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**Imaging COX-2 expression in cancer using PET/SPECT radioligands: Current status and future directions**

**Short title:- PET/SPECT imaging of COX-2 expression**

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### **Abstract**

The role of COX-2 as a driving force in early tumourigenesis and the current interest in the combination of COX-2 inhibitors with standard therapy in clinical trials creates an urgent need to establish clinically relevant diagnostic tests for COX-2 expression. Molecular imaging using small molecule probes radiolabelled for both PET and SPECT offer the potential to meet this need, providing a minimally invasive readout for the whole disease burden. This review summarises current approaches to the radiolabelling of small molecule COX-2 inhibitors and their analogues for PET and SPECT imaging, and gives an overview of their biological evaluation and likely success of clinical application.

### **Introduction**

Prostaglandins are an important class of inflammatory signalling molecules with pleiotropic effects in tumourigenesis across several of the hallmarks of cancer. They are synthesised from arachidonic acid by the cyclooxygenases (COXs), COX-1 and 2, with the former being constitutively expressed in most mammalian tissues whilst COX-2 is induced in response to variety of pro-inflammatory stimuli. COX-2 is thus expressed in a variety of pathological conditions with inflammatory components, including neurodegenerative disorders and cancer [1-3]. The lack of basal COX-2 in most normal tissue makes it an attractive biomarker for detection via molecular imaging with Positron Emission Tomography (PET) imaging.

Chronic inflammation has been linked to the development of the majority of cancers, and COX-2 expression has been demonstrated in a number of pre-malignant inflammatory conditions [4, 5]. The importance of COX-2 expression in the early stages of colon cancer was demonstrated when the treatment of patients with familial adenomatous polyposis (FAP) with COX-2 inhibitors resulted in the development of significantly fewer adenomas [6]; as COX-2 is also overexpressed in several cancers including lung, breast, colon, prostate, head and neck, pancreatic and brain [7] the molecular imaging of this target has strong potential for the early detection of cancer. Additionally, as expression levels of COX-2 have been found to be prognostic for progression from ductal carcinoma in situ to invasive cancer in the breast

and associated with progression and metastasis in prostate cancer [8, 9], imaging COX-2 may improve the staging of cancer and thus patient management. Importantly, improved therapeutic outcomes resulting from the addition of COX-2 inhibitors to standard chemotherapy regimens have been correlated to pre-treatment COX-2 levels in several malignancies [10-12] with levels of COX-2 also linked to conventional chemoradiation responsiveness [13]; thus molecular imaging of COX-2 may allow the stratification of patients for COX-2 therapy.

This review focuses on previous studies of radiolabelled small-molecule COX-2 inhibitors and, where available, summarises the biological evaluation of their potential use as imaging agents. Where data are available for COX-1/COX-2 probe affinity and selectivity these are summarised in Table 1, together with calculated logP values.

## Radiolabelling Strategies

### [<sup>11</sup>C] radiolabelling

An early foray into COX-2 probe development was by Prabhakaran *et al*, who reported the synthesis of [<sup>11</sup>C]celecoxib **1** (Figure 1) as a potential marker for COX-2 overexpression [14]. Radiosynthesis via a Stille reaction of a tributylstannyl precursor with [<sup>11</sup>C]methyl iodide gave **1** in ~8 % radiochemical yield (RCY). However, no further biological data were provided. An alternative palladium catalysed [<sup>11</sup>C]methylation of celecoxib **1** was carried out by Hirano *et al* in 63 % RCY (d.c.) [15]. The authors further converted [<sup>11</sup>C]celecoxib to the known metabolite [<sup>11</sup>C]SC-62807 **2** by oxidation of the tolyl carbon using potassium permanganate. The two radioligands were then evaluated *in vivo* as potential radiotracers for imaging hepatobiliary excretion via drug transporters. [<sup>11</sup>C]celecoxib demonstrated poor suitability for imaging this process due to slow blood clearance and the fact that signal will consist of both parent and metabolite, but [<sup>11</sup>C]SC-62807 showing potentially favourable pharmacokinetics. No assessment of [<sup>11</sup>C]celecoxib as a COX-2 imaging agent was made.

### Figure 1 here

Gao and co-workers synthesised a library of celecoxib derivatives that were radiolabelled using [<sup>11</sup>C]methyl triflate at either the phenolic methyl ether (**3**) or as the methyl ester (**4-7**) [16]. The non-radiolabelled versions of these compounds were tested in an MTS (cell proliferation/viability) assay and were found to inhibit growth of MDA-MB-435 cells with similar potency to celecoxib. Crucially, no further biological data were forthcoming in the report or any subsequent manuscript to date.

### Table 1 here

An elegant generic approach to radiolabel COX-2 motifs at the methyl sulfone functionality was demonstrated with structures such as etoricoxib (**8**) and rofecoxib (**9**). The approach involved a thiobutyrate ester as radiolabelling precursor that was deprotected *in situ* and then alkylated using [<sup>11</sup>C]methyl iodide, followed by oxidation to the methyl sulfone with oxone, in high reported RCY (20-30% end of bombardment (EOB)) [17]. A similar strategy

was adopted by de Vries *et al.* to radiolabel rofecoxib (**9**), in this case refined to require just a single step alkylation with [<sup>11</sup>C]methyl iodide, resulting in ~60% RCY and moderate specific activity (14 GBq/μmol) [18]. These authors assessed [<sup>11</sup>C]rofecoxib (**9**) uptake in the brain of healthy rats, and demonstrated that this correlated with regions of high basal COX-2 mRNA expression; furthermore this accumulation was displaceable by the COX-2 inhibitor NS398. [<sup>11</sup>C]rofecoxib (**9**) uptake could not be demonstrated in pre-clinical models of inflammation based on HSV encephalitis or carageenan-induced paw inflammation, albeit that where characterised, there was no significant difference in COX-2 peroxidase activity.

Tanaka *et al* synthesised and evaluated three [<sup>11</sup>C]labelled diaryl-substituted indole and imidazole derivatives **10-12** (Figure 1)[19]. The three compounds showed high COX2/COX1 selectivity and lower logP than standard COX2 radiotracers (Table 1). The compounds were demonstrated to have acceptable metabolic stability with approximately 90% parent remaining in rat plasma at one hour post-injection for [<sup>11</sup>C]**11** and [<sup>11</sup>C]**12**, although [<sup>11</sup>C]**10** showed reduced metabolic stability with ~50% parent at this time-point. Despite low uptake into the brain, all compounds showed differential regional uptake to some extent as measured by autoradiography of brain slices; crucially however binding to these and other organs was not displaceable by COX-2 inhibitors. [<sup>11</sup>C]**10** was shown to be a P-gp substrate but pre-treatment with the P-gp inhibitor cyclosporine A did not modulate brain uptake *in vivo*. None of the compounds showed uptake into a rat AH109A hepatoma model, and the authors concluded that [<sup>11</sup>C]**10-12** were sub-optimal COX-2 imaging agents, as a result of non-specific binding.

### [<sup>18</sup>F]radiolabelling

The vicinal 1,2-diaryl heterocycle core structure exemplified by celecoxib has provided a convenient starting point for many fluorine-18 based COX-2 probes. Prabharakan *et al* radiolabelled celecoxib at the trifluoromethyl position by S<sub>N</sub>2 displacement of a bromo-difluoromethyl functionality. The formulated radiotracer [<sup>18</sup>F]**13** (Figure 2) defluorinated in the product vial over time however (*ca.* 6% in 4 hours), with significant defluorination (qualitatively assessed by observing bone uptake) also occurring *in vivo* during imaging of 2 hours' duration. The authors pointed out that defluorination was slower in baboons than in rats and could therefore be slower still in humans [20], however [<sup>18</sup>F]**13** was also rapidly metabolised in baboons with only 20% parent radiotracer remaining in plasma after 60 minutes. Toyokuni and co-workers synthesised a similar analogue of valdecoxib, [<sup>18</sup>F]**14**, that also exhibited rapid *in vivo* defluorination. Taken together these results suggest that the 3-pyrazole/isoxazole position is suboptimal for radiolabelling [21].

McCarthy and co-workers reported the radiosynthesis [<sup>18</sup>F]-labelled SC58125 **15**, by direct nucleophilic displacement of the corresponding trimethylammonium triflate, with extensive biological evaluation [22, 23]. Although *in vitro* cell studies showed selective uptake of [<sup>18</sup>F]-SC58125 **15** that was reversed by pre-incubation with cold SC58125, blocking studies *in vivo* were unsuccessful in rats. As [<sup>18</sup>F]-SC58125 showed high retention in rat brain

however, baboon studies were carried out although no pattern of cerebral uptake could be discerned that correlated with the known distribution of COX-2. This coupled with the high uptake into regions at the base of the brain suggested non-specific binding, again questioning the applicability to the clinical setting.

Desbromo-DuP-697 (**[<sup>18</sup>F]16**), a selective COX-2 inhibitor was radiolabelled via nucleophilic substitution on the corresponding nitro precursor <sup>[24]</sup>. Biodistribution studies in rat showed displaceable uptake in lung, kidney and heart, all sites where COX-2 is known to be expressed. Intestines and fat showed high and non-displaceable uptake, possibly due to biliary excretion and to the lipophilicity of the tracer ( $\log P = 3.72 \pm 0.16$ ) respectively. In studies performed on carrageenan-induced inflammation in rats the inflamed and control paws showed similar uptake that was not displaceable by NS-398 or indomethacin; however it is worth noting again that COX-2 activity as measured by peroxidase assay was not different in the inflamed/non-inflamed paws in this model. The high levels of nonspecific binding in the abdomen renders this tracer non suitable for PET-imaging of this area; however, the high lipophilicity and consequent ability to cross the blood-brain barrier may indicate utility in the measurement of COX-2 levels in neuroinflammation or neurodegenerative disorders.

Uddin *et al* reported an extensive library of fluorinated indomethacin and celecoxib derivatives as potential PET probes <sup>[25]</sup>. A lead candidate (**[<sup>18</sup>F]17**) was radiolabelled by simple nucleophilic substitution of a tosylate precursor and then evaluated *in vivo* using a carrageenan-induced inflammation model in male rats, demonstrating higher uptake in the inflamed versus control paw. Further evaluation in tumour models showed displaceable uptake in HSNCC 1483 xenografts with high COX-2 expression and negligible uptake HCT116 xenografts with low COX-2 expression. Stability analysis for the lead radiotracer **[<sup>18</sup>F]17** showed defluorination (~9% over 2 hours *in vivo*) in line with observed defluorination for similar radiotracers discussed above.

Kniess and co-workers studied the indole based radiotracer **[<sup>18</sup>F]18**, radiolabelled by substitution of a trimethylammonium salt precursor followed by McMurry cyclisation in 10% decay corrected RCY and 70-90 GBq/ $\mu$ mol specific activity <sup>[26]</sup>. The radiotracer showed uptake in COX-2 expressing cell lines that was blocked by pre-incubation with cold **18**. Evaluation *in vivo* showed good metabolic stability with 75% parent radiotracer intact after 60 min in rat plasma; however evaluation in a mouse HT-29 xenograft model showed no significant tumour uptake, leading the authors to conclude that this was likely due to the lower affinity of the probe.

The 5-membered heterocyclic core common to most COX-2 inhibitors invites comparison with the 1,2,3-triazole ring typical of Huisgen 1,3-dipolar cycloaddition or “click” chemistry<sup>[27]</sup>. Wuest and co-workers have studied COX-2 inhibitors incorporating a triazole core, synthesised by cycloaddition with a copper catalyst to give 1,4-disubstituted triazoles or with a ruthenium catalyst to give the more conventional 1,5-disubstitution favoured by COX-2 inhibitors <sup>[28]</sup>. Representative lead compounds from both series are shown in Figure 2 (**19** and **20**). The 1,4 disubstituted triazoles showed a surprising high affinity for COX-2 given the established SAR around this pharmacophore class; however, as expected, the 1,5-

disubstituted triazoles showed greater COX-2 affinity in general. A subsequent paper by the same group investigated the use of tetrazoles (eg.g. **21**) as the core heterocyclic structure but the library of compounds examined showed reduced COX-2 affinity in comparison to the lead triazole series exemplified by **19** [29]. Although these approaches may result in improved imaging agents, there has been no biological evaluation so far of this class of compounds.

## Figure 2 here

### *[<sup>123</sup>I]- and [<sup>125</sup>I]-radiolabelling*

Kuge and co-workers synthesised two related iodocelecoxib derivatives, varying in the presence of a methyl sulfone or sulfonamide moiety functionalisation (**22** and **23** in Figure 3) by halogenation exchange from a bromine precursor with iodine-125 [30]. The rationale for this study was to investigate the possibility that sulfonamide binding to carbonic anhydrases in erythrocytes slowed blood clearance for the radiotracers. *In vivo* evaluation of [<sup>125</sup>I]**22** and [<sup>125</sup>I]**23** showed that the sulfonamide derivative [<sup>125</sup>I]**22** cleared more slowly from blood than the sulfone counterpart. Further analysis of blood uptake demonstrated that uptake of [<sup>125</sup>I]**22** was blocked by incubation with carbonic anhydrase inhibitors such as acetazolamide whereas blood uptake of sulfone [<sup>125</sup>I]**23** was unaffected under the same conditions.

The same group also investigated a radioiodinated derivative of lumaricoxib, [<sup>125</sup>I]**24**; a weakly acidic COX-2 inhibitor. Introduction of the iodine has a slight effect on COX-2 affinity (Table 1) but COX-2/COX-1 selectivity is preserved [31]. [<sup>125</sup>I]**24** was radiolabelled by iodo-destannylation followed by base-induced hydrolysis of an amide precursor to give the final benzoic acid. [<sup>125</sup>I]**24** showed uptake in an *in vitro* inflammation-induced macrophage model that was blocked by incubation with cold **24**. Biodistribution in normal rats showed rapid clearance from blood but time dependent accumulation in intestines, precluding imaging in the abdomen. Competition/blocking experiments to assess the specificity of binding *in vivo* were not carried out in this or the previous study however.

Uddin and co-workers synthesised a library of sulfonamide and methyl sulfone celecoxib derivatives with meta or para iodo substitution on the vicinal aryl ring and various substituents at the 3-pyrazole position [32]. A lead compound **23** was chosen based on COX-2 inhibitory profile and radiolabelled with iodine-123 by iodo-destannylation to give [<sup>123</sup>I]**23**, an alternative radiolabelled version of [<sup>125</sup>I]**23** above and was purified by a simple aqueous/organic extraction/phase separation. The radiotracer [<sup>123</sup>I]**23** showed selective and displaceable uptake in a carrageenan-induced model of inflammation in the rat paw.

The same research group also explored the synthesis of indomethacin derivatives in order to radiolabel them with iodine-123 [33]. From a COX-2 enzyme assay and a COX-2 inhibition assay in intact cell compounds **25** and **26** (Figure 3) were selected for further investigation. Radiolabelling via iodo-destannylation gave the desired radiotracers [<sup>123</sup>I]**25** and [<sup>123</sup>I]**26**. The lead compound [<sup>123</sup>I]**26** was metabolically stable *in vivo* and was able to accumulate in a COX-2 expressing tumour *in vivo* at 3 hours post-injection, although full biodistribution data were not reported in this non-radiolabelled study.

## Figure 3 here

## Conclusion

Preclinical and clinical studies suggest that measurement of COX-2 expression may have application across multiple pathologies. Most imaging strategies to date have focused on small molecule probes derived from COX-2 targeted therapeutic agents. Presumably this is a result of the intracellular localisation of the COX-2 enzyme, creating the requirement for both diffusion of the imaging agent from the extracellular medium to the intracellular compartment and efflux from cells that do not contain a COX-2 binding site; characteristics routinely optimised for small-molecule therapeutic agents. Of further note, at least one group has investigated a peptide based approach to COX-2 imaging, albeit with limited success, presumably as a result of poor intracellular localisation of the peptide [34].

Although this review has focused on applications in oncology, COX-2 imaging probes have potential utility in a range of neurological conditions, due to the impossibility of biopsy. In oncology, the detection of pre- or early stage malignant lesions as well as the potential for improved staging of disease or stratification of patients for COX-2 inhibitor therapy are indicated as major potential applications. Molecular imaging also offers the possibility to assess the entire disease burden *in vivo*, avoiding problems associated with the lability of COX-2 protein and mRNA.

Radiolabelling with [<sup>11</sup>C] offers the ability to produce probes from existing and well-validated COX-2 inhibitors; however, this approach has shown few translatable results to date, perhaps because therapeutic agents are commonly optimised to have slow clearance from the blood which is contraindicative for their use as imaging agents with short-lived isotopes [35]. The necessity of an on-site cyclotron to produce [<sup>11</sup>C]-labelled tracers also limits the clinical application of such tracers. Although this difficulty is removed when labelling with isotopes for SPECT, the decreased sensitivity of this technique may also limit the clinical success of this approach. Thus the most promising data so far has been derived from the use of [<sup>18</sup>F]-labelled coxib analogues. The design of such agents should take into account the observation that primary sulfonamides such as that found on celecoxib also show binding affinity to carbonic anhydrases, which may be the underlying cause for many of the specificity problems observed with COX-2 probes to date [30, 36]; care should be taken to design COX-2 probes that are poor substrates for drug efflux proteins as COX-2 expression is often correlated with a resistant phenotype that also expresses multi-drug resistance (MDR) proteins such as P-glycoprotein in many cancers.

It is also worth noting the care necessary to establish reliable models for validation of COX-2 imaging agents, especially *in vivo*. For example, it is difficult to assess candidate probe uptake in inflammatory models where COX-2 upregulation cannot be demonstrated [17,23]. Similarly, metabolic differences between rodent species may explain a lack of tumour uptake where metabolism and target binding have been assessed in rats and mice [25]; such differences have been postulated to account for the differential uptake of the TSPO ligand [<sup>18</sup>F]-DPA-714 (Zheng et al, presented at the World Molecular Imaging Conference, Dublin, Ireland 5-8<sup>th</sup> September 2012 and C.Cawthorne, unpublished data). Finally, the gold

standard for specific *in vivo* binding is the ability to reduce tumoural uptake by the addition of cold compound (ideally not the same molecule) known to bind to the target site.

An open question is whether the COX-2 expression levels seen in malignancy are amenable to detection with radionuclide imaging, especially SPECT. However low receptor density targets have been successfully imaged in the brain for many years using PET (e.g. dopamine D2 receptor), and low basal expression potentially facilitates a wider dynamic range. Another issue is the perceived difficulty of imaging intracellular targets, with recent criteria suggested for selecting imaging biomarkers giving high weight to the accessibility of target on the cell surface<sup>[37]</sup>. The imaging of intracellular target expression/activity with non-substrate tracers considerably widens the range of candidate biomarkers in oncology however, and clinical proof-of-concept has been provided with a small molecule inhibitor of EGFR<sup>[38]</sup>. Further investigation is required to prove the utility of this approach.

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**For submission to Journal of Labelled Compounds and Radiopharmaceuticals**

**Imaging COX-2 expression in cancer using PET/SPECT radioligands: Current status and future directions**

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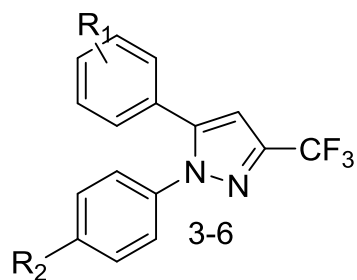
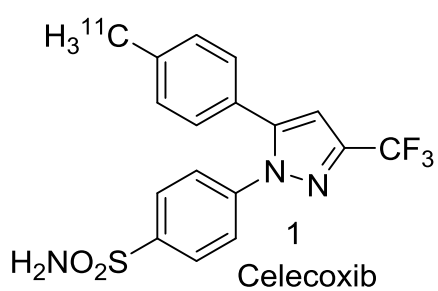
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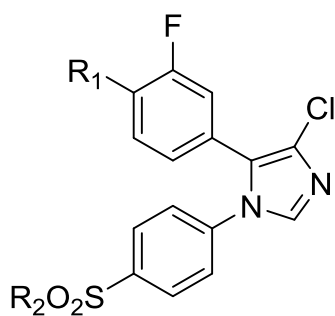
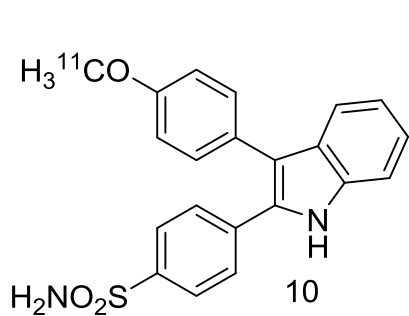
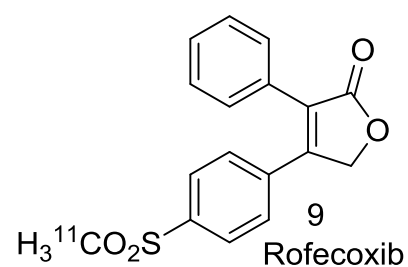
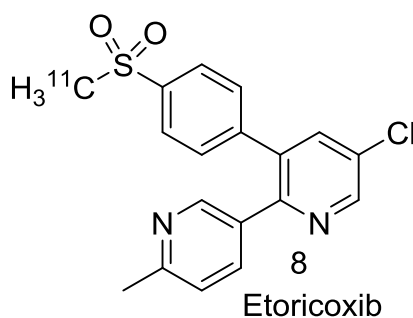
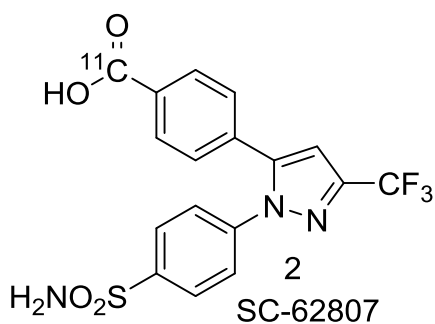
**Table 1.**

Table 1: IC<sub>50</sub> and logP values of several inhibitors. \*: LogP values have been calculated based on ACDLabs predictions. (ACDLabs Release 12.00, product version 12.01) . <sup>a</sup> [30], <sup>b</sup> [32]

Compound	IC <sub>50</sub> (μM)		logP
	COX-1	COX-2	
1 (Celecoxib) [24]	>4	0.03	4.21±1.49 *
2 (SC-62807)			3.53±1.51*
8 (Etoricoxib) [33]	116±18	1.1±0.1	2.21±0.43 *
9 (Rofecoxib) [32]	18.8±0.9	0.53±0.02	1.34±0.46 *
10 [18]			2.33
11 [18]			2.21
12 [18]			1.89
16 (Desbromo-DuP-697) [23]			3.72±0.16
17 [24]	>4	0.16	3.06±1.49 *
18 [25]	6.6	1.2	2.3 ± 0.7
20 [26]	20	0.15	1.84±0.85 *
21 [26]	0.81	0.11	1.84±0.85 *
22 [28]	>100	8.20±1.43	4.76±1.50 *
23 <sup>a, b</sup>	>100 >4	5.16±2.83 0.05	4.93±1.50 *
24[29]	>446±317	2.46±0.78	5.56±1.01 *
25 [31]	>66	0.12	4.99±0.59 *
26 [31]	>66	0.40	4.94±0.40 *
Valdecoxib [34]	26.1	0.87	1.71±0.56 *
Lumiracoxib [29]	164±75	0.77±0.21	3.90±0.50 *
Indomethacin [24]	0.05	0.75	4.27 [35]

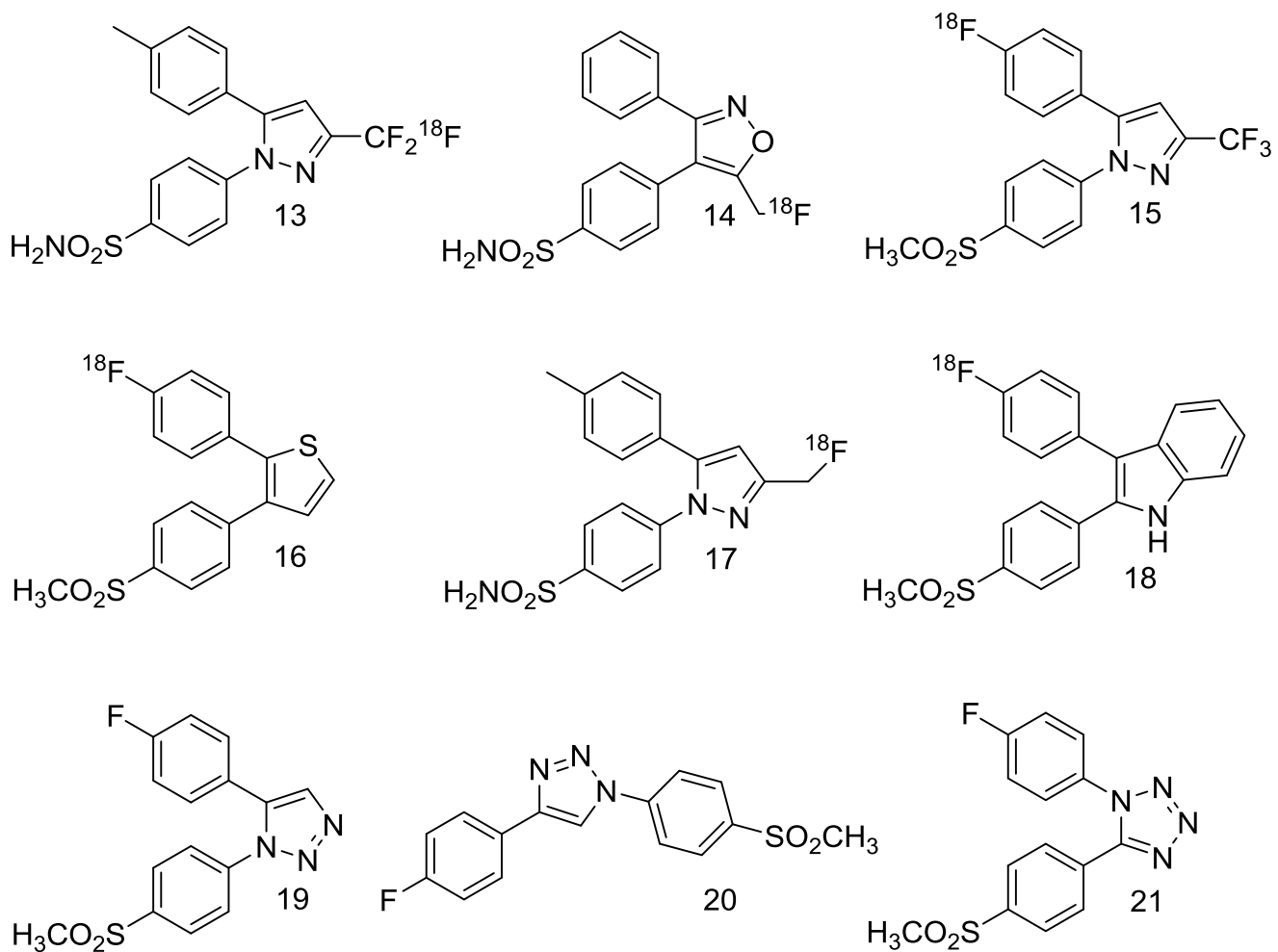


[<sup>11</sup>C]3: R1= 3-O<sup>11</sup>CH<sub>3</sub>; R2=SO<sub>2</sub>NH<sub>2</sub>  
 [<sup>11</sup>C]4: R1=2-OCH<sub>3</sub>, R2=COO<sup>11</sup>CH<sub>3</sub>  
 [<sup>11</sup>C]5: R1=3-OCH<sub>3</sub>, R2=COO<sup>11</sup>CH<sub>3</sub>  
 [<sup>11</sup>C]6: R1=4-OCH<sub>3</sub>, R2=COO<sup>11</sup>CH<sub>3</sub>  
 [<sup>11</sup>C]7: R1=2-CH<sub>3</sub>, R2=COO<sup>11</sup>CH<sub>3</sub>

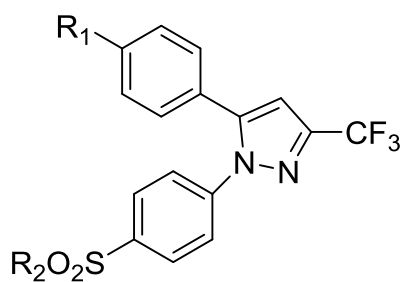


[<sup>11</sup>C]11:R1=O<sup>11</sup>CH<sub>3</sub> R2=CH<sub>3</sub>  
 [<sup>11</sup>C]12:R1=O<sup>11</sup>CH<sub>3</sub> R2=NH<sub>2</sub>

**Figure 1.** [<sup>11</sup>C]radiolabelled COX-2 inhibitors. The structures summarised above are mostly derived from previously reported therapeutic lead compounds and radiolabelling was in all cases carried out by [<sup>11</sup>C]methylation.

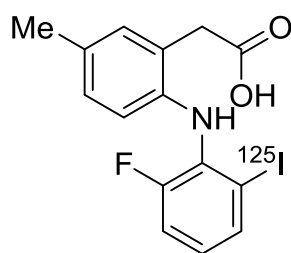


**Figure 2.** [ $^{18}\text{F}$ ]radiolabelled COX-2 inhibitors. As with the [ $^{11}\text{C}$ ]radiolabelled counterparts these are primarily derived from therapeutic lead compounds with isolated exceptions.

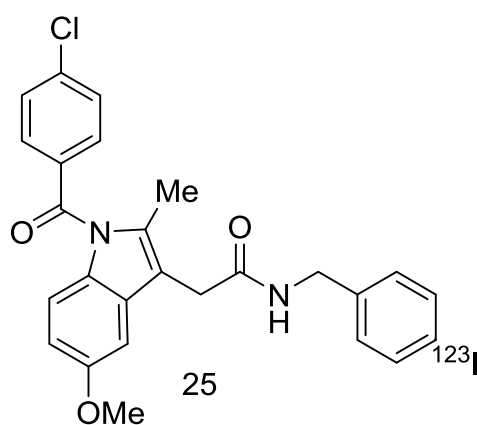


22: R1=[<sup>123</sup>I] or [<sup>125</sup>I], R2=NH<sub>2</sub>

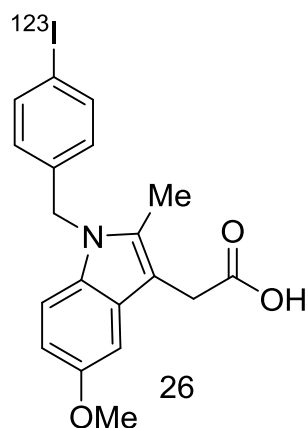
23: R1=[<sup>123</sup>I] or [<sup>125</sup>I], R2=CH<sub>3</sub>



24



25



26

**Figure 3.** Radioiodinated COX-2 inhibitors. Radiolabelling has focused on SPECT applications but the structures and radiochemistry employed is also readily amenable to PET radiolabelling with iodine-124.