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Synthesis of three ^{18}F-labelled cyclooxygenase-2 (COX-2) inhibitors based on a pyrimidine scaffold

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Cyclooxygenase (COX) is the key enzyme within the complex conversion of arachidonic acid into prostaglandins (PGs). Inhibitors of this enzyme represent a particularly promising class of compounds for chemoprevention and cancer therapy. The experimental data on the involvement of COX isoform COX-2 in tumour development and progression, as well as the observed overexpression of COX-2 in a variety of human cancers provide the rationale for targeting COX-2 for molecular imaging and therapy of cancer. A series of trifluoromethyl-substituted pyrimidines was prepared as a novel class of selective COX-2 inhibitors, based on the lead structure **1a**. All compounds were tested in cyclooxygenase (COX) assays *in vitro* to determine COX-1 and COX-2 inhibitory potency and selectivity. Molecular docking studies using the catalytic site of COX-1 and COX-2, respectively, provided complementary theoretical support for the obtained experimental biological structure–activity relationship data of three highly potent and selective fluorobenzyl-containing COX-2 inhibitors. Selected fluorobenzyl-substituted pyrimidine derivatives were further developed as ^{18}F-labelled radiotracers ([^{18}F]**1a**, [^{18}F]**2a**, [^{18}F]**3a**). Radiotracers [^{18}F]**1a** and [^{18}F]**2a** were radiolabelled using 4-[^{18}F]fluorobenzylamine ([^{18}F]FBA) as a building block. Radiotracer [^{18}F]**3a** was radiofluorinated directly using a nucleophilic aromatic substitution reaction with no-carrier-added (n.c.a.) [^{18}F]fluoride on an iodylaryl compound as a labelling precursor.

Introduction

Cyclooxygenases (COXs) control the complex conversion of arachidonic acid (AA) into prostaglandins and thromboxanes, which function as locally active messenger molecules and trigger important physiological and pathophysiological processes. The COX enzyme family consists of a constitutively expressed isoform (COX-1) and an inducible isoform (COX-2). Recently, a third isoform, believed to be a COX-1 splice variant, has been reported as COX-3.[1] Both COX-1 and COX-2 convert arachidonic acid to prostaglandin H_2. In the first step of the reaction COXs cyclise AA to prostaglandin G_2 which then is rapidly converted into PGH_2 by a peroxidase reaction at a second catalytic site in the same enzyme. Most COX inhibitors derive their therapeutic potential by blocking the first catalytic site of the COX enzymes. PGH_2 is converted to a variety of different prostaglandins by a number of downstream enzymes. These prostaglandins mediate various physiological processes by binding to G-protein coupled receptors.[2]

The conformations of the two COX isoforms are very similar, as are the amino acid sequences that constitute the substrate-binding pockets.[3] Given these similarities, the development of COX-2 selective inhibitors has been a great challenge.[4] Since the identification of the COX-2 isoform in the early 1990s, a number of selective inhibitors have been developed. A selection of these molecules has been used extensively in clinical application. In 2005, most of these drugs were withdrawn from the market following concerns over their cardiac safety profile.

Among the most widely used selective COX-2 inhibitors (coxibs) were celecoxib, rofecoxib and valdecoxib.[5] More recently, the underlining biochemical mechanisms of the cardiac toxicity of the coxib compound class are starting to be unravelled.[6,7]

COX-1 functions as a housekeeping enzyme and is expressed in most resting tissues; its responsibilities include the maintenance of gastric and renal integrity.[8] COX-2 is expressed in the resting tissue of the brain and the kidney, but virtually absent in all other tissue types.[9,10] COX-2 expression is induced in response to various acute and chronic inflammatory conditions. Expression can be triggered in fibroblast, epithelial, endothelial, macrophage, and smooth muscle cells in response to growth factors, cytokines, and pro-inflammatory stimuli.[11] Several studies have suggested that COX-2 is also

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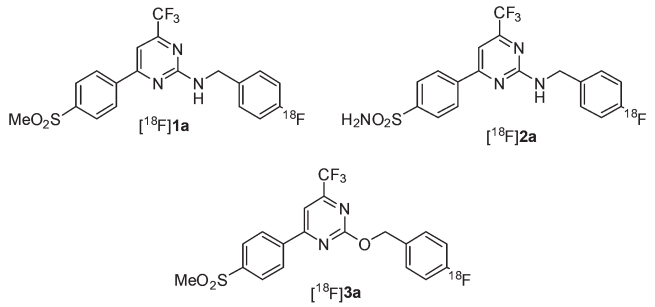


Fig. 1 Structures of radiotracers [^{18}F]**1a**, [^{18}F]**2a** and [^{18}F]**3a**.

involved in neurodegenerative diseases such as Parkinson's and Alzheimer's.[12] Elevated COX-2 expression has furthermore been found in a variety of human cancers, most prominently colorectal, gastric, and breast cancers.[13–15] Some of the key signalling pathways involving effects of COX-2 in inflammation and tumorigenesis have been dissected.[16] There are, however, discrepancies between potent anticancer effects of COX-2 inhibitors *in vitro* and their failure in the majority of clinical trials.[17]

The role that the COX-2 enzyme plays in the development and progression of various diseases appears to be very complex and requires more basic research on COX-2 pharmacology.

An exact and accurate assessment of COX-2 expression levels and activity in tissues under different disease conditions is of vital importance to these efforts. To date, an exact assessment of COX-2 expression can only be achieved by *ex vivo* analysis. This type of analysis is relatively complex due to the instability of COX-2 mRNA *ex vivo*.[18]

Non-invasive monitoring of COX-2 expression *in vivo* would further advance efforts into elucidating basic pharmacology of the enzyme. Over the past decade a number of COX-2 inhibitors have been radiolabelled with ^{11}C, ^{18}F, ^{99m}Tc, ^{123}I and ^{125}I to assess COX-2 expression *in vivo* using positron emission tomography (PET) and single photon emission computed tomography (SPECT). Among the radiolabelled compounds were celecoxib,[19–27] rofecoxib[28–30] and a number of novel structures.[31–34] However, despite the large number of radiolabelled COX-2 inhibitors reported in the literature, COX-2-mediated uptake of a radiotracer in an *in vivo* model has not yet been convincingly demonstrated, mainly due to insufficient metabolic stability and high degree of non-specific binding.

The aim of this study is to develop a novel class of highly potent and selective ^{18}F-labelled COX-2 inhibitors as radiotracers for molecular imaging of COX-2 expression *in vivo* (Fig. 1). The lead structure for this investigation is compound **1a**, which was originally reported by Swarbrick and co-workers.[35]

Results and discussion

Chemistry

An important compound within the synthesis route of compounds **1a** and **3a** is 2-(methylsulfonyl)-4-(4-(methylsulfonyl)-phenyl)-6-(trifluoromethyl)pyrimidine **6** as a labelling precursor.

The synthetic strategy for the preparation of compound **6** follows the route outlined by Swarbrick *et al.*[35] (Fig. 2). Claisen condensation of 1-(4-(methylthio)-phenyl)ethanone with ethyl trifluoroacetate yielded trifluoro-substituted dione **4** in a high yield of 92%. Pyrimidine ring formation using condensation with *S*-methylisothiourea afforded compound **5** in almost quantitative yield. Methylthioether groups in **5** were oxidized using Oxone to afford compound **6** in overall yield of 58% for the three step reaction sequence.

COX-2 inhibitors **1a–p** were prepared by heating corresponding primary amines and in the presence of compound **6** in acetonitrile in sealed vials at 140 °C. Methylsulfonyl group in the 2-position of the pyrimidine ring acts as a good leaving group upon attack with primary amines. Upon completion of the reaction, excess of compound **6** and amine could easily be removed by diluting the mixture with hydrochloric acid. The desired product precipitated and was collected by filtration. Impurities were removed using purification with column chromatography. Syntheses using benzylamine hydrochloride salts, such as 4-nitrobenzylamine, were carried out by adding triethylamine as the auxiliary base.

To synthesize compound **3a**, it was necessary to replace the secondary amine linker with an ether moiety. To this end, 4-fluorobenzyl alcohol and sodium hydride were dissolved in dry THF under a nitrogen atmosphere, and the mixture was cooled to 0 °C. Addition of compound **6** allowed for the formation of fluorobenzyl ether compound **3a** (Fig. 2). This compound has been reported in a patent by the Glaxo Group.[36]

However, to the best of our knowledge, COX-2 potency and selectivity of this substance has not been published to date. The synthesis of methylsulfones **1a–j**, **1k–p**, and **3a** are depicted in Fig. 2.

Many selective COX-2 inhibitors carry a methylsulfonyl group as a common COX-2 pharmacophore on one of the aryl rings. It is thought that this moiety is indispensable for high binding potency and selectively.[2] However, sulfonamide moieties are also frequently used as COX-2 pharmacophores.[37,38] They are thought to yield compounds with comparable or even higher potency. Prominent examples of sulfonamide carrying selective COX-2 inhibitors include celecoxib and valdecoxib. Replacing a methylsulfonyl group with a sulfonamide can have significant effects on the pharmacokinetic profile of the drug. We identified sulfonamide **2a** as a potential candidate for ^{18}F radiolabelling in addition to two methylsulfonyl group-containing compounds **1a** and **3a**. The synthesis of sulfonamide-containing structures generally requires protection of the amine group to enable efficient and high yielding synthesis.

A very elegant method published by Mahalingam *et al.*[39] described the protection of the sulfonamide groups *via tert*-butyl groups, which can easily be removed under mild conditions using catalytic amounts of Lewis acid scandium triflate. To synthesize the *tert*-butyl protected precursor **8**, we first attempted to reproduce the synthetic template that was used for the preparation of compound **6**. Reaction of

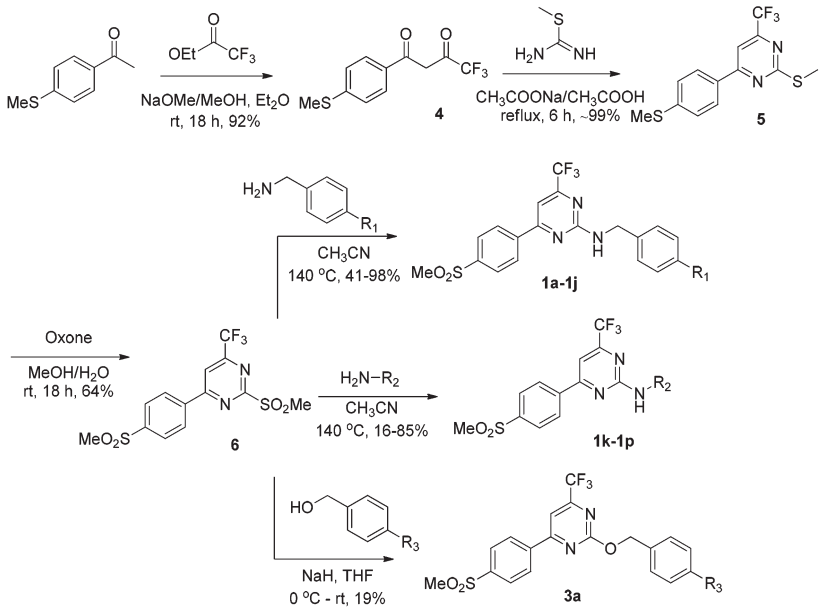


Fig. 2 Synthesis of compounds **1a–1p**, **3a**.

4-acetylbenzene sulfonylchloride with *tert*-butylamine gave *tert*-butyl-protected sulfonamide. However, subsequent Claisen condensation with ethyl trifluoroacetate failed presumably due to the basic reaction conditions. Basic reaction conditions led to the abstraction of the acidic sulfonamide proton leading to the formation of poorly soluble salt complexes.

To omit Claisen condensation, we applied the Suzuki coupling reaction between commercially available 4-chloro-2-(methylsulphonyl)-6-(trifluoromethyl)pyrimidine with *tert*-butyl 4-boronobenzenesulfonamide to form compound **7**. The methyl–sulfonyl group in compound **7** was oxidized using Oxone to afford compound **8**. Removal of the *tert*-butyl group was achieved through treatment of compound **8** with scandium triflate to give compound **9** in a total yield of 53% for the three steps. Treatment of sulfonamide **9** with various primary amines gave compounds **2a–e** in 57–91% yield. The synthesis of sulfonamides **2a–e** is summarized in Fig. 3.

In vitro COX-1 and COX-2 enzyme inhibition

Compounds **1a–p**, **2a–e** and **3a** were evaluated for their COX-2 inhibitory potency and selectivity profile. The determined enzyme inhibition data are summarized in Tables 1–4. Celecoxib was included in all assays as an internal reference compound for comparison. Celecoxib showed IC_{50} values of 40 nM against COX-2 and 15 μM against COX-1, which is in good agreement with previously reported literature values.[40] The lead structure **1a** displayed excellent COX-2 inhibitory potency (IC_{50} (COX-2) = 7 nM) and did not show COX-1 inhibition in the concentration range tested. This makes structure **1a** more potent and many times more selective than celecoxib. In the original report, compound **1a** displayed an IC_{50} (COX-2) of 0.28 nM,[35] which is an order of magnitude lower than the value we have obtained. This is likely due to differences in the inhibition assay used to determine the IC_{50} values. Compounds **1b** to **1j** are representative of our effort to

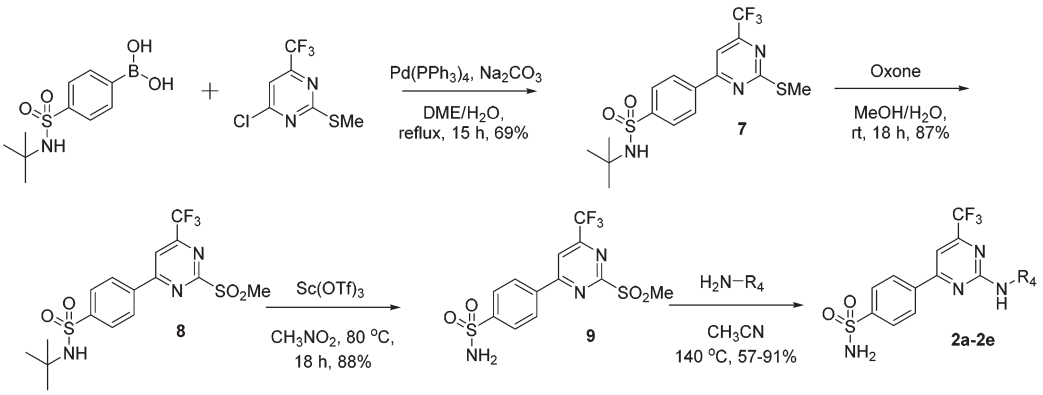
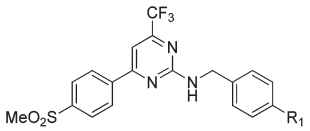


Fig. 3 Synthesis of compounds **2a–2e**.

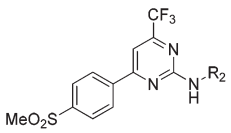
Table 1 IC_{50} (COX-1) and IC_{50} (COX-2) values for compounds **1a–1j**



	R_1	IC_{50} [a] (μM) COX-1	COX-2
Celecoxib		15	0.040
1a	F	>100	0.007
1b	Cl	>100	0.006
1c	Br	>100	0.018
1d	CF_3	>100	0.017
1e	H	>100	0.016
1f	Me	>100	0.005
1g	Ph	>100	>10
1h	*t*-Bu	>100	>10
1i	OMe	>100	0.007
1j	NO_2	>100	0.086

[a] Values are means of two determinations.

Table 2 IC_{50} (COX-1) and IC_{50} (COX-2) values for compounds **1k–1o**



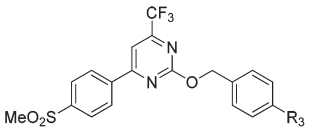
	R_2	IC_{50} [a] (μM) COX-1	COX-2
Celecoxib		15	0.040
1k	*N*-(4-Pyridyl)-CH_2–	>100	0.30
1l	*N*-(3-Pyridyl)-CH_2–	>100	0.080
1m	*N*-(2-Pyridyl)-CH_2–	>100	0.050
1n	*N*-(2-Fluoroethyl)	>100	2.0
1o	*N*-Butyl	>100	0.020

[a] Values are means of two determinations.

determine how structural changes to the second aryl ring effect inhibitory potency and selectivity (Table 1).

We found that changes at the 4-position of the aryl ring are usually well tolerated. Most compounds displayed better COX-2 inhibitory potency than celecoxib with IC_{50} values for COX-2 inhibition below 20 nM while possessing high selectivity over COX-1. The 4-bromine substituted compound (**1c**) is especially notable. **1c** Shows good COX-2 inhibitory potency (IC_{50} = 18 nM), despite the considerable steric bulk of bromine as a substituent. In contrast, more bulky substituents, such as *tert*-butyl and phenyl (**1h**, **1g**) show no COX-2 inhibition at the concentrations tested. It can be concluded that too much steric bulk at 4-position of the second aryl ring is detrimental to COX-2 binding potency. Despite the relatively modest steric bulk of the 4-position of the nitro-substituted compound (**1j**), we found its potency decreased by an order of magnitude in comparison to **1a**. This is likely to be due to a combination of

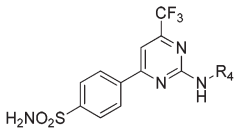
Table 3 IC_{50} (COX-1) and IC_{50} (COX-2) values for compound **3a**



	R_3	IC_{50} [a] (μM) COX-1	COX-2
Celecoxib		15	0.040
3a	F	>100	0.020

[a] Values are means of two determinations.

Table 4 IC_{50} (COX-1) and IC_{50} (COX-2) values for compound **2a–2e**



	R_4	IC_{50} [a] (μM) COX-1	COX-2
Celecoxib		15	0.040
2a	*N*-(4-Fluorobenzyl)	>100	0.039
2b	*N*-Benzyl	>100	0.034
2c	*N*-(4-Methylbenzyl)	>100	0.028
2d	*N*-(4-Methoxybenzyl)	>100	0.014
2e	*N*-Butyl	>100	0.031

[a] Values are means of two determinations.

the strongly electron withdrawing nature and modest steric bulk of the nitro group, which has a negative effect on binding potency. Compound **1i** was found to have similar COX-2 inhibitory potency and selectivity as **1a** (IC_{50} = 7 nM). Compound **1i** carries a methoxy group, which makes this compound a good candidate for ^{11}C labelling. ^{11}C labelling can be carried out *via* *O*-methylation of free alcohols using $^{11}CH_3I$ or [^{11}C]methyltriflate.[32] Compound **1i** would be a good radiolabelling candidate for a ^{11}C-based COX-2 radiotracer study.

We found the lead compound **1a** to be relatively lipophilic (log *P* = 3.37; determined experimentally by partition in octanol–water using a ^{18}F-labelled compound). High lipophilicity of a PET radiotracer is likely to cause high non-specific binding and high intestine uptake. Compounds **1k** to **1m** as displayed in Table 2 are representative of our efforts to synthesize structures that are less lipophilic by substitution of the second aryl ring.

The most promising candidate in this library is pyridine compound **1m**. Compound **1m** was found to have a COX-2 inhibitory potency (IC_{50} = 50 nM) comparable to that of celecoxib, but a much better COX-2 selectivity. Compound **1m** is more water soluble than **1a** and might be developed as a ^{18}F radiotracer by including fluorine-18 at the 4-position of the pyridine ring. Various ^{18}F-labelled fluoropyridines have been

reported as highly efficient radiotracers and radiopharmaceuticals.[51,52] Efforts to synthesize alkyl substituted compounds resulted in only one high potency compound (**1o**). Compound **1o** displayed COX-2 inhibitory potency that exceeded that of celecoxibs (IC_{50} = 20 nM).

Oxygen-containing compound **3a** (IC_{50} = 20 nM) was found to be slightly less potent than the corresponding amine compound **1a** (Table 3). Thus, replacing nitrogen with oxygen reduces slightly binding potency, but binding potency of compound **3a** is still high when compared with an internal reference compound celecoxib.

Inhibitory potencies of sulfonamide compounds **2a** to **2e** are summarized in Table 4. All five compounds were slightly less potent than their corresponding methylsulfone counterparts. We can conclude that for the particular structural pyrimidine-based backbone, methylsulfone-containing compounds seem to display higher COX-2 inhibitory potency than the corresponding sulfonamides. Nonetheless, the potential candidate for ^{18}F radiolabelling **2a**, displayed inhibitory potency similar to that of celecoxib (IC_{50} = 39 nM) while showing higher COX-2 selectivity. Compound **2e**, similar to **1i**, carries a methoxy group and might therefore serve as a good ^{11}C radiolabelling candidate. The COX-2 inhibitory potency of **2e** is comparable to that of **1i** (IC_{50} = 14 nM).

Compounds **1a**, **2a** and **3a** displayed COX-2 inhibitory potency and selectivity rendering all three compounds suitable for development as ^{18}F-labelled radiotracers.

Molecular docking studies

Molecular docking experiments were performed using X-ray crystal structure data for COX-1 and COX-2 obtained from the protein data bank to explore possible interaction of compounds **1a**, **2a** and **3a** with the active site of COX-1 and COX-2 enzymes. High inhibitory potency of compound **1a** (COX-2, IC_{50} = 7 nM) suggests a favourable orientation within the COX-2 binding site. The SO_2CH_3 group in compound **1a** completely enters into the secondary pocket region of the COX-2 active site, where it is oriented towards Q192, R513, H90, and A516 residues (Fig. 4, left). One of the oxygen atoms of the SO_2CH_3 group undergoes hydrogen bonding interactions with the nitrogen atom of H90 (S═O···N = 2.64 Å). The other oxygen atom indicates hydrogen bonding interactions with the nitrogen atom of Q192 amino acid residue (S═O···N = 2.23 Å). Moreover, the phenyl ring bearing a fluorine atom is placed in the vicinity of R120, A527 and V349 amino acid residues. On the other side, docking studies of compound **1a** into COX-1 enzyme indicated that compound **1a** was not able to enter into the COX-1 active site completely (Fig. 4, right). The fluorine-containing phenyl ring is situated near A527, S530 and L531 residues while the SO_2CH_3 group is positioned outside the active site of COX-1 enzyme. This finding is in good agreement with the determined high inhibitory potency and selectivity of compound **1a** towards COX-2.

Docking studies with compound **2a** (COX-2, IC_{50} = 39 nM) indicated that the phenyl ring bearing the SO_2NH_2 group is inserted into the secondary pocket region of the COX-2 active site where it is surrounded by H90, Q192, A516 and R513 residues (Fig. 5, left). The nitrogen atom of the SO_2NH_2 group displays hydrogen bonding to carbonyl oxygen of Q192 (N···O═C = 2.85 Å), and to carbonyl oxygen of L352 (N···O═C = 2.78 Å). One oxygen atom of the SO_2NH_2 group is hydrogen bonded to the nitrogen atom of H90 residue (S═O···N = 2.57 Å).

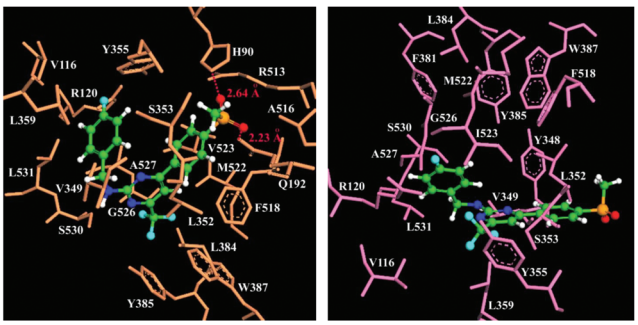


Fig. 4 (Left) Molecular docking of compound **1a** (carbon atoms in green) positioned in the binding site of COX-2 (PDB ID: 6COX; $E_{intermolecular}$ = −10.70 kcal mol^{-1}) and (right) COX-1 (PDB ID: 1EQG; $E_{intermolecular}$ = −8.03 kcal mol^{-1}). Hydrogen atoms of amino acid residues have been removed for clarity.

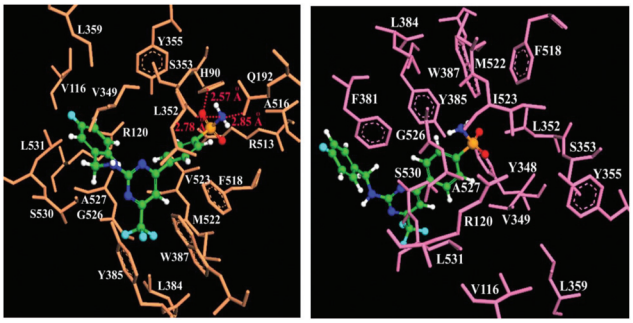


Fig. 5 (Left) Molecular docking of compound **2a** (carbon atoms in green) positioned in the binding site of COX-2 (PDB ID: 6COX; $E_{intermolecular}$ = −10.55 kcal mol^{-1}) and (right) COX-1 (PDB ID: 1EQG; $E_{intermolecular}$ = −8.39 kcal mol^{-1}). Hydrogen atoms of amino acid residues have been removed for clarity.

The 4-fluoro phenyl ring of compound **2a** is positioned in the vicinity of R120, V349, V116, L531 and A527 amino acid residues. The CF_3 group of compound **2a** is positioned at the entrance of COX-2 hydrophobic pocket constituted by W387, Y385 and F518 residues. Compound **2a** shows only partial entry into the COX-1 enzyme active site and did therefore not exhibit significant interactions with the COX-1 active site residues (Fig. 5, right). This is also in agreement with the determined COX-2 inhibitory potency and selectivity of compound **2a**.

The top scored docking pose of compound **3a** (COX-2 IC_{50} = 20 nM) displays a favorable orientation into the COX-2 active site wherein the SO_2CH_3 group is sloping towards the secondary pocket region of COX-2 active site lined by H90, R513, S353 and L352 residues (Fig. 6, left). One of the oxygen atoms of the SO_2CH_3 group is hydrogen bonded to the nitrogen atom of H90 residue (S═O···N = 2.64 Å), and the other oxygen atom shows hydrogen bonding interactions with nitrogen atom of

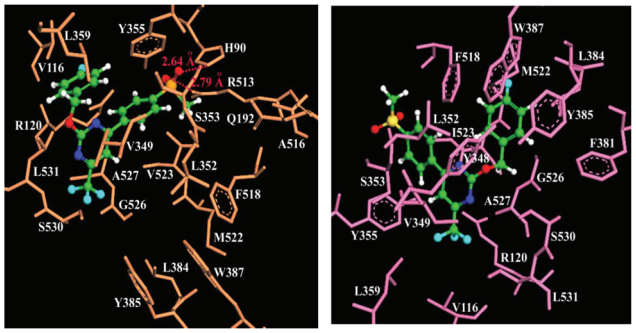


Fig. 6 (Left) Molecular modeling (docking) of compound **3a** (carbon atoms in green) positioned in the binding site of COX-2 (PDB ID: 6COX; $E_{intermolecular}$ = −10.12 kcal mol^{-1}) and (right) COX-1 (PDB ID: 1EQG; $E_{intermolecular}$ = −9.03 kcal mol^{-1}). Hydrogen atoms of amino acid residues have been removed for clarity.

R513 amino acid residue (S═O···N = 2.79 Å). The CF_3 group of compound **3a** is located in close proximity of the S530 residue where the measured distance between one of the F atom of the CF_3 group and the –OH group of S530 is 1.36 Å. The 4-fluoro phenyl ring of compound **3a** is positioned in the vicinity of L359, V116 and R120 amino acid residues. However, docking of compound **3a** within the COX-1 active site clearly indicates that the SO_2CH_3 pharmacophore, which probably contributes to potent COX-2 inhibition, is not able to enter into the COX-1 active site, whereas the F-phenyl ring is sandwiched between the Y385 and W387 amino acid residues of the hydrophobic region.

Radiochemistry

Radiotracers [^{18}F]**1a** and [^{18}F]**2a** were synthesized using a 4-[^{18}F]fluorobenzylamine ([^{18}F]FBA) as a building block. [^{18}F]-FBA was synthesized using a method recently described by our group[41,42] (Fig. 7). 4-Cyano-*N*,*N*,*N*-trimethylanilinium trifluoromethansulfonate as a labelling precursor was radio-fluorinated using nucleophilic no-carrier-added (n.c.a.) [^{18}F]KF in the presence of Kryptofix K_{222} in dry DMSO at elevated temperature.

The resulting 4-[^{18}F]fluorobenzonitrile ([^{18}F]FBN) was reduced to [^{18}F]FBA using transition metal-assisted $NaBH_4$ reduction. A fully automated [^{18}F]FBA synthesis was recently developed by Way *et al.*[42] This work also describes the usefulness of [^{18}F]FBA as a building block for the synthesis of a variety of ^{18}F-labelled compounds like prosthetic groups for peptide labelling or built-up synthesis of complex molecules as ^{18}F-labelled Hsp90 inhibitor geldanamycin.

Radiotracer [^{18}F]**1a** was prepared through the reaction of compound **6** with [^{18}F]FBA. The reaction was carried out in THF at 140 °C for 20 min (Fig. 5). Total synthesis time for the preparation of [^{18}F]**1a**, including HPLC purification, was

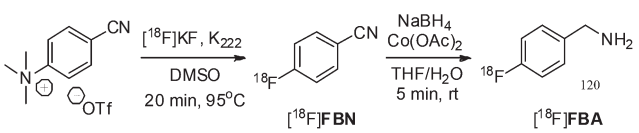


Fig. 7 Radiosynthesis of 4-[^{18}F]fluorobenzylamine **[^{18}F]FBA**.

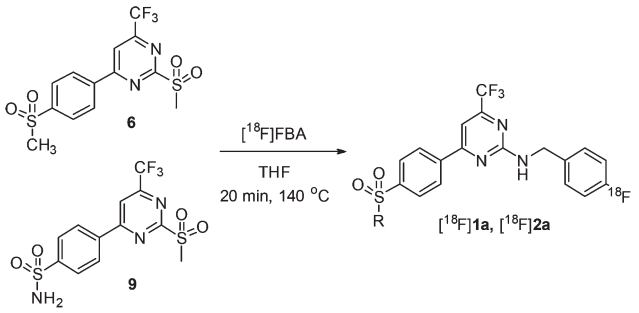


Fig. 8 Radiosynthesis of [^{18}F]**1a** (R = CH_3) and [^{18}F]**2a** (R = NH_2).

95 min. Decay-corrected radiochemical yield based on [^{18}F]FBA was 27 ± 11%. In a typical experiment, starting from 1 GBq of [^{18}F]FBA prepared on an automated synthesis unit provided 160 MBq of radiotracer [^{18}F]**1a**. Specific activity of [^{18}F]**1a** at the end of synthesis was determined to be greater than 40 GBq μmol^{-1}.

Synthesis of radiotracer [^{18}F]**2a** was accomplished by the same methodology described for [^{18}F]**1a** but using labelling precursor **9** as the starting material. Radiosynthesis was carried out within 110 min with a decay-corrected radiochemical yield of 23% ± 1%. Specific activity of [^{18}F]**2a** was greater than 40 GBq μmol^{-1} at the end of synthesis. Radiosyntheses of compounds of [^{18}F]**2a** and of [^{18}F]**2b** are depicted in Fig. 8.

We also attempted to synthesize radiotracer [^{18}F]**3a** by expanding the concept of nucleophilic substitution reactions with compound **6** as the labelling precursor. To replace the nitrogen present in compound [^{18}F]**1a** with an oxygen, we performed radiosyntheses with 4-[^{18}F]fluorobenzyl alcohol ([^{18}F]-FBAlc) as a building block. This strategy would allow the extension of feasible radiochemistry as exemplified for compounds [^{18}F]**1a** and [^{18}F]**2a** to the readily available ^{18}F building block [^{18}F]FBAlc.

As described in the chemistry section, we successfully synthesized reference compound **3a** by the reaction of 4-fluorobenzyl alcohol with compound **6** in the presence of sodium hydride. Radiosynthesis of [^{18}F]FBAlc as a ^{18}F building block was accomplished following a protocol developed by Donohue *et al.*[43] (4-Trimethylamino)benzaldehyde trifluoromethanesulfonate was treated with a powerful radiofluorination agent [^{18}F]KF in the presence of Kryptofix K_{222} in dry acetonitrile at elevated temperature to yield 4-[^{18}F]fluorobenzaldehyde. 4-[^{18}F]Fluorobenzaldehyde was reduced to [^{18}F]FBAlc using $NaBH_4$. $NaBH_4$ was dissolved in water and passed through a solid phase extraction cartridge containing 4-[^{18}F]-fluorobenzaldehyde.

[^{18}F]FBAlc was purified using HPLC, and the solvent was evaporated under reduced pressure. Dried [^{18}F]FBAlc was redissolved in dry solvent to be used in subsequent reaction steps. Despite extensive efforts to promote the substitution reaction between [^{18}F]FBAlc and labelling precursor **6**, it was not possible to obtain reasonable amounts of the desired product [^{18}F]**3a**. Radiochemical yields were below 1% as indicated by radio-TLC analysis of the reaction mixture.

Radiosynthesis of [^{18}F]**3a** based on the reaction between [^{18}F]FBAlc and compound **6** was performed using different reaction conditions. This included variation of reaction temperature (0 °C to 180 °C) use of different bases (no base, triethylamine, potassium *tert*-butoxide, sodium hydride), and the use of different solvents (THF, CH_3CN, DMF). To our disappointment, no product formation of **3a** was achieved as confirmed by radio-TLC and radio-HPLC analyses. We hypothesize that the ratio of [^{18}F]FBAlc to a base and a precursor is crucial for the success of the reaction. The reaction does not proceed in the absence of a base. During the radiosynthesis only trace amounts of [^{18}F]FBAlc are present in the reaction mixture, therefore any added base will be in large stoichiometric excess. We believe that the excess of base interferes with the reaction. Moreover, the reaction tended to be very water sensitive. Although most residual water can be removed during the radiosynthesis, even trace amounts of water seem to have a detrimental effect on the reaction using tracer concentrations of 4-fluorobenzyl alcohol.

As a result, it seems to be very challenging to fine-tune the amount of base required for successful radiosynthesis of compound [^{18}F]**3a** based on the reaction of labelling precursor **6** with [^{18}F]FBAlc.

The difficulties to prepare compound [^{18}F]**3a** according to the synthesis method of cold reference compound **3a** (Fig. 2) prompted us to envisage an alternative synthesis route. Direct nucleophilic aromatic radiofluorination has been reported using iodylbenzene derivatives as labelling precursors. Iodylbenzene derivatives substituted with electron donating as well as electron withdrawing groups on the aromatic ring were shown to readily undergo radiofluorination reaction with n.c.a. [^{18}F]fluoride as exemplified for various compounds described in a recent patent publication.[44] Synthesis of the iodyl group-containing compound **11** as a labelling precursor for the preparation of radiotracer [^{18}F]**3a** is given in Fig. 9.

Synthesis of iodylaryl compound **11** was achieved by the reaction of compound **6** with 4-iodobenzyl alcohol and sodium hydride, applying similar reaction conditions described for the synthesis of **3a** to form compound **10** in 71% yield. 4-Iodobenzyl compound **10** was oxidized to iodyl compound **11** using Oxone in a mixture of water and methanol, while being gently

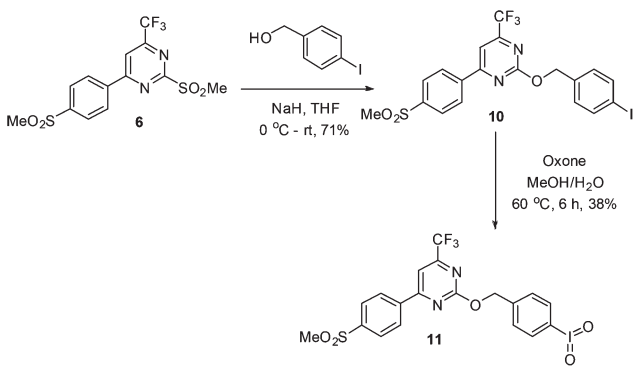


Fig. 9 Synthesis of iodylaryl compound **11**.

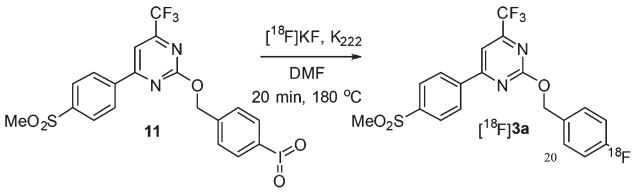


Fig. 10 Radiosynthesis of [^{18}F]**3a** from precursor **11**.

heated. Dilution of the reaction mixture with water and collection of the formed precipitate by filtration yielded iodyl compound **15**. The product also contained small amounts of starting material **11** and the mono-oxidized iodine intermediate. Both small impurities could be removed by column chromatography to give pure iodyl compound **11** in 38% yield.

Radiolabelling with n.c.a. [^{18}F]fluoride was achieved by heating 1 mg of iodylaryl compound **11** in 300 μl of dry DMF in the presence of Kryptofix K_{222} at 180 °C for 20 min (Fig. 10).

A range of different solvents (DMF, DMSO, NMP), temperatures (80 °C to 180 °C) and different precursor concentrations were tested. It was found that optimal radiochemical yields of 5 to 10% (as determined by radio-TLC) could be achieved by using 1 mg of iodyl compound **11** in DMF at 180 °C. To prepare [^{18}F]**3a** in radiopharmaceutical quality for *in vitro* and *in vivo* work, the reaction mixture was diluted in 0.1 M NaOAc buffer (pH 5.3) and passed through a solid phase extraction cartridge. The cartridge was washed with water and product [^{18}F]**3a** was eluted with CH_3CN. The radiotracer was further purified using HPLC. After evaporation of the solvent, the radiotracer was redissolved in 10% EtOH–saline for further radiopharmacological evaluation. Total synthesis was accomplished in 120 min. A starting activity of 300 MBq [^{18}F]fluoride typically yielded 3–5 MBq of purified product [^{18}F]**3a**. The identity of the radiotracer was confirmed by HPLC co-injection with the non-radiolabelled reference compound.

Upon further reaction optimization of the reaction conditions, the iodylaryl-based direct radiofluorination approach could prove to be a versatile strategy for the site specific radiolabelling of more complex drug-like molecules in sufficient radiochemical yields.

Other direct radiolabelling strategies using nucleophilic aromatic substitution reactions with [^{18}F]KF on non-activated aromatic systems involve iodonium and sulfonium salts. However, syntheses of iodonium and sulfonium salts of structurally more complex compounds tend to be challenging, making them generally less versatile with respect to their incorporation of n.c.a. fluorine-18 at specific sites in a given molecule.[45–47]

Summary and conclusion

We have prepared a series of novel COX-2 inhibitors based on a pyrimidine scaffold in continuation to the original work reported by Swarbrick and co-workers.[35] *In vitro* COX-1 and COX-2 enzyme inhibition studies revealed the great potential of pyrimidine-based compounds as highly potent and selective COX-2 inhibitors. Except for bulky substituents such as phenyl

and *tert*-butyl groups attached to the *para* position of the benzyl ring (compounds **1g** and **1h**), COX-2 enzyme seems to accept a broad variety of electron-donating (Me, OMe,) and electron-withdrawing (F, Cl, Br, CF_3, NO_2) groups. Molecular docking studies confirmed the determined high COX-2 inhibitory potency and selectivity of fluorine-containing compounds **1a**, **2a** and **3a**. The high COX-2 inhibitory potency and selectivity of fluorine-containing compounds **1a**, **2a** and **3a** make them interesting compounds for the development of corresponding ^{18}F-labelled radiotracers. Radiolabelling was achieved through two different routes, using the indirect labelling method with 4-[^{18}F]fluoro-benzylamine as the building block, and the direct radiolabelling method with iodylaryl derivative **11** as the labelling precursor. All radiotracers could be prepared in radiochemical yields and radiopharmaceutical quality suitable for subsequent radiopharmacological evaluation. First results on radiopharmacological evaluation of radiotracers [^{18}F]**1a**, [^{18}F]**2a**, and [^{18}F]**3a** have been reported during the 20th International Symposium on Radiopharmaceutical Sciences 2013 in Jeju, Korea.[50]

Experimental

Chemistry

General methods. All reagents and solvents were obtained from Sigma-Aldrich, unless otherwise stated and used without further purification. Nuclear magnetic resonance spectra were recorded on a 400 MHz Varian unit and a 600 MHz Burker unit. ^{1}H-NMR and ^{13}C-NMR chemical shifts are recorded in ppm relative to tetramethylsilane (TMS). ^{19}F-NMR chemical shifts are recorded in ppm relative to trichlorofluoromethane. Low resolution mass spectra were obtained using an Agilent Technologies 6220 TOF instrument. Column chromatography was conducted using Merck silica gel (mesh size 230–400 ASTM). Thin-layer chromatography (TLC) was performed using Merck silica gel F-254 aluminum plates, with visualization under UV light (254 nm). High performance liquid chromatography (HPLC) purifications and analysis were performed using a Phenomenex LUNA® C18 column (100 Å, 250 × 10 mm, 10 μm) using a Gilson 322 Pump module fitted with a 171 Diode Array and a radio detector. Compounds **4**, **5** and **6** were prepared according to the literature procedure.[35] Compounds **1a**, **1e** and **1f** have been described by Swarbrick *et al.*[35]

General procedure for the synthesis of compounds 1a–1p. Compound **6** (50 mg, 0.13 mmol) was dissolved in CH_3CN (0.75–1.5 ml) and the corresponding amine (0.65 mmol, 5.0 eq.) was added. The reaction vessel was sealed and heated at 140 °C for 2–6 h. The reaction mixture was cooled to room temperature, and 8 ml of 1 N HCl was added. The reaction mixture was stirred, and the precipitating solid was filtered off. The product was thoroughly washed with water.

N-*(4-Fluorobenzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoro-methyl)-pyrimidin-2-amine (**1a**).* Compound **1a** (90.6 mg, 82% yield) was obtained as a pale yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.10 (s, 3H, SO_2CH_3); 4.72 (s, 2H, CH_2); 5.85 (s, 1H, N–H); 7.04 (m, J = 8.8 Hz, 2H, Ar–H); 7.33 (s, 1H, Ar–H); 7.37 (m, J = 5.27 Hz, J = 3.22 Hz, 2H, Ar–H); 8.07 (m, J = 8.8 Hz, 2H, Ar–H); 8.22 (m, J = 8.8 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.5; 45.1; 102.6; 115.7 (d, ^2J(C–F) = 21 Hz); 122.0 (q, ^1J(C–F) = 275 Hz); 128.0; 128.2; 129.2 (d, ^3J(C–F) = 8 Hz); 134.1 (d, ^4J(C–F) = 3 Hz); 141.4; 142.8; 162.2 (d, ^1J(C–F) = 245 Hz); 162.7 (q, ^2J(C–F) = 36 Hz); 165.4; 165.5. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.78 (s, 3F, CF_3); −114.83 (m, 1F, Ar–F). LR-MS: 448.1 [M + Na].

N-*(4-Chlorobenzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoro-methyl)pyrimidin-2-amine (**1b**).* Compound **1b** (48.4 mg, 84% yield) was obtained as a pale yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.04 (s, 3H, SO_2CH_3); 4.67 (s, 2H, CH_2); 5.82 (s, 1H, N–H); 7.20 (s, 1H, Ar–H); 7.27 (m, 4H, Ar–H); 8.00 (m, J = 8.8 Hz, 2H, Ar–H); 8.14 (m, J = 8.8 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.5; 45.1; 102.7; 122.0 (q, ^1J(C–F) = 275 Hz); 128.0; 128.2; 128.9; 129.5; 133.4; 136.9; 141.4; 142.8; 162.2 (q, ^2J(C–F) = 36 Hz); 165.5; 165.6. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.45 (s, 3F, CF_3). LR-MS: 464.0 [M + Na].

N-*(4-Bromobenzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoro-methyl)pyrimidin-2-amine (**1c**).* Compound **1c** (51.6 mg, 82% yield) was obtained as a pale yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.10 (s, 3H, SO_2CH_3); 4.71 (s, 2H, CH_2); 5.87 (s, 1H, N–H); 7.28 (m, J = 8.5 Hz, 2H, Ar–H); 7.33 (s, 1H, Ar–H); 7.48 (m, J = 8.5 Hz, 2H, Ar–H); 8.07 (m, J = 8.8 Hz, 2H, Ar–H); 8.20 (m, J = 8.8 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.5; 45.1; 102.7; 121.4 (q, ^1J(C–F) = 275 Hz); 128.0; 128.2; 129.7; 130.9; 132.5; 137.4; 141.4; 142.8; 162.4 (q, ^2J(C–F) = 36 Hz); 165.5; 165.6. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.7 (s, 3F, CF_3). LR-MS: 510.0 [M + Na].

N-*(4-Trifluorobenzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoro-methyl)pyrimidin-2-amine (**1d**).* Compound **1d** (42.3 mg, 68% yield) was obtained as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.10 (s, 3H, SO_2CH_3); 4.83 (s, 2H, CH_2); 5.95 (s, 1H, N–H); 7.35 (s, 1H, Ar–H); 7.52 (m, J = 8.2 Hz, 2H, Ar–H); 7.61 (m, J = 8.2 Hz, 2H, Ar–H); 8.06 (m, J = 8.5 Hz, 2H, Ar–H); 8.19 (m, J = 8.2 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.5; 45.3; 102.9; 122.1 (q, ^1J(C–F) = 275 Hz); 124.0 (q, ^1J(C–F) = 271 Hz); 125.7; 127.6; 128.0; 128.2; 129.8 (q, ^2J(C–F) = 32 Hz); 141.3; 142.5; 162.5 (q, ^2J(C–F) = 36 Hz); 165.5; 165.6. ^{19}F-NMR (375 MHz, $CDCl_3$): −62.5 (s, 3F, CF_3); −70.7 (s, 3F, CF_3). LR-MS: 498.1 [M + Na].

N-*(Benzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)-pyrimidin-2-amine (**1e**).* Compound **1e** (42.9 mg, 81% yield) was obtained as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.10 (s, 3H, SO_2CH_3); 4.76 (s, 2H, CH_2); 5.86 (s, 1H, N–H); 7.30 (m, 1H, Ar–H) 7.32 (s, 1H, Ar–H); 7.36 (m, 2H, Ar–H); 7.40 (m, 2H, Ar–H); 8.10 (m, J = 8.8 Hz, 2H, Ar–H); 8.22 (m, J = 8.5 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.5; 45.8; 102.4; 121.1 (q, ^1J(C–F) = 275 Hz); 127.5; 127.6; 128.0; 128.2; 128.7; 138.3; 141.5; 142.7; 162.4 (q, ^2J(C–F) = 36 Hz); 165.4; 165.5. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.46 (s, 3F, CF_3). LR-MS: 430.1 [M + Na].

N-*(4-Methylbenzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)pyrimidin-2-amine (**1f**).* Compound **1f** (42.6 mg, 78% yield) was obtained as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 2.35 (s, 3H, CH_3–Ar); 3.10 (s, 3H, SO_2CH_3); 4.71 (s, 2H, CH_2); 5.83 (s, 1H, N–H); 7.16 (m, J = 7.9 Hz, 2H, Ar–H); 7.29 (m, J = 8.2 Hz, 2H, Ar–H); 7.30 (s, 1H, Ar–H); 8.06 (m, J = 8.5 Hz, 2H, Ar–H); 8.22 (m, J = 8.2 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 21.1; 44.5; 45.5; 102.3; 122.2 (q, ^1J(C–F) = 275 Hz); 127.6; 128.0; 128.2; 129.4; 135.2; 137.3; 141.6; 142.6; 162.1 (q, ^2J(C–F) = 36 Hz); 165.4; 165.5. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.7 (s, 3F, CF_3). LR-MS: 444.1 [M + Na].

N-*(4-Phenylbenzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)pyrimidin-2-amine (**1g**).* Compound **1g** (31.0 mg, 49% yield) was obtained as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.10 (s, 3H, SO_2CH_3); 4.80 (s, 2H, CH_2); 5.91 (s, 1H, N–H); 7.33 (s, 1H, Ar–H); 7.36 (m, J = 7.3 Hz, 1H, Ar–H); 7.46 (m, 4H, Ar–H); 7.59 (m, 4H, Ar–H); 8.07 (m, J = 8.8 Hz, 2H, Ar–H); 8.24 (m, J = 8.2 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.5; 45.1; 103.2; 120.7 (q, ^1J(C–F) = 275 Hz); 123.9; 128.0; 128.2; 128.5; 129.0; 130.0; 130.5; 141.2; 142.9; 146.1; 146.2; 147.4; 162.5 (q, ^2J(C–F) = 36 Hz); 165.2; 165.3. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.6 (s, 3F, CF_3). LR-MS: 506.1 [M + Na].

N-*(4-*tert*-Butylbenzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)pyrimidin-2-amine (**1h**).* Compound **1h** (23.9 mg, 40% yield) was obtained as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 1.32 (s, 9H, $C(CH_3)_3$); 3.11 (s, 3H, SO_2CH_3); 4.75 (s, 2H, CH_2); 5.90 (s, 1H, N–H); 7.30 (s, 1H, Ar–H); 7.35 (m, J = 7.9 Hz, 2H, Ar–H); 7.38 (m, J = 7.9 Hz, 2H, Ar–H); 8.09 (m, J = 7.9 Hz, 2H, Ar–H); 8.26 (m, J = 7.9 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 31.3; 34.6; 44.5; 45.4; 102.3; 122.5 (q, ^1J(C–F) = 275 Hz); 125.6; 128.0; 128.2; 128.8; 135.3; 141.6; 142.6; 150.7; 162.7 (q, ^2J(C–F) = 36 Hz); 165.4; 165.5. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.7 (s, 3F, CF_3).

N-*(4-Methoxybenzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)pyrimidin-2-amine (**1i**).* Compound **1i** (111.6 mg, 98% yield) was obtained as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.10 (s, 3H, SO_2CH_3); 3.81 (s, 3H, CH_3–O); 4.68 (s, 2H, CH_2); 5.80 (s, 1H, N–H); 6.89 (m, J = 8.5 Hz, 2H, Ar–H); 7.30 (s, 1H, Ar–H); 7.33 (m, J = 8.8 Hz, 2H, Ar–H); 8.07 (m, J = 8.5 Hz, 2H, Ar–H); 8.24 (m, J = 7.6 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.5; 45.3; 55.3; 102.3; 121.5 (q, ^1J(C–F) = 275 Hz); 128.0; 128.2; 129.4; 129.8; 130.3; 141.6; 142.6; 159.1; 162.1 (q, ^2J(C–F) = 36 Hz); 165.4; 165.5. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.7 (s, 3F, CF_3). LR-MS: 460.1 [M + Na].

N-*(4-Nitrobenzyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)-pyrimidin-2-amine (**1j**).* The synthesis follows the general method described; with the exception that triethylamine (4.9 eq.) was added to the reaction mixture. Compound **1j** (24.1 mg, 41% yield) was obtained as a yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.10 (s, 3H, SO_2CH_3); 4.88 (s, 2H, CH_2); 6.03 (s, 1H, N–H); 7.37 (s, 1H, Ar–H); 7.57 (m, J = 8.5 Hz, 2H, Ar–H); 8.07 (m, J = 8.5 Hz, 2H, Ar–H); 8.20 (m, 4H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.5; 45.1; 102.2; 120.1; 122.0 (q, ^1J(C–F) = 275 Hz); 128.0; 128.2; 129.7; 135.2; 141.6; 142.6; 158.5; 162.9 (q, ^2J(C–F) = 36 Hz); 165.5; 165.6. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.7 (s, 3F, CF_3). LR-MS: 475.1 [M + Na].

N-*(4-Pyridyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)-pyrimidin-2-amine (**1k**).* Compound **1k** (30.7 mg, 58% yield) was obtained as an off-white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.11 (s, 3H, SO_2CH_3); 5.01 (s, 2H, CH_2); 6.74 (s, 1H, N–H); 7.43 (s, 1H, Ar–H); 7.95 (m, J = 5.6 Hz, 2H, Ar–H); 8.07 (m, J = 8.2 Hz, 2H, Ar–H); 8.28 (m, J = 8.2 Hz, 2H, Ar–H);8.72 (m, J = 5.6 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.4; 45.0; 103.9; 113.8; 121.3 (q, ^1J(C–F) = 275 Hz); 128.1; 128.2; 128.7; 134.3; 141.2; 143.1; 162.2 (q, ^2J(C–F) = 36 Hz); 165.3; 165.4. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.3 (s, 3F, CF_3). LR-MS: 431.1 [M + Na].

N-*(3-Pyridyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)-pyrimidin-2-amine (**1l**).* Compound **1l** (23.8 mg, 45% yield) was obtained as a pale yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.10 (s, 3H, SO_2CH_3); 4.92 (s, 2H, CH_2); 6.91 (s, 1H, N–H); 7.36 (s, 1H, Ar–H); 7.69 (m, 1H, Ar–H); 8.05 (m, J = 7.6 Hz, 2H, Ar–H);8.16 (m, J = 8.5 Hz, 2H, Ar–H);8.34 (s, 1H, Ar–H); 8.62 (m, J = 5.2 Hz, 1H, Ar–H); 9.09 (m, 1H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 42.9; 44.5; 103.3; 111.0; 121.1; 121.3 (q, ^1J(C–F) = 275 Hz); 125.1; 128.1; 128.2; 128.3; 133.3; 141.1; 142.9; 162.2 (q, ^2J(C–F) = 36 Hz); 165.4; 165.5. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.4 (s, 3F, CF_3). LR-MS: 431.1 [M + Na].

N-*(2-Pyridyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)-pyrimidin-2-amine (**1m**).* Compound **1m** (8.5 mg, 16% yield) was obtained as a pale yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.11 (s, 3H, SO_2CH_3); 3.13 (m, 2H, CH_2); 5.17 (s, 1H, N–H); 7.36 (s, 1H, Ar–H); 7.81 (m, J = 6.4 Hz, 1H, Ar–H); 8.00 (m, J = 7.6 Hz, 2H, Ar–H); 8.14 (m, J = 7.6 Hz, 2H, Ar–H); 8.24 (m, J = 7.3 Hz, 1H, Ar–H); 8.37 (m, J = 7.3 Hz, 1H, Ar–H); 8.67 (m, J = 6.4 Hz, 1H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 42.6; 44.5; 103.5; 113.9; 118.9; 121.5 (q, ^1J(C–F) = 275 Hz); 125.3; 128.0; 128.2; 128.9; 134.5; 140.8; 143.0; 161.8 (q, ^2J(C–F) = 36 Hz); 165.5; 165.6. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.4 (s, 3F, CF_3). LR-MS: 431.1 [M + Na].

N-*(2-Fluoroethyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)-pyrimidin-2-amine (**1n**).* The synthesis follows the general method described; with the exception that triethylamine (4.9 eq.) was added to the reaction mixture. Compound **1n** (26.1 mg, 52% yield) was obtained as a silvery white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 3.12 (s, 3H, SO_2CH_3); 3.92 (m, J = 5.5 Hz, 2H, CH_2); 4.67 (m, J = 5.0 Hz, 2H, CH_2); 5.78 (s, 1H, N–H); 7.34 (s, 1H, Ar–H); 8.10 (m, J = 7.6 Hz, 2H, Ar–H); 8.25 (m, J = 7.6 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 42.1 (d, ^2J(C–F) = 20 Hz); 44.5; 82.0 (d, ^1J(C–F) = 167 Hz); 102.7; 121.3 (q, ^1J(C–F) = 275 Hz); 128.0; 128.3; 139.5; 142.8; 162.1 (q, ^2J(C–F) = 36 Hz); 165.4; 165.5. ^{19}F-NMR (375 MHz, $CDCl_3$): −67.9 (s, 1F, C–F); −70.8 (s, 3F, CF_3). LR-MS: 386.1 [M + Na].

N-*(Butyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)-pyrimidin-2-amine (**1o**).* Compound **1o** (29.5 mg 61% yield) was obtained as a pale yellow solid.

^{1}H-NMR (400 MHz, $CDCl_3$): 0.98 (m, J = 7.3 Hz, 3H, CH_3); 1.47 (m, J = 7.0, 7.6 Hz, 2H, CH_2); 1.67 (m, J = 7.0, 7.6 Hz, 2H,

CH_2); 3.11 (s, 3H, SO_2CH_3); 3.59 (m, J = 6.1, 6.4 Hz, 2H, CH_2); 4.83 (s, 2H, CH_2); 5.48 (s, 1H, N–H); 7.26 (s, 1H, Ar–H); 8.10 (m, J = 8.2 Hz, 2H, Ar–H); 8.27 (m, J = 8.2 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 13.8; 20.0; 31.4; 41.5; 44.5; 101.7; 121.5 (q, ^1J(C–F) = 275 Hz); 128.2; 128.5; 140.3; 143.4; 161.2 (q, ^2J(C–F) = 36 Hz); 165.3; 165.4. ^{19}F-NMR (375 MHz, $CDCl_3$): −70.7 (s, 3F, CF_3). LR-MS: 396.1 [M + Na].

*2-[(4-Fluorobenzyl)oxy]-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)pyrimidine (**3a**).* To a solution of 33 mg 4-fluorobenzyl alcohol (0.26 mmol) in 3 ml of dry THF under an inert atmosphere and at 0 °C was added 11 mg of 60% NaH suspension. After 5 min, 100 mg of 2-(methylsulfonyl)-4-[4-(methylsulfonyl)-phenyl]-6-(trifluoromethyl)pyrimidine **6** (0.26 mmol) was added in small portions. The reaction mixture was stirred at 0 °C for 1 hour and quenched by adding an excess of 1 N HCl. Compound **3a** was extracted in ethyl acetate. The organic layer was washed with water, dried over sodium sulfate and solvent removed *in vacuo*. Compound **3a** was purified using a silica column eluting at 1% MeOH–CH_2Cl_2 and obtained as a white solid (78.6 mg, 71% yield).

^{1}H-NMR (600 MHz, $CDCl_3$): 3.05 (s, 3H, SO_2CH_3); 5.47 (s, 2H, CH_2); 7.01 (m, J = 8.2 Hz, 2H, Ar–H); 7.46 (m, J = 8.2 Hz, 2H, Ar–H); 7.65 (s, 1H, Ar–H); 8.04 (m, J = 8.4 Hz, 2H, Ar–H); 8.24 (m, J = 8.4 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, $CDCl_3$): 44.4; 69.7; 107.1; 115.5 (d, ^2J(C–F) = 21 Hz); 121.1 (q, ^1J(C–F) = 275 Hz); 128.2; 128.5; 130.7 (d, ^3J(C–F) = 9 Hz); 131.3 (d, ^4J(C–F) = 3 Hz); 140.3; 143.4; 159.1 (q, ^2J(C–F) = 36 Hz); 162.7 (d, ^1J(C–F) = 247 Hz); 165.6; 167.4. ^{19}F-NMR (565 MHz, $CDCl_3$): −71.1 (s, 3F, CF_3); −114.1 (m, 1F, Ar–F). LR-MS: 449.1 [M + Na].

N-tert-*Butyl-4-[2-(methylsulfanyl)-6-(trifluoromethyl)-pyrimidin-4-yl]benzenesulfonamide (**7**).* 4-Chloro-2-(methylsulfonyl)-6-(trifluoromethyl)pyrimidine (Key Organics Ltd, Camelford, UK) (1.0 g, 4.42 mmol), *tert*-butyl 4-boronobenzenesulfonamide (Combi-Blocks Inc., San Diego, USA) (1.25 g, 4.8 mmol) and tetrakis-triphenylphosphinepalladium(0) (100 mg) were dissolved in 70 ml of DME and 1.15 g of sodium carbonate in water (11 ml) added dropwise. The reaction mixture was heated under reflux for 15 hours. The solvent was reduced on a rotary evaporator and the residue partitioned between ethyl acetate and water; the organic phase was dried using sodium sulfate and concentrated *in vacuo*. The compound was purified using a silica column, eluting at 30% ethyl acetate–hexanes. The title compound **11** (1.27 g, 69% yield) was obtained as a pale yellow solid. LR-MS: 444.1 [M + Na]. Low resolution mass spectrometry indicates that the product is sulfoxide. However, the product can be used directly in the next step involving oxidation with Oxone.

N-tert-*Butyl-4-[2-(methylsulfonyl)-6-(trifluoromethyl)-pyrimidin-4-yl]benzenesulfonamide (**8**).* Compound **7** (1.2 g, 2.85 mmol) was dissolved in 100 ml of dichloromethane. A solution of Oxone (4.37 g, 7.13 mmol) in 50 ml of water was added in small portions, and the mixture was stirred at room temperature. After 18 h, solvent was reduced on a rotary evaporator and the residue partitioned between ethyl acetate and water; the organic phase was dried over sodium sulfate and concentrated *in vacuo*. The title compound **8** (1.08 g, 87% yield) was obtained as a white solid.

^{1}H-NMR (600 MHz, DMSO-d_6): 1.18 (s, 9H, $(CH_3)_3$); 3.64 (s, 3H, SO_2CH_3); 7.87 (s, 1H, NH); 8.13 (m, J = 9.0, 2H, Ar–H); 8.69 (m, J = 9.0 Hz, 2H, Ar–H); 9.04 (s, 1H, Ar–H). ^{13}C-NMR (150 MHz, DMSO-d_6): 30.2; 39.6; 54.0; 117.5; 121.6 (q, ^1J(C–F) = 275 Hz); 127.5; 129.5; 137.0; 148.6; 158.7 (q, ^2J(C–F) = 36 Hz); 166.4; 167.2. ^{19}F-NMR (565 MHz, DMSO-d_6): −69.02 (s, 3F, CF_3). LR-MS: 460.1 [M + Na].

*4-[2-(Methylsulfonyl)-6-(trifluoromethyl)pyrimidin-4-yl]benzenesulfonamide (**9**).* 110 mg of compound **8** (0.252 mmol) was dissolved in 4 ml of nitromethane and 50 mg of scandium triflate was added. The mixture was heated at 80 °C overnight and subsequently concentrated *in vacuo*. The residue was partitioned between ethyl acetate and water; the organic phase was dried using sodium sulfate and concentrated *in vacuo*. Compound **9** (85 mg, 88% yield) was obtained as a light brown solid.

^{1}H-NMR (600 MHz, DMSO-d_6): 3.64 (s, 3H, SO_2CH_3); 7.68 (s, 2H, NH_2); 8.12 (m, J = 8.4, 2H, Ar–H); 8.70 (m, J = 8.4 Hz, 2H, Ar–H); 9.05 (s, 1H, Ar–H). ^{13}C-NMR (150 MHz, DMSO-d_6): 39.6; 117.5; 121.6 (q, ^1J(C–F) = 275 Hz); 126.8; 129.5; 137.0; 148.2; 156.7 (q, ^2J(C–F) = 36 Hz); 166.4; 167.3. ^{19}F-NMR (565 MHz, DMSO-d_6): −69.00 (s, 3F, CF_3). LR-MS: 404.0 [M + Na].

General procedure for the synthesis of compounds 2a–2e. Compound **9** (50 mg, 0.13 mmol) was dissolved in 1.5 ml of acetonitrile and corresponding amine (0.65 mmol, 5.0 eq.) was added. The reaction vessel was sealed and heated at 140 °C for 2 to 6 hours. Solvent was removed on a rotary evaporator and the residue partitioned between ethyl acetate and 1 N HCl. The organic layer was washed dried over sodium sulfate and the solvent removed *in vacuo*. Further impurities were removed by purification on a silica column.

*4-{2-[(4-Fluorobenzyl)amino]-6-(trifluoromethyl)pyrimidin-4-yl}-benzenesulfonamide (**2a**).* Compound **2a** (90.6 mg, 82% yield) was obtained as a yellow solid.

^{1}H-NMR (600 MHz, DMSO-d_6): 4.70 (s, 2H, CH_2); 7.21 (m, J = 8.8 Hz, 2H, Ar–H); 7.51 (m, J = 8.8 Hz, 2H, Ar–H); 7.57 (s, 2H, SO_2NH_2); 7.71 (s, 1H, Ar–H); 8.43 (m, J = 8.8 Hz, 2H, Ar–H); 8.62 (m, J = 8.8 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, DMSO-d_6): 44.1; 101.8; 115.5 (d, ^2J(C–F) = 23 Hz); 121.5 (q, ^1J(C–F) = 275 Hz); 126.6; 128.4; 129.8 (d, ^3J(C–F) = 8 Hz); 136.4 (d, ^4J(C–F) = 3 Hz); 139.2; 146.9; 157.1 (q, ^2J(C–F) = 36 Hz); 161.6 (d, ^1J(C–F) = 243 Hz); 162.5; 166.1. ^{19}F-NMR (565 MHz, DMSO-d_6): −70.03 (s, 3F, CF_3); −117.08 (m, 1F, Ar–F). LR-MS: 449.1 [M + Na].

*4-[2-(Benzylamino)-6-(trifluoromethyl)pyrimidin-4-yl]benzenesulfonamide (**2b**).* Compound **2b** (48.4 mg, 84% yield) was obtained as a dark yellow solid.

^{1}H-NMR (600 MHz, DMSO-d_6): 4.71 (s, 2H, CH_2); 7.28 (m, 1H, Ar–H); 7.39 (m, J = 8.8 Hz, 2H, Ar–H); 7.47 (m, J = 8.8 Hz, 2H, Ar–H); 7.57 (s, 2H, SO_2NH_2); 7.71 (s, 1H, Ar–H); 8.03 (m, J = 8.8 Hz, 2H, Ar–H); 8.42 (m, J = 8.8 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, DMSO-d_6): 44.8; 101.7; 121.2 (q, ^1J(C–F) = 275 Hz); 126.6; 127.3; 128.3; 128.8; 129.4; 139.3; 140.2; 146.9; 157.1 (q, ^2J(C–F) = 36 Hz); 162.7; 166.1. ^{19}F-NMR (565 MHz, DMSO-d_6): −70.03 (s, 3F, CF_3). LR-MS: 431.1 [M + Na].

*4-{2-[(4-Methylbenzyl)amino]-6-(trifluoromethyl)pyrimidin-4-yl}-benzenesulfonamide (**2c**).* Compound **2c** (49.8 mg, 91% yield) was obtained as a brown solid.

^{1}H-NMR (600 MHz, DMSO-d_6): 2.31 (s, 3H, CH_3); 4.66 (s, 2H, CH_2); 7.18 (m, J = 8.8 Hz, 2H, Ar–H); 7.33 (m, J = 8.8 Hz, 2H, Ar–H); 7.57 (s, 2H, SO_2NH_2); 7.70 (s, 1H, Ar–H); 8.03 (m, J = 8.8 Hz, 2H, Ar–H); 8.43 (m, J = 8.8 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, DMSO-d_6): 38.5; 44.5; 101.6; 121.2 (q, ^1J(C–F) = 275 Hz); 126.6; 127.8; 128.4; 129.3; 136.7; 137.2; 139.3; 146.9; 157.1 (q, ^2J(C–F) = 36 Hz); 162.7; 166.1. ^{19}F-NMR (565 MHz, DMSO-d_6): −70.04 (s, 3F, CF_3). LR-MS: 445.1 [M + Na].

*4-{2-[(4-Methoxybenzyl)amino]-6-(trifluoromethyl)pyrimidin-4-yl}-benzenesulfonamide (**2d**).* Compound **2d** (32.5 mg, 57% yield) was obtained as a dark yellow solid.

^{1}H-NMR (600 MHz, DMSO-d_6): 3.72 (s, 1H, CH_3); 4.60 (s, 2H, CH_2); 6.89 (m, J = 8.8 Hz, 2H, Ar–H); 7.34 (m, J = 8.8 Hz, 2H, Ar–H); 7.52 (s, 2H, SO_2NH_2); 7.64 (s, 1H, Ar–H); 7.97 (m, J = 8.8 Hz, 2H, Ar–H); 8.38 (m, J = 8.8 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, DMSO-d_6): 44.2; 55.5; 101.6; 115.6; 121.1 (q, ^1J(C–F) = 275 Hz); 126.6; 128.8; 129.2; 132.1; 139.3; 146.9; 157.1 (q, ^2J(C–F) = 36 Hz); 158.7; 162.7; 165.6. ^{19}F-NMR (565 MHz, DMSO-d_6): −70.05 (s, 3F, CF_3). LR-MS: 461.1 [M + Na].

*4-[2-(Butylamino)-6-(trifluoromethyl)pyrimidin-4-yl]benzenesulfonamide (**2e**).* Compound **2e** (31.4 mg, 65%) was obtained as a dark yellow solid.

^{1}H-NMR (600 MHz, DMSO-d_6): 0.98 (m, J = 7.2 Hz, 4H, $(CH_2)_2$); 1.44 (m, J = 7.2 Hz, 3H, CH_3); 1.64 (m, J = 7.2 Hz, 2H, CH_2); 7.57 (s, 2H, SO_2NH_2); 7.65 (s, 1H, Ar–H); 8.05 (m, J = 8.8 Hz, 2H, Ar–H); 8.43 (m, J = 8.8 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, DMSO-d_6): 14.2; 20.0; 31.2; 41.0; 101.6; 121.1 (q, ^1J(C–F) = 275 Hz); 126.6; 128.9; 139.5; 146.8; 157.1 (q, ^2J(C–F) = 36 Hz); 162.8; 165.7. ^{19}F-NMR (565 MHz, DMSO-d_6): −70.08 (s, 3F, CF_3). LR-MS: 397.1 [M + Na].

*2-[(4-Iodobenzyl)oxy]-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)pyrimidine (**10**).* 677 mg of 4-iodobenzyl alcohol (2.89 mmol) was dissolved in 25 ml of dry THF under an inert gas atmosphere and at 0 °C, 115 mg of 60% NaH (2.89 mmol) was added to the stirring mixture. After 5 min, 1.0 g of compound **6** (2.63 mmol) was added. The reaction mixture was stirred at 0 °C for 1 h and quenched by adding an excess of 1 N HCl. The title compound was extracted in ethyl acetate. The organic layer was washed with water, dried over sodium sulfate and solvent removed *in vacuo*. Compound **10** was purified using a silica column eluting at 1% MeOH–CH_2Cl_2 and obtained as a pale yellow solid (1.0 g, 71% yield).

^{1}H-NMR (600 MHz, DMSO-d_6): 3.15 (s, 3H, SO_2CH_3); 5.55 (s, 2H, CH_2); 7.32 (m, J = 8.4 Hz, 2H, Ar–H); 7.75 (s, 1H, Ar–H); 7.75 (m, J = 8.4 Hz, 2H, Ar–H); 8.14 (m, J = 8.4 Hz, 2H, Ar–H); 8.33 (m, J = 8.4 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, DMSO-d_6): 43.7; 69.3; 95.0; 108.5; 121.7 (q, ^1J(C–F) = 275 Hz); 123.6; 128.1; 129.2; 131.0; 137.2; 139.8; 143.7; 157.9 (q, ^2J(C–F) = 36 Hz); 165.3; 167.7. ^{19}F-NMR (375 MHz, DMSO-d_6): −71.0 (s, 3F, CF_3). LR-MS: 557.0 [M + Na].

*2-[(4-Iodylbenzyl)oxy]-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)pyrimidine (**11**).* 500 mg of compound **10** (0.93 mmol) was dissolved in 100 ml of MeOH and Oxone (2.8 g, 4.65 mmol) in 40 ml of H_2O added drop-wise. The reaction mixture was heated at 60 °C for 6 hours, cooled on ice and diluted in excess water. The forming precipitate was filtered off, washed with water and dried under vacuum. The compound was purified on a silica column eluting from 10 to 50% MeOH–CH_2Cl_2. Compound **11** was obtained as a white solid (353 mg, 67% yield).

^{1}H-NMR (600 MHz, DMSO-d_6): 3.30 (s, 3H, SO_2CH_3); 5.67 (s, 2H, CH_2); 7.76 (m, J = 8.4 Hz, 2H, Ar–H); 8.00 (m, J = 8.4 Hz, 2H, Ar–H); 8.14 (m, J = 8.4 Hz, 2H, Ar–H); 8.38 (s, 1H, Ar–H); 8.59 (m, J = 8.4 Hz, 2H, Ar–H). ^{13}C-NMR (150 MHz, DMSO-d_6): 43.7; 69.3; 108.6; 121.7 (q, ^1J(C–F) = 275 Hz); 127.2; 128.1; 129.3; 131.0; 139.7; 139.8; 144.2; 151.2; 158.0 (q, ^2J(C–F) = 36 Hz); 165.3; 167.7. ^{19}F-NMR (560 MHz, DMSO-d_6): −68.5 (s, 3F, CF_3). LR-MS: 589.0 [M + Na].

In vitro COX inhibition assay

The ability of celecoxib and compounds **1a–1p**, **2a–2e**, **3a** to inhibit ovine COX-1 and recombinant human COX-2 was determined using a COX fluorescence inhibitor assay (Cayman Chemical, Ann Arbor, USA; catalog #: 700100) according to the manufacturers protocol. Compounds were assayed in a concentration range of 10^{-9} –10^{-3} M. PRISM5 software was used to calculate IC_{50} values.

Molecular docking studies

The molecular docking experiments were performed using crystal coordinates from the X-ray crystal structure of COX-1 (ovine, 1EQG, ibuprofen bound in the active site) and COX-2 (murine, 6COX, SC558 bound in the active site) were obtained from the protein data bank.[48,49] Compounds were built using the builder toolkit of the software package ArgusLab 4.0.1 (Mark, A. ArgusLab, Version 4.0.1; Thompson Planaria Software LLC: Seattle, WA) and energy minimized using the semi-empirical quantum mechanical method PM3. The monomeric structure of the enzyme was chosen and the active site was defined around the ligand. The molecule to be docked in the active site of the enzyme was inserted in the work space carrying the structure of the enzyme. The docking program implements an efficient grid based docking algorithm which approximates an exhaustive search within the free volume of the binding site cavity. The conformational space was surveyed by the geometry optimization of the flexible ligand (rings are treated as rigid) in combination with the incremental construction of the ligand torsions. Thus, docking occurred between the flexible ligand parts of the compound and enzyme. The ligand orientation was determined by a shape scoring function based on Ascore and the final positions were ranked by lowest interaction energy values. The interaction is the sum of the energies involved in H-bond interactions, hydrophobic interactions and van der Waal's interactions. H-bond and hydrophobic interactions between the compound and the enzyme were explored by distance measurements.

Radiochemistry

No-carrier-added (n.c.a.) [^{18}F]fluoride was produced *via* the ^{18}O(p,n)^{18}F nuclear exchange reaction from [^{18}O]H_2O (Rotem

Industries, Hyox oxygen-18 enriched water) on an ACSI TR19/9 Cyclotron (Advanced Cyclotron Systems Inc., Burnaby, Canada).

[^{18}F]fluoride was trapped on a Waters SepPak® light QMA anion exchange cartridge. Radiosynthesis of 4-[^{18}F]fluorobenzylamine ([^{18}F]FBA) followed the procedure published by Way *et al.*[42] Please refer to the publication for a detailed description of the procedure.

Synthesis of [^{18}F]1a. To 6 mg of compound **6** was added [^{18}F]FBA in 1 ml of THF (typically 1 GBq of [^{18}F]FBA) in a sealed vessel. The reaction vessel was heated at 140 °C for 30 min. The mixture was diluted in 10 ml water and passed onto a SepPak® C18 cartridge, the cartridge was washed with 5 ml water and the title compound eluted using 3 ml of CH_3CN. The volume of the solvent was reduced on a rotary evaporator to prepare a 1 ml 70/30 CH_3CN–H_2O formulation for HPLC injection. The compound was purified using HPLC (HPLC conditions: isocratic 70/30 CH_3CN–H_2O; flow rate 3 ml min^{-1}) and the product collected at retention time of 12.6 min. The solvent was evaporated using a rotary evaporator under vacuum at 30 °C.

Synthesis of [^{18}F]2a. The synthesis of [^{18}F]**2a** follows the same procedure as the synthesis of [^{18}F]**1a**, except that [^{18}F]FBA was heated with 6 mg of compound **9** and that the desired product elutes of the HPLC after 11.1 min.

Synthesis of [^{18}F]3a. N.c.a. [^{18}F]fluoride was eluted off the QMA cartridge in Kryptofix K_{222} and K_2CO_3 in CH_3CN. [^{18}F]fluoride was dried under azotropic conditions, using a steady stream of nitrogen at 95 °C while adding 5 ml of CH_3CN to the mixture. To the dried [^{18}F]fluoride was added compound **11** (1 mg) dissolved in DMF (300 μl). The mixture was heated at 180 °C for 20 min, 0.1 M pH 5.3 NaOAc buffer (10 ml) was added and the mixture passed through a SepPak® C18 cartridge. The cartridge was washed with water (5 ml) and [^{18}F]**3a** was eluted using acetonitrile (3 ml). The volume of the solvent was reduced on a rotary evaporator to prepare a 1 ml 70/30 CH_3CN–H_2O formulation for HPLC injection. The compound was purified using HPLC (HPLC conditions: isocratic 70/30 CH_3CN–H_2O; flowrate 3 ml min^{-1}) and the product collected at retention time of 13.4 min. The solvent was evaporated using a rotary evaporator under vacuum at 30 °C.

Octanol–water partition coefficient

The lipophilicity of compound [^{18}F]**1a** was determined by adding 1 MBq of compound and the respective radiotracer in a mixture of 1 ml octanol and 1 ml phosphate-buffered saline (PBS) at pH 7.4. The mixture was shaken for 30 min and the layers separated. A 10 μl sample of the organic layer and a 1 ml sample of the aqueous layer were taken and the radioactivity measured using a gamma counter.

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