(benzyloxy)phenyl)-1-(4-methoxyphenyl)-1*H*-1,2,4-triazol-3-ol (**17**). Alkylation of **17** with 2-chloro-1,1,1-trifluoro-ethane gave **18**. Treatment of **18** with boron trichloride selectively removed the *O*-benzyl group to give the phenol **19**, which later served as a precursor for radiolabeling. Inhibitor **20** was then obtained by treatment of **19** with iodomethane under basic conditions (Scheme 2). All steps proceeded in at least moderately high yields.

Inhibitors **24** and **26–28** are new. Inhibitor **24** was obtained in three steps starting with the addition of 2,2,2-trifluoroacetimidamide to (4-methoxyphenyl)hydrazine hydrochloride (**21**) to give crude (*Z*)-2,2,2-trifluoro-*N'*-(4-methoxyphenyl)-acetohydrazonamide (**22**). Crude **22** was then treated with 6-chloronicotinoyl chloride to effect cyclization to the chloro intermediate **23** in good yield. Fluoro for chloro exchange in **23** then gave **24** in moderate yield (Scheme 3).

Treatment of 1,5-bis(4-methoxyphenyl)-1*H*-1,2,4-triazol-3-thiol (**25**)²⁴ with fluoroiodomethane in the presence of sodium hydride gave the fluoromethyl thioether inhibitor **26** in good yield (Scheme 4). Time- and stoichiometry-controlled oxidations of **26** then gave the sulfoxide **27**, and finally the sulfone **28** in high yields (Scheme 4).

Inhibitors **31** and **32** were obtained in three steps, each starting from 2-(4-methoxyphenyl)hydrazinecarboxamide (**15**) (Scheme 5). Treatment of **15** with benzoyl chloride gave the amide **29** in good yield. Cyclization of **29** with alcoholic potassium hydroxide gave the hydroxy intermediate 1-(4-methoxyphenyl)-5-phenyl-1*H*-1,2,4-triazol-3-ol (**30**) in high yield. Treatment of **30** with iodomethane or fluoroiodomethane under basic conditions gave the methoxy and fluoromethoxy ethers **31** and **32**, respectively, in moderate yields.

COX-1 and COX-2 Inhibition Assays.

The patent²⁴ first describing the synthesis of COX-1 inhibitors **6–14** had not specified human COX-1 inhibitory potencies beyond stating that the IC_{50} 's were less than 10 nM. Because PET radioligand binding potential (B_{max}/K_D) is directly proportional to radioligand affinity ($1/K_D$) and low nanomolar affinities are often required in successful PET radioligands,¹⁹ more precise measures of human COX-1 inhibitory potency were needed. Moreover, because radioligands must be evaluated with PET in animals preceding any evaluation in human subjects, we also needed to measure COX-1 inhibitory potencies in a species available to us for PET imaging, namely rat or rhesus monkey. We also needed to be sure that inhibitory potencies in the selected preclinical species would be close to those for human. In addition to high affinity for the imaging target, PET radioligands should show much lower affinity for off-target sites. Of main concern was that many COX inhibitors do not show selectivity for binding to either isoform because of their close structural homology.²⁶ Therefore, in selecting candidate PET radioligands, we sought very low potency for inhibiting human COX-2, tantamount to over 100-fold greater potency for inhibition of COX-1 over COX-2. Consequently, we also assayed our set of compounds for inhibitory potency on monkey and human blood COX-2. The assay kit for COX-2 could not however be used for rat blood.

Results from the testing of **4**, the 17 triazole-based inhibitors, and the COX-2 selective inhibitor **34** for inhibitory potencies in COX-1 and COX-2 enzymatic immunoassays¹³ using fresh blood from human and monkey are shown in Table 1. Compound **4**, a nontriazole, showed only moderate potency for inhibition of human COX-1, as previously reported,¹³ and only moderate selectivity for inhibition of this isoform over human COX-2. The results that we obtained for inhibition of human whole blood COX-1 ($IC_{50} = 5$ nM) and human whole blood COX-2 ($IC_{50} > 1000$ nM) by the triazole **5 (FK881)** (Table 1) also agreed well with those already reported (4.9 and 3200 nM, respectively).²³ All the other prepared triazoles, except **14**, showed sub-10-nM potency for inhibition of human COX-1 and above 100-nM potency for inhibition of human COX-2, in accord with the patent report²⁴ for inhibitors **6–14** and **20**. Among all the tested inhibitors, **14** has the bulkiest 3-substituent and was found to have the lowest COX-1 inhibitory potency (IC_{50} , 42 nM). The nontriazole **34**²⁷ was confirmed to be a highly potent and selective COX-2 inhibitor. Generally, the potencies of the triazoles for inhibiting human COX-1 resembled their potencies for inhibiting rhesus monkey COX-1; in just a few cases were somewhat higher (**7**, **8**, **24**, and **28**). Therefore, monkey appeared to be an excellent animal model for assisting the selection of candidate radioligands that might be later evaluated in human.

Inhibitors **4–6** and **20** were also tested in a COX-1 enzymatic immunoassay using fresh rat blood. However, these compounds showed much less potency for inhibiting COX-1 from rat than for inhibiting COX-1 from monkey or human (Table 1). Therefore, we concluded that rat was not useful for the preclinical evaluation of candidate COX-1 radioligands with PET.

Selection of compounds for radiolabeling was based on various considerations. First, compounds with monkey and/or human COX-1 IC_{50} 's greater than 5 nM were omitted from consideration (**8**, **12–14**, **24**, **26**, **28**, **32**), except **7**, which showed equally highest inhibitory

potency for human COX-1 ($IC_{50} \sim 1$ nM). Compound **11** was omitted from consideration because of less than 100-fold selectivity for inhibiting human COX-1 over human COX-2. Two compounds, **6** and **27**, with potential for labeling with fluorine-18, presented quite similar inhibitory potencies. Compound **6** was preferred because of its potential to be labeled in a single-step with [d_2 - ^{18}F]-fluorobromomethane as labeling agent, whereas the labeling of **27** would have required an extra step of thioether oxidation. Compounds **5**, **7**, **9**, **10**, **20**, and **31** showed high inhibitory potency for human COX-1 with **5**, **7**, **9**, **20**, and **31** showing well over 100-fold less inhibitory potency for human COX-2. From this group, we selected **5** and **20** for labeling with carbon-11 after we had considered other factors, such as access to labeling precursor.

Pharmacological Screening.

COX-1 inhibitors **5–9**, **11–14**, **20**, **24**, and **26–28** were found to have K_i values exceeding 10 μ M for a wide range of human recombinant receptors and binding sites (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT₃, 5-HT_{5A}, 5-HT₆, and 5-HT₇, α_{1A} , α_{1B} , α_{1d} , α_{2A} , α_{2B} , β_1 , β_2 , β_3 D₁, D₂, D₄, D₅, DAT, DOR, GABA, H₁, H₂, H₃, H₄, KOR, M₁, M₂, M₄, M₅, MOR, NET, PBR, SERT, σ_1 , σ_2 , σ_2 PC12, and the rat brain benzodiazepine site). These results attest to the general specificity of this class of compounds for inhibition of human COX-1 versus interactions with other binding sites and receptors.

Radiochemistry.

[^{11}C]**5** was prepared by methylation of the hydroxy triazole **33** with no-carrier-added (NCA) [^{11}C]-iodomethane in DMF under basic conditions at room temperature for 5 min. [d_2 - ^{18}F]**6** was obtained by treating **33** with NCA [d_2 - ^{18}F]-fluorobromomethane, cesium carbonate, and 18-crown-6 at 80°C for 10 min. [^{11}C]**20** was obtained by treating the phenol precursor **19** with NCA [^{11}C]-iodomethane in DMSO under basic conditions at 70°C for 3 min. (Scheme 6). These labeling methods were fully automated and extended to include first pass HPLC separation and formulation for intravenous injection. Each radioligand was readily obtained in activities sufficient for subsequent PET experiments in monkey. The formulated radioligands had high radiochemical purities, moderately high molar activities, and negligible chemical impurities (see the Supporting Information).

CONCLUSIONS

Among the evaluated 3-substituted 1,5-diaryl-1*H*-1,2,4-triazoles, inhibitors **5**, **6**, and **20** showed the most favorable pharmacology for further evaluation as potential PET radioligands for imaging brain COX-1 in monkey and human. Each of these inhibitors was readily labeled with either carbon-11 or fluorine-18 in moderately high molar activity and was readily purified and formulated for intravenous injection. Part 2 describes the evaluation of these three candidate radioligands in monkey with PET for the quantitative imaging of COX-1 in brain.²⁸

METHODS

Materials and Methods.

(*S*)-Ketoprofen methyl ester (**3**) was prepared²⁹ from (*S*)-ketoprofen (**4**), purchased from Sigma-Aldrich (St. Louis, MO). The following compounds were prepared as previously described: inhibitors **5–14**;²⁴ starting materials 2-(4-methoxyphenyl)hydrazinehydrochloride (**15**);²⁵ and (4-methoxyphenyl)hydrazine hydrochloride (**21**);²⁴ and the labeling precursors 1,5-bis(4-methoxyphenyl)-1*H*-1,2,4-triazol-3-thiol (**25**)²⁵ and 1,5-bis(4-methoxyphenyl)-1*H*-1,2,4-triazol-3-ol (**33**).²⁴ [2-(4-Methanesulfonylphenyl)-6-methoxypyrimidin-4-yl]thiophen-2-ylmethylamine (**34**), which is highly selective for inhibiting human COX-2 over human COX-1, was prepared as previously described.²⁷

COX-1 and COX-2 enzymatic immunoassay kits were purchased from Cayman Chemical (Ann Arbor, MI). IC_{50} values were obtained with a detection platform spectrophotometer (SpectraMax i3x Multi-Mode; Molecular Devices LLC; CA).

Reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further treatment. Air-sensitive reagents were stored under nitrogen in a glovebox. Precoated silica gel layers (0.2 mm; Grace; Deerfield, IL) were used for TLC and compounds were observed under 254 nm light. Synthesized compounds were isolated with a Teledyne R_F+ chromatography apparatus using silica gel cartridges (24 g, Redi-SepR_F; Isco: Lincoln, NE) with hexane and ethyl acetate as mobile phases (general method A). To achieve greater than 95% purity, some compounds were purified with preparative reversed phase HPLC (Beckman Coulter; Fullerton, CA) on an XBridge C18 column (19 × 150 mm, 10 μm; Waters; Milford MA) eluted at 6 mL/min with a gradient mobile phase composed of aq. ammonium hydroxide (0.25 mM) (A) and acetonitrile (B) starting at 20% B rising linearly to 80% B at 30 min (general method B). Eluate was monitored for absorbance at 254 nm. Melting points were determined on a SMP20 apparatus (Stuart; Staffordshire, UK). ¹H NMR (400.13 MHz), ¹³C NMR (100.62 MHz), and ¹⁹F NMR (376.46 MHz) spectra were recorded on an Avance 400 instrument (Bruker; Billerica, MA). ¹H NMR and ¹³C NMR chemical shifts are reported in δ units (ppm) downfield relative to the chemical shift for tetramethylsilane. Abbreviations bs, d, m, s, and t denote broad singlet, doublet, multiplet, singlet, and triplet, respectively. LC-MS (ESI) was performed on a Velos Pro instrument (Thermo Scientific, San Jose, CA) using reversed phase chromatography on a Luna C18 column (50 × 2 mm, 3 μm; Phenomenex; Torrance, CA) eluted at 200 μL/min with a gradient mobile phase composed of water–methanol–acetic acid (90:10:0.5 by vol.; C) and methanol–acetic acid (100:0.5, v/v; D), starting with C:D at 80:20 for 0.25 min, changed linearly to 20:80 over 3 min, and then held at 20:80 for 3.5 min (method 1) or starting with C:D 80:20 for 0.25 min, changed linearly to 10:90 over 3 min, and then held at 10:90 for 3.5 min (method 2). HRMS data were obtained at the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois at Urbana–Champaign (Urbana, IL) under electron ionization conditions using a double-focusing high-resolution mass spectrometer (Micromass, Waters; Milford, MA). The chemical purities of all prepared inhibitors were shown to be >95% with analytical reversed phase HPLC on a Luna C18 (2) column (250 × 4.6 mm, 10 μm; Phenomenex; Torrance, CA) eluted isocratically with ammonium formate

(10 mM)–acetonitrile (50:50) at 2 mL/min (general method C). Eluate was monitored for absorbance at 254 nm (see the Supporting Information).

γ -Radioactivity from carbon-11 or fluorine-18 was measured with a calibrated dose calibrator (Atomlab 300, Biodex Medical Systems, Shirley, NY) or an automatic γ -counter (Wizard 3", 1480; PerkinElmer; Waltham, MA). Radioactivity measurements were corrected for physical decay. All radiochemistry was performed in a lead-shielded hot-cell for personnel protection from radiation. The molar activities³⁰ of prepared radioligands were obtained by HPLC measurement of carrier associated with a measured activity of analyte. The HPLC system response (absorbance at 254 or 274 nm per mass of ligand) was calibrated by injections of reference compound of known amounts.

cLog *D* values and pK_a values were computed with Pallas for Windows software version 3.8 in default option (CompuDrug International; Bal Harbor, FL). tPSAs were estimated with ChemDraw version 10.

Grouped data are reported as mean $\pm$ SD.

Synthesis.

2-(4-(Benzyloxy)benzoyl)-2-(4-methoxyphenyl)-hydrazinecarboxamide (16).—A mixture of 2-(4-methoxyphenyl)-hydrazinecarboxamide (**15**; 2.0 g, 1.1 mmol), 4-(benzyloxy)benzoyl chloride (3.40 g, 13.8 mmol, 1.25 equiv), and pyridine (1.1 g, 13.8 mmol, 1.25 equiv) in toluene (30 mL) was heated for 1 h at 110°C. Ethyl acetate (450 mL), THF (50 mL), and water (100 mL) were added, and the reaction mixture was sonicated for 20 min. The resultant solid was filtered off, washed with cold water, and dried under reduced pressure to give **16** as a white solid (3.87 g; 90%). All characterization data agree with literature values.²⁵

5-(4-(Benzyloxy)phenyl)-1-(4-methoxyphenyl)-1H-1,2,4-triazol-3-ol (17).—A mixture of **16** (0.40 g, 1.02 mmol), aq KOH solution (10% w/v; 2.7 mL) and anhydrous ethanol (99.5%; 200 proof; 1.35 mL) was heated at 65°C for 1.5 h and then cooled to room temperature. Solvent was removed under reduced pressure. Hydrochloric acid (2 M) was then added dropwise to the residue until the pH became 2. The resultant white solid was filtered off, washed with cold water, and dried under vacuum to give **17** (0.30 g; 78%). All characterization data agree with literature values.²⁵

5-(4-(Benzyloxy)phenyl)-1-(4-methoxyphenyl)-3-(2,2,2-trifluoroethoxy)-1H-1,2,4-triazole (18).—Compound **17** (0.5 g, 1.34 mmol), 2-chloro-1,1,1-trifluoroethane (0.8 g, 6.7 mmol, 5 equiv), KI (0.67 g, 4.02 mmol, 3 equiv), and K₂CO₃ (0.93 g, 6.7 mmol, 5 equiv) were mixed together in DMF (5 mL) and stirred at room temperature for 24 h. Water (25 mL) and ethyl acetate (20 mL) were poured into the reaction mixture. The organic layer was separated off, washed with brine (25 mL $\times$ 2) and dried (MgSO₄). Solvent was removed under reduced pressure and the residue was purified with flash chromatography (20–50% ethyl acetate in hexane; general method A) to give **18** as a white solid (0.47 g; 77%). All characterization data agree with literature values.²⁵

4-(1-(4-Methoxyphenyl)-3-(2,2,2-trifluoroethoxy)-1H-1,2,4-triazol-5-yl)phenol (19).—A solution of BCl_3 (1.0 M) in dichloromethane (0.5 mL) was added dropwise to a solution of **18** (100 mg, 0.22 mmol) in dichloromethane (6 mL) at -20°C under inert atmosphere. The resulting mixture was stirred for 3 h at -20°C , and then for 1 h at 0°C . Solvent and unreacted BCl_3 were removed under reduced pressure. The residue was purified with flash chromatography (10–20% ethyl acetate in hexane; general method A) to give **19** as a white solid (51 mg; 64%). All characterization data agree with literature values.²⁵

1,5-Bis(4-methoxyphenyl)-3-(2,2,2-trifluoroethoxy)-1H-1,2,4-triazole (20).—Compound **19** (1.0 g, 3.4 mmol), iodomethane (2.4 g, 17 mmol, 5 equiv), K_2CO_3 (1.4 g, 10.2 mmol, 3 equiv) and DMF (5 mL) were mixed in a one-neck round-bottom flask and stirred at room temperature. Reaction progress was monitored with TLC (silica gel; ethyl acetate–hexane; 1:1 v/v). After 16 h, the reaction mixture was poured into water (50 mL) and extracted with ethyl acetate (40 mL $\times$ 3). The organic layers were combined, washed with brine–water (1:1 v/v; 40 mL $\times$ 2) and dried (MgSO_4). Solvent was removed under reduced pressure, and the residue was purified with flash chromatography (20–50% ethyl acetate in hexane; general method A), followed by preparative HPLC (general method B) to give **20** as a white solid (0.97 g; 75%). Mp $91.4\text{--}93.2^\circ\text{C}$. ^1H NMR (CD_3OD , δ) 7.40–7.37 (m, 2H), 7.31–7.26 (m, 2H), 7.03–6.99 (m, 2H), 6.95–6.88 (m, 2H), 4.87 (q, $J = 8.5$ Hz, 2H), 3.85 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (CDCl_3 , δ) 166.27, 161.01, 159.86, 153.48, 131.07, 130.28, 127.05, 124.36, 121.60, 119.68, 118.84, 114.62, 113.96, 66.25, 65.89, 65.52, 65.16, 55.57, 55.31. ^{19}F NMR ($\text{DMSO}-d_6$, δ) -76.10 . HPLC (t_R (general method B) = 7.5 min; 99% purity). HRMS-ESI (m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{16}\text{F}_3\text{N}_3\text{O}_3$, 380.1222; found, 380.1221.

(Z)-2,2,2-Trifluoro-N'-(6-methoxypyridin-3-yl)-acetohydrazonamide (22).—(4-Methoxyphenyl)hydrazine hydrochloride (**21**, 2.52 g, 14.4 mmol) was treated with 2,2,2-trifluoroacetimidamide (1.79 g, 15.9 mmol, 1.11 equiv) in methanol (10 mL) in the presence of triethylamine (2.8 g, 21.6 mmol, 1.5 equiv) under an inert atmosphere, at room temperature for 18 h. The reaction mixture was then poured into a mixture of water (10 mL) and ethyl acetate–THF (9:1 v/v; 25 mL). The organic layer was separated off, washed with brine (20 mL $\times$ 2), and dried (MgSO_4). The solvent was removed under reduced pressure and the resultant thick black liquid (**22**) was used without further purification to prepare **23**.

2-Chloro-5-(1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)pyridine (23).—Compound **22** (1.0 g, 4.3 mmol) and pyridine (0.5 mL) were mixed in 1,4-dioxane (11 mL) in a two-necked round-bottom flask. 6-Chloronicotinoyl chloride (0.83 g, 4.7 mmol, 1.1 equiv) was dissolved separately in 1,4-dioxane (11 mL) and added dropwise to the reaction mixture. The resulted mixture was refluxed at 101°C for 4 h. After cooling the mixture to room temperature, hydrochloric acid (0.1 M; 6.4 mL) was added with stirring to neutralize the unreacted base. This mixture was then poured into a mixture of ethyl acetate (50 mL) and water (50 mL). The organic layer was separated off, washed with brine (20 mL $\times$ 2), and dried (MgSO_4). Solvent was then removed under reduced pressure. The residue was purified with flash chromatography (30–60% ethyl acetate in hexane; general method A) to give **23** as a light brown solid (1.06 g; 70%). Mp $103.0\text{--}105.3^\circ\text{C}$. ^1H

NMR (CDCl₃, δ): 8.48 (dd, J = 2.5, 0.8 Hz, 1H), 7.89 (dd, J = 8.4, 2.5 Hz, 1H), 7.37 (dd, J = 8.4, 0.8 Hz, 1H), 7.35–7.27 (m, 2H), 7.05–6.96 (m, 2H), 3.88 (s, 3H). ¹³C NMR (CDCl₃, δ): 161.04, 154.32, 153.92, 153.58, 152.23, 149.33, 138.71, 129.42, 127.01, 124.44, 121.97, 120.40, 115.25, 55.71. ¹⁹F NMR (DMSO-*d*₆, δ) = –63.72. HRMS-ESI (m/z): [M + H]⁺ calcd for C₁₄H₉ClF₃N₄O, 355.0573; found, 355.0573.

2-Fluoro-5-(1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)pyridine (24).—Compound **23** (0.50 g, 1.4 mmol), potassium fluoride (0.12 g, 2.1 mmol, 1.5 equiv), 18-crown-6 (0.37 g, 1.4 mmol) and DMSO (2 mL) were mixed together under an inert atmosphere in a pressure tube and heated at 100°C for 24 h. Water (20 mL) and ethyl acetate (20 mL) were added. The organic layer was separated off, washed with brine (10 mL $\times$ 2), dried (MgSO₄), and concentrated to give the crude product, which was purified with preparative HPLC (general method B) to afford **24** as a white solid (0.02 g; 43%). Mp 176.7–178.0°C. ¹H NMR (CD₃OD, δ) 8.39–8.33 (m, 1H), 8.03 (ddd, J = 8.6, 7.4, 2.5 Hz, 1H), 7.46–7.36 (m, 2H), 7.13 (ddd, J = 8.6, 7.4, 2.5 Hz, 1H), 7.11–7.06 (m, 2H), 3.87 (s, 3H). ¹³C NMR (CD₃OD, δ) 166.91, 164.49, 162.62, 154.21, 149.51, 149.35, 143.61, 143.52, 130.86, 128.57, 122.97, 122.92, 116.11, 111.16, 110.79, 56.24. HPLC (general method C; t_R = 8.68 min; 98.6% purity). HRMS-ESI (m/z): [M + H]⁺ calcd for C₁₅H₁₀F₄N₄O, 366.1063; found, 366.1063.

3-(Fluoromethylthio)-1,5-bis(4-methoxyphenyl)-1H-1,2,4-triazole (26).—1,5-Bis(4-methoxyphenyl)-1H-1,2,4-triazol-3-thiol (**25**; 0.53 g, 1.68 mmol) was heated at 100°C for 3.5 h with fluoroiodomethane (1.3 g, 8.4 mmol, 5 equiv) and potassium carbonate (0.7 g, 5.04 mmol, 3 equiv) in DMF (5 mL) and cooled. After 3.5 h, the reaction mixture was cooled to room temperature. Water (25 mL) and ethyl acetate (20 mL) were poured into the reaction mixture. The organic layer was separated off, washed with brine (20 mL $\times$ 2), and dried (MgSO₄). Solvent was removed under reduced pressure and the residue was purified with flash chromatography (30–60% ethyl acetate in hexane; (general method A) followed by preparative HPLC (general method B) to give **26** as a white solid (0.43 g; 74%). Mp 93.4–95.6°C. ¹H NMR (CDCl₃, δ) = 7.45–7.41 (m, 2H), 7.29–7.25 (m, 2H), 6.94–6.90 (m, 2H), 6.85–6.81 (m, 2H), 6.17 (s, 1H), 6.04 (s, 1H), 3.84 (s, 3H), 3.80 (s, 3H). ¹³C NMR (CD₃OD, δ) = 162.94, 161.90, 158.60, 158.57, 157.02, 131.94, 131.59, 128.44, 120.27, 115.78, 115.19, 87.08, 84.91, 56.18, 55.95. ¹⁹F NMR (DMSO-*d*₆, δ) = –188.39. HPLC (general method C; t_R = 10.4 min; 99.8% purity). HRMS-ESI (m/z): [M + H]⁺: calcd for C₁₇H₁₆FN₃O₂S, 346.1026; found, 346.1019

3-(Fluoromethylsulfinyl)-1,5-bis(4-methoxyphenyl)-1H-1,2,4-triazole (27).—A mixture of **26** (100 mg, 0.31 mmol) and *m*CPBA (76 mg, 0.46 mmol, 1.5 equiv) in dichloromethane (1 mL) was stirred at room temperature for 3 h. Dichloromethane (20 mL) and saturated sodium bicarbonate solution (10 mL) were poured into the mixture. The organic layer was separated off and dried (MgSO₄). The solvent was removed under reduced pressure, and the residue was purified with preparative HPLC (general method B) to give **27** as a light yellow oil, which solidified on storage at 4°C (98 mg; 88%). Mp 116.6–118.4°C. ¹H NMR (CDCl₃, δ) 7.50–7.45 (m, 2H), 7.32–7.28 (m, 2H), 6.98–6.94 (m, 2H), 6.86–6.84 (m, 2H), 5.82–5.77 (m, 1H), 5.71–5.65 (m, 1H), 3.86 (s, 3H), 3.82 (s, 3H). ¹³C NMR

(CDCl₃, δ) 161.50, 161.18, 161.08, 160.44, 156.33, 130.56, 130.42, 126.95, 118.68, 114.77, 114.17, 97.65, 95.50, 55.63, 55.39. ¹⁹F NMR (DMSO-*d*₆, δ) –183.07. HPLC (general method C; *t_R* = 12.2 min; 99.2% purity). HRMS-ESI (*m/z*): [M + H]⁺, calcd for C₁₇H₁₆FN₃O₃S, 362.0975; found, 362.0969.

3-(Fluoromethylsulfonyl)-1,5-bis(4-methoxyphenyl)-1H-1,2,4-triazole (28).—A mixture of **26** (100 mg, 0.31 mmol) and *m*CPBA (200 mg, 1.16 mmol, 4 equiv) in dichloromethane (1 mL), was stirred at room temperature for 5 h. The solvent was then removed under reduced pressure. The residue was purified with preparative HPLC (general method B) to give **28** as a white solid (91 mg; 78%). Mp 127.3–128.1°C. ¹H NMR (CD₃OD, δ) 7.41–7.37 (m, 2H), 7.32–7.28 (m, 2H), 7.01–6.97 (m, 2H), 6.89–6.84 (m, 2H), 5.67 (s, 1H), 5.56 (s, 1H), 3.79 (s, 3H), 3.74 (s, 3H). ¹³C NMR (CD₃OD, δ) 163.41, 162.46, 159.86, 158.28, 131.89, 131.48, 128.52, 119.52, 115.94, 115.30, 93.43, 91.27, 56.21, 55.97. ¹⁹F NMR (DMSO-*d*₆, δ) –187.84. HPLC (general method C; *t_R* = 7.9 min, > 99.9% purity). HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₁₇H₁₆FN₃O₄S, 378.0924; found, 378.0919

2-Benzoyl-2-(4-methoxyphenyl)hydrazine-1-carboxamide (29).—A mixture of 2-(4-methoxyphenyl)hydrazinecarboxamide (**15**; 1.0 g, 5.5 mmol), benzoyl chloride (0.96 g, 6.87 mmol, 1.25 equiv), and pyridine (1.55 g, 6.9 mmol, 1.25 equiv) in toluene (30 mL) was heated for 4 h at 110°C. A mixture of ethyl acetate (450 mL), THF (50 mL) and water (100 mL) was added and sonicated for 20 min. The white precipitate was filtered off, washed with cold water and dried under reduced pressure to give **29** (1.07 g; 68%). Mp 130.3–132.1°C. ¹H NMR (DMSO-*d*₆, δ) 8.89 (s, 1H), 7.52 (s, 2H), 7.40–7.28 (m, 5H), 6.90 (d, *J* = 8.02 Hz), 3.74 (s, 2H), 3.41 (bs, 2H). ¹³C NMR (DMSO-*d*₆, δ) 157.84, 136.43, 130.21, 128.13, 126.99, 114.08, 55.74, 49.06. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₁₈H₁₆F₃N₃O₃, 308.1011; found, 308.1006.

1-(4-Methoxyphenyl)-5-phenyl-1H-1,2,4-triazol-3-ol (30).—A mixture of **29** (0.62 g, 2.17 mmol), aq KOH solution (10% w/v; 8 mL) and ethanol (99.9%; 200 proof; 4 mL) was heated at 60°C for 2 h and then cooled. Solvent was removed under reduced pressure. Hydrochloric acid (2 M) was then added dropwise until the reaction mixture reached pH 2. The white precipitate was filtered off, washed with cold water, and dried under vacuum to give **30** (0.48 g; 83%). Mp 191.0–192.2°C. ¹H NMR (DMSO-*d*₆, δ) 7.44–7.34 (m, 5H), 7.30–7.25 (m, 2H), 7.03–6.97 (m, 2H), 3.79 (s, 3H). ¹³C NMR (CD₃OD, δ) 161.73, 131.79, 131.62, 129.92, 129.76, 128.46, 115.64, 56.09. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₁₅H₁₃N₃O₂, 268.1086; found, 268.1094

3-Methoxy-1-(4-methoxyphenyl)-5-phenyl-1H-1,2,4-triazole (31).—Compound **30** (0.9 g, 3.4 mmol), iodomethane (2.41 g, 17 mmol, 5 equiv), K₂CO₃ (1.4 g, 10.2 mmol, 3 equiv) and DMF (5 mL) were mixed together in a one-neck round-bottom flask and stirred at room temperature for 16 h. Reaction progress was monitored with TLC (silica gel; ethyl acetate–hexane, 1:1 v/v). The mixture was poured into water (50 mL) and extracted with ethyl acetate (50 mL × 2). The combined organic layers were washed with brine–water (1:1 v/v; 50 mL) and dried (MgSO₄). Solvent was removed under reduced pressure. The residue was purified with flash chromatography (general method A; 40–60% ethyl acetate in

hexane) followed by preparative HPLC (general method B) to give **31** as a white solid (0.55 g; 57%). Mp 125.1–126.2°C. ¹H NMR (CD₃OD, δ) 7.44–7.38 (m, 3H), 7.36–7.30 (m, 2H), 7.27–7.22 (m, 2H), 6.99–6.93 (m, 2H), 4.01 (s, 3H), 3.80 (s, 3H). ¹³C NMR (CD₃OD, δ) 169.26, 161.65, 154.82, 132.02, 131.50, 129.95, 129.73, 128.59, 128.49, 115.65, 57.51, 56.14. HPLC (general method C; t_R = 2.3 min, 97.2% purity). HRMS-ESI (m/z): [M + H]⁺ calcd for C₁₆H₁₅N₃O₂, 282.1243; found, 282.1244

3-(Fluoromethoxy)-1-(4-methoxyphenyl)-5-phenyl-1H-1,2,4-triazole (**32**).—

Compound **30** (0.45 g, 1.68 mmol) was heated at 100°C for 3.5 h with fluoriodomethane (1.3 g, 8.4 mmol, 5 equiv) and potassium carbonate (0.7 g, 5.04 mmol, 3 equiv) in DMF (5 mL) and then cooled. Water (20 mL) and ethyl acetate (20 mL) were poured into the reaction mixture. The organic layer was separated off, washed with brine (20 mL $\times$ 2), and dried (MgSO₄). Solvent was removed under reduced pressure. The residue was purified with flash chromatography (general method A; 40–60% ethyl acetate in hexane) followed by preparative HPLC (general method B) to give **32** as a white solid (0.25 g; 50%). Mp 52.8–53.5°C. ¹H NMR (CDCl₃, δ) 7.5–7.48 (m, 2H), 7.39–7.26 (m, 5H), 6.92–6.9 (m, 2H), 6.07 (s, 1H), 5.9 (s, 1H), 3.84 (s, 3H). ¹³C NMR (CD₃OD, δ) 165.86, 165.83, 153.36, 130.88, 130.18, 128.82, 128.53, 127.41, 127.01, 114.56, 100.15, 97.93, 55.56. HPLC (general method C; t_R = 10.3 min; >99.9% purity). HRMS-ESI (m/z): [M + H]⁺ calcd for C₁₆H₁₄FN₃O₂, 300.1148; found, 300.1145.

COX-1 and COX-2 Inhibition Assays.

Whole-blood enzymatic assays were carried out in two steps, as previously described,¹³ with minor modifications. All incubations were performed in a light-shielded shaker at 500 rpm and 37°C. In the first step, blood (rat, monkey, or human) was collected into heparinized tubes. For the COX-1 inhibition assay, blood (220 μ L) was aliquoted into each well of 96-well plates, treated with test agent (5 μ L) to give various final concentrations (10 pM to 100 μ M), and then incubated for 60 min. To stimulate COX-1, Ca²⁺ ionophore (50 μ M; A23187) was then added, and incubated for 30 min. For COX-2 inhibition assay, aspirin solution (4 mM; 6 μ L) was added to blood (214 μ L) and incubated for 6 h. Test agent (5 μ L) was added, followed by lipopolysaccharides (10 μ g/mL; LPS from *Escherichia coli* O26:B6), and incubated for 18 h. To terminate either the COX-1 or COX-2 reaction, heparinized diclofenac was added to a final concentration of 107 μ M, left for 5 min, and centrifuged for 5 min at 4°C. Plasma (100 μ L) was obtained from each well for enzymatic assay (Step 2).

In the second step, competitive assays were performed using a thromboxane (Tx) B₂ (COX-1) or prostaglandin (PG) E₂ (COX-2) enzymatic immunoassay (EIA) kit (Cayman Chemical, USA). Concentrations of TxB₂ or PGE₂ in the plasma samples were determined according to the kit instructions. Inhibitory potencies (IC_{50} s) of the test agents to each COX isozyme were calculated using a 4-parameter logistic fit.³¹ Assays were performed in triplicate.

Pharmacological Screening.

Inhibitors **5–9**, **11–14**, **20**, **24**, and **26–28** were submitted to the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP) for assay of binding affinities

to a wide range of human recombinant receptors and binding sites, and the rat brain benzodiazepine site. Detailed assay protocols are available at the NIMH-PDSP Web site (<http://pdsp.cwru.edu>).

Radiochemistry.

Radiochemistry was performed in lead-shielded hot-cells with automated radiosynthesis apparatus for personnel protection from radiation.

Production of [^{11}C]Carbon Dioxide.—NCA [^{11}C]carbon dioxide (~85 GBq) was produced with a PETtrace cyclotron (GE Medical Systems; Milwaukee, WI) according to the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ reaction by irradiation of nitrogen gas³² (initial pressure, 160 psi; volume, 75 mL) containing oxygen (1%) with a proton beam (16.5 MeV, 45 μA) for 40 min.

Production of [^{11}C]Iodomethane.—NCA [^{11}C]iodomethane was produced from NCA [^{11}C]carbon dioxide in a PETtrace MeI Process Module (GE Medical Systems: Severna Park, MD). Thus, at the end of proton irradiation, [^{11}C]carbon dioxide was delivered to the apparatus through stainless tubing (OD 1/8 in, ID 1/16 in) over 3 min, trapped on molecular sieves (13 $\times$), released for reduction to [^{11}C]methane, and recirculated over iodine at 720°C.³³ The generated [^{11}C]iodomethane was trapped on Porapak Q held in the recirculation path.

Radiosynthesis of [^{11}C]5.—Precursor **33** (0.7 mg, 2.3 μmol) was dissolved in DMF (80 μL). Tetra-*n*-butylammonium hydroxide (TBAH) in MeOH (0.5 M, 4.0 μL) was added to this solution and then injected into the loop of an AutoLoop apparatus (Bioscan; Washington, DC). [^{11}C]Iodomethane was released from the PETtrace module and into the loop, which was then kept at room temperature for 5 min. [^{11}C]5 was isolated with reversed phase HPLC on a Luna C18 column (10 $\times$ 250 mm, 10 μm ; Phenomenex) eluted isocratically with MeCN–10 mM aq HCOONH₄ (45:55 v/v) at 6 mL/min (t_{R} = 14 min) (see the Supporting Information). After removal of mobile phase, [^{11}C]5 was formulated for intravenous injection in sterile ethanol-saline (10:90 v/v), and sterile-filtered (Millex-MP 0.22 μm , 25 mm; Merck Millipore; Burlington, MA). The identity of the [^{11}C]5 was confirmed with reversed phase HPLC on a Luna C18 column (4.6 mm $\times$ 250 mm, 10 μm ; Phenomenex) eluted isocratically with MeCN–10 mM aq HCOONH₄ (55:45 v/v) at 2 mL/min (t_{R} = 5.4 min) (see the Supporting Information), and by LC-MS (ESI) (method 1) of associated carrier [t_{R} = 4.80 min, (m/z) [$\text{M} + \text{H}$]⁺: 312.4]. [^{11}C] **5** was obtained in 20 $\pm$ 3% yield from [^{11}C]carbon dioxide in >99% radiochemical purity and with a molar activity of 125 $\pm$ 62 GBq/ μmol (n = 12).

Radiosynthesis of [^{11}C]20.—[^{11}C]Iodomethane was trapped in a crimp-sealed V-vial containing **19** (0.7 mg, 1.9 μmol) and solid KOH (5 mg) in DMSO (0.4 mL) and heated at 70°C for 3 min. [^{11}C]20 was isolated with reversed phase HPLC on a Luna C18 column (10 $\times$ 250 mm, 10 μm ; Phenomenex) eluted isocratically with MeCN–100 mM aq HCOONH₄ (65:35 v/v) at 6 mL/min (t_{R} = 9.5 min) (see the Supporting Information). After removal of mobile phase, [^{11}C]20 was formulated for intravenous injection in sterile ethanol-saline (10:90 v/v) containing Tween 80 (12 mg) and sterile-filtered (Millex-MP 0.22 μm , 25 mm).

The identity of [^{11}C]**20** was confirmed with reversed phase HPLC on a Luna C18 column (4.6 mm $\times$ 250 mm, 10 μm ; Phenomenex) eluted isocratically with MeCN–100 mM aq HCOONH_4 (65:35 v/v) at 2 mL/min (t_R = 6.1 min) (see the Supporting Information), and with LC-MS (ESI) (method 2) of associated carrier [t_R = 5.31 min, (m/z) [$\text{M} + \text{H}$] $^+$: 380.1]. [^{11}C]**20** was obtained in $9.8 \pm 3.5\%$ yield from [^{11}C]carbon dioxide with >99% radiochemical purity and with a molar activity of $176 \pm 73 \text{ GBq}/\mu\text{mol}$ (n = 100).

Production of [^{18}F]Fluoride Ion.—NCA [^{18}F]fluoride ion was produced with the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ reaction by irradiating ^{18}O -enriched water (95 atom %; 1.8 mL) with a beam of protons (14.1 MeV; 20–25 μA) from a PETtrace cyclotron (GE Medical System).

Radiosynthesis of [d_2 - ^{18}F]6**.**—Cyclotron-produced [^{18}F]fluoride ion (7.4 GBq) in [^{18}O]water was delivered into a 5 mL glass V-vial equipped with a screw-on cap and liner (20/400 PTFE/silicone; Alltech Associates; Deerfield, IL), containing K 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane; 5.0 mg, 13.3 μmol) and potassium carbonate (0.50 mg, 3.6 μmol) in MeCN– H_2O (0.1 mL, 9:1 v/v). This solution was transferred to TRACERlab FX $_{\text{FN}}$ module (GE) that had been modified as previously described³⁴ and diluted with MeCN (2 mL). The mixture was evaporated to dryness at 90°C under reduced pressure while being flushed with nitrogen. MeCN (2 mL) was again added and then evaporated to dryness. The vessel was sealed and then CD_2Br_2 (100 μL) in MeCN (1.0 mL) was added to the dry [^{18}F]fluoride ion- K^+ -K 2.2.2 complex, which was then heated at 95°C for 15 min. The reaction vessel was then cooled to 35°C. Nitrogen gas was used to transfer the volatile [d_2 - ^{18}F]-fluorobromomethane through a series of four silica gel cartridges (SepPak Plus; Waters Milford, MA), and then into a septum-sealed 1-mL V-vial (Alltech, Deerfield, IL) containing **33** (0.5 mg, 1.7 μmol), CS_2CO_3 (~2 mg, ~6.1 μmol), and 18-crown-6 (~5 mg, ~19 μmol) in DMF (0.5 mL). The mixture was heated at 80°C for 10 min. [d_2 - ^{18}F]**6** was isolated with reversed phase HPLC on a Luna C18 column (10 $\times$ 250 mm, 10 μm ; Phenomenex) eluted isocratically with MeCN–10 mM aq HCOONH_4 (35:65 v/v) at 6 mL/min (t_R = 39 min) (see the Supporting Information). After removal of mobile phase, the residue of [d_2 - ^{18}F]**6** was formulated for intravenous injection in sterile ethanol–saline (10:90 v/v), and then sterile filtered (Millex-MP 0.22 μm , 25 mm). The identity of the [d_2 - ^{18}F]**6** was confirmed with reversed phase HPLC on a Luna C18 column (4.6 $\times$ 250 mm; Phenomenex) eluted isocratically with MeCN–10 mM aq HCOONH_4 (50:50 v/v) at 2 mL/min (t_R = 7.8 min) (see the Supporting Information), and with LC-MS (ESI) (method 1) of associated carrier [t_R = 4.75 min, (m/z) [$\text{M} + \text{H}$] $^+$: 332.3]. [d_2 - ^{18}F]**6** was obtained in $14 \pm 4\%$ yield in >99% radiochemical purity and with a molar activity of $53 \pm 17 \text{ GBq}/\mu\text{mol}$ (n = 8).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

DAT	dopamine transporter
DOR	δ -opiate receptor
GABA	γ -aminobutyric acid
H	histamine
K 2.2.2	7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane
KOR	κ -opiate receptor
MOR	μ -opiate receptor
PDSP	Psychoactive Drug Screening Program
PBR	peripheral benzodiazepine receptor
NCA	no-carrier-added
NET	noradrenaline transporter
NIMH	National Institute of Mental Health
SERT	serotonin transporter
tPSA	total polar surface area

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**$[\text{}^{18}\text{F}]1$; ($[\text{}^{18}\text{F}]\text{SC63217}$), $\text{X} = \text{}^{18}\text{F}$, $\text{Y} = \text{OMe}$
 $[\text{}^{11}\text{C}]2$; $\text{X} = \text{Cl}$, $\text{Y} = \text{O}^{11}\text{CH}_3$**

Chart 1.

Former Candidate PET Radioligands for COX-1 Based on 1,5-Diarylpyrazoles

Chart 2.
Structure of 5 (FK881)

Scheme 1.

Structure of Prodrug Type Radioligand $[^{11}\text{C}](S)$ -Ketoprofen Methyl Ester ($[^{11}\text{C}]3$) and Hydrolysis of $[^{11}\text{C}]3$ to COX-1 Radioligand $[^{11}\text{C}]4$

Scheme 2. Syntheses of Labeling Precursor 19 and COX-1 Inhibitor 20^a

^aReagents and conditions: (i) 4-(benzyloxy)benzoyl chloride, toluene, pyridine, 110°C, 1 h; (ii) 10% aq KOH, EtOH, 60°C, 1.5 h; (iii) 2-chloro-1,1,1-trifluoroethane, KI, K₂CO₃, DMF, room temperature, 24 h; (iv) 1 M BCl₃, CH₂Cl₂, -20°C, 3 h, then 0°C for 1 h; (v) MeI, K₂CO₃, DMF, room temperature, 16 h.

Scheme 3. Synthesis of COX-1 Inhibitor **24^a**

^aReagents and conditions: (i) 2,2,2-trifluoroacetimidamide, Et₃N, MeOH, room temperature, 18 h; (ii) 6-chloronicotinoyl chloride, pyridine, 1,4-dioxane, 101°C, 4 h; (iii) KF, 18-crown-6, DMSO, 100°C, 24 h.

Scheme 4. Syntheses of COX-1 Inhibitors 26–28^a

^aReagents and conditions: (i) ICH₂F, K₂CO₃, DMF, 100°C, 3.5 h; (ii) mCPBA (1.5 equiv), CH₂Cl₂, room temperature, 3 h; (iii) mCPBA (4 equiv), CH₂Cl₂, room temperature, 5 h.

Scheme 5. Syntheses of COX-1 Inhibitors 31 and 32^a

^aReagents and conditions: (i) benzoyl chloride, pyridine, toluene, 110°C, 4 h; (ii) 10% KOH, EtOH, 60°C, 2 h; (iii) MeI, K₂CO₃, DMF, room temperature, 16 h; (iv) ICH₂F, K₂CO₃, DMF, 100°C, 3.5 h.

Scheme 6. Syntheses of Radioligands [¹¹C]5, [d₂-¹⁸F]6, and [¹¹C]20^a

^aReagents and conditions: (i) [¹¹C]iodomethane, DMF, TBAH, room temperature, 5 min; (ii) [¹⁸F]FCD₂Br, Cs₂CO₃, 18-crown-6, 80°C, 10 min; (iii) [¹¹C]iodomethane, DMSO, KOH, 70°C, 3 min.

Table 1.

Structures, IC_{50} s, and cLog D Values for COX-1 Inhibitors

Inhibitor	R ¹	R ²	R ³	clogD	IC_{50} (nM) ^{a,b}			
					COX-1		COX-2	
					Monkey	Human	Monkey	Human
4				2.76	40 ± 17	20 ± 7	400 ± 113	264 ± 110
5	OMe	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	3.72	4.6 ± 1.3	5.0 ± 5.2	> 1000	> 1000
6	OCH ₂ F	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	3.84	3.6 ± 1.0	4.0 ± 2.0	> 1000	841 ± 102
7	SMe	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	4.01	10.0 ± 0.5	1.0 ± 3.6	n.d. ^c	> 1000
8	SO ₂ Me	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	2.99	11 ± 1	6.0 ± 0.2	> 1000	> 1000
9	SOMe	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	2.88	3.6 ± 1.8	2.0 ± 0.9	n.d.	> 1000
10	OMe	Ph	4-CNC ₆ H ₄	3.62	n.d.	1.0 ± 1.4	200 ± 45	n.d.
11	CF ₃	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	3.72	5.0 ± 3.0	3.0 ± 3.2	n.d.	247 ± 614
12	CF ₃	4-MeOC ₆ H ₄	2-MeO-pyridin-5-yl	2.75	5.1 ± 1.5	4.0 ± 5.9	125 ± 34	> 1000
13	CF ₃	2-MeO-pyridin-5-yl	4-MeOC ₆ H ₄	2.74	n.d.	6.0 ± 3.0	n.d.	776 ± 71

Inhibitor	R ¹	R ²	R ³	clogD	IC ₅₀ (nM) ^{a,b}			
					COX-1		COX-2	
					Monkey	Human	Monkey	Human
14		4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	3.71	125 ± 2	42 ± 8	n.d.	> 1000
20	OCH ₂ CF ₃	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	3.59	1.0 ± 0.3	1.0 ± 0.2	> 1000	> 1000
24	CF ₃	2-F-pyridin-5-yl	4-MeOC ₆ H ₄	3.38	10 ± 3.0	2.0 ± 0.1	n.d.	> 1000
26	SCH ₂ F	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	3.56	6.4 ± 0.8	5.0 ± 3.9	n.d.	> 1000
27	SOCH ₂ F	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	2.33	5.0 ± 2.0	3.0 ± 1.7	545 ± 102	> 1000
28	SO ₂ CH ₂ F	4-MeOC ₆ H ₄	4-MeOC ₆ H ₄	3.17	25 ± 3	3.0 ± 1.2	n.d.	> 1000
31	OMe	Ph	4-MeOC ₆ H ₄	3.76	3.0 ± 2.5	3.00 ± 0.06	n.d.	> 1000
32	OCH ₂ F	Ph	4-MeOC ₆ H ₄	3.96	100	1.7 ± 0.6	n.d.	100 ± 25
34			4-MeOC ₆ H ₄	2.80	> 1000	> 1000	1.0 ± 0.5	1.0 ± 0.1

^a All data are mean ± SD ($n = 3$).

^b COX-1 rat whole blood IC₅₀s for **4**, **5**, **6**, and **20**, were 130, 418, 280, and 15 nM, respectively.

n.d. = not determined.
c

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