11C-Labeled Analogs of Indomethacin Esters and Amides for Brain Cyclooxygenase-2 Imaging: Radiosynthesis, in Vitro Evaluation and in Vivo Characteristics in Mice

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There is great potential in the use of positron emission tomography (PET) and suitable radiotracers for the study of cyclooxygenase type 2 (COX-2) enzyme in living subjects. In the present study, we prepared and evaluated five 11C-labeled ester and amide analogs derived from indomethacin as potential PET imaging agents for the in vivo visualization of the brain COX-2 enzyme. Five 11C-labeled COX-2 inhibitors, with different lipophilicities and moderate COX-2 inhibitory activity, were prepared by treatment of the corresponding O-desmethyl precursors with [11C]methyl triflate and purified by HPLC (radiochemical yields of 55–71%, radiochemical purity of >93%, and the specific activities of 22–331 GBq/μmol). In mice, radioactivity in the brain for all radiotracers was low, with very low brain-to-blood ratios. A clear inverse relationship was observed between brain uptake at 1 min postinjection and the lipophilicity (experimental log \( P \)) of the studied 11C-radiotracers. Pretreatment of mice with cyclosporine A to block P-glycoproteins caused a significant increase in brain uptake of radioactivity following injection of the 11C-radiotracer compared to control. HPLC analysis showed that each radiotracer was rapidly metabolized, and a few metabolites, which were more polar than the original radiotracers, were found in both plasma and brain. No specific binding of the tracers towards the COX-2 enzyme in the brain was clearly revealed by in vivo blocking study. Further structural refinement of the tracer agent is necessary for better enhancement of brain uptake and for sufficient metabolic stability.

Key words cyclooxygenase type 2; indomethacin; carbon-11; brain; biodistribution; mouse

Cyclooxygenase (COX) is the crucial enzyme in the conversion of arachidonic acid to prostaglandins. It exists at least in two isoforms, a constitutive form (COX-1) and an inducible form (COX-2), although a third distinct COX isozyme has also been reported. The COX-1 enzyme is responsible for maintaining homeostasis whereas COX-2 is induced during inflammation by various stimuli and, in some tissues including the brain, kidney and placenta, is also constitutively expressed. Recent studies indicate that elevated expression of COX-2 has been implicated in many pathological events, including rheumatoid arthritis, cancer, heart disease, and neurodegenerative disorders.

In the brain, COX-2 is mainly expressed in the cortex, hypothalamus, and hippocampus, and is upregulated in neurological disorders such as Parkinson’s disease and Alzheimer’s disease, although the functions of COX-2 in pathophysiological processes are not yet well-understood. There is great potential that COX-2-targeted imaging by positron emission tomography (PET) may provide useful information on the role of this enzyme, especially in various neurological disorders, and also may help to evaluate the efficacy of selective COX-2 inhibitors. Thus, many COX-2 inhibitors have been labeled with a suitable radionuclide for mapping the enzymes in vivo. However, previously evaluated PET radiotracers, including 11C-labeled COX inhibitors of the diarylheterocyclic class which were synthesized by us, have only achieved limited success, due to a lack of sufficient specific-binding to the COX-2 enzyme and/or sufficient brain penetration, in spite of promising in vitro pharmacological data. There is a need for systematic studies correlating the physicochemical properties of agents with their in vivo behavior for a better design of COX-2 imaging probes.

Currently, many selective COX-2 inhibitors with various structural features, as exemplified by vicinal diaryl heterocycles such as celecoxib and refecoxib, have been developed as non-steroidal anti-inflammatory drugs (NSAIDs) with improved gastric safety profiles. Most of them have high lipophilicity. Indomethacin is a traditional non-selective inhibitor of the COX isozyme which is widely used as a NSAID, but with poor brain penetration largely due to the presence of the carboxylic acid moiety. Recently, recent studies concerning ester and amide derivatives of indomethacin have found very high COX-2 inhibition potency and a high degree of COX-2-selectivity, Marnett and colleagues synthesized \(^{123}\)I-labeled \(N\)-iodobenzyl-containing amide derivative of indomethacin as a COX-2 imaging agent, which exhibited sufficient stability in COX-2 expressing nude mouse tumor. More recently, using organic fluorophores tethered to indomethacin through an amide linkage, the feasibility of specific in vivo targeting of COX-2 in inflammatory lesions in mice has been demonstrated by the same group.

The purpose of this study is to find suitable radioligands for imaging brain COX-2 in vivo by PET. We initially chose two potent and selective COX-2 inhibitors of indomethacin ester analog for \(^{11}\)C-radiolabeling: \(N\)-Pentyl-(1-\(p\)-chlorobenzoyl-5-methoxy-2-methylindolino)-3-acetate (1) (COX-1, \(IC_{50}=66 \mu M\); COX-2, \(IC_{50}=50 \mu M\)) and \(N\)-octyl-(1-\(p\)-chlorobenzoyl-5-methoxy-2-methylindolino)-3-acetate (2) (COX-1, \(IC_{50}=66 \mu M\); COX-2, \(IC_{50}=40 \mu M\)). Furthermore, on the basis of the reported structure–activity studies concerning the ester and amide derivatives of indomethacin, we...
next designed and synthesized one additional amide derivative, two amide compounds with a –OCH₂– linkage, and one carbamate ester derivative of indomethacin, in order to examine the effects of different functional groups on COX-2 inhibitory potency and lipophilicity. As a consequence, five ¹¹C-labeled COX-inhibitors were prepared via demethyl precursors, and examined for in vivo characteristics in mice.

Results and Discussion

**Synthesis** Compounds 1 and 2 were prepared as previously described. The target compounds (3—6) for HPLC standards and in vitro studies were obtained in satisfactory yields by treatment of indomethacin with the appropriate amine or alcohol according to a similar literature procedure, as illustrated in Chart 1. Additionally, the carbamate butyl ester (4) of indomethacin was obtained by Curtius rearrangement of the indomethacin azide, followed by treatment of intermediate isocyanate with 1-butanol. The phenolic precursors (7—10) for ¹¹C-radiolabeling were prepared in reasonable yields by demethylation of the corresponding indomethacin derivatives (1—4) using BBr₃ as a dealkylating agent. On the other hand, two phenolic compounds, 12 and 13, with an alkoxy moiety in the side chain were obtained in a two-step procedure, in which demethylation of the methoxy group in indomethacin was achieved by treating it with BBr₃, and subsequent condensation of the carboxyl group with 2-ethoxyethylamine or 3-methoxypropylamine gave the desired compounds (Chart 1). All of the synthetic compounds gave satisfactory spectroscopic data, which were in full accordance with their depicted structures.

**COX Inhibitory Activity** The inhibitory activity of the compounds (2—6) for the COX enzyme and selectivity index (SI) was determined in vitro, including celecoxib and indomethacin as a reference COX inhibitor, using a colorimetric COX (ovine) inhibitor screening assay, in which arachidonic acid was used as the substrate and N,N,N',N'-tetramethylphenylenediamine (TMPD) as the cosubstrate. The IC₅₀ value was calculated from the concentration–inhibition response curve. Additionally, the ratio of IC₅₀ value of indomethacin as a reference compound-to-IC₅₀ values of the test compounds was calculated to know the relative COX-2 inhibitory activity. In vitro inhibition studies showed that compound 3 had the highest affinity and selectivity for COX-2, followed by compound 2 (Table 1). These two compounds
have higher activity than celecoxib and indomethacin. The introduction of a –OCH$_2$ linkage instead of the methylene moiety in the side chain of indomethacin amide 3 significantly decreased the COX-2 inhibition potency with reduced selectivity (compounds 5, 6), which indicates that ether-oxygen may be detrimental to COX inhibition. Furthermore, compound 4 showed a significant loss of inhibition activity against both COX isoforms.

**Radiolabeling** Five $^{11}$C-labeled indomethacin analogs [$^{11}$C]1—3, 5, 6 were prepared by [$^{11}$C]-O-methylation of the corresponding demethyl precursors in acetone with [$^{11}$C]methyl triflate in the presence of NaOH as the base at room temperature, followed by purification with HPLC, as shown in Chart 2. Table 2 summarizes the results of the radiosyntheses. The presence of NaOH was essential for an efficient procedure for the desired methylation; however, for [14$^C$]-O-methylation of precursor 7, the use of an excess amount of NaOH led to a more polar radioactive byproduct, consistent with the identity of 14$^C$-labeled indomethacin. Effective methylation of precursor 7 proceeded by the use of an equimolar amount of NaOH.$^{11}$ For [14$^C$]-O-methylations of the other four precursors, the use of a larger amount of NaOH successfully gave the required products. The absence of any residual of starting demethyl precursors in the purified product was verified by HPLC analysis.

**Lipophilicity** The partition coefficient of five $^{11}$C-labeled compounds between 1-octanol and phosphate buffered saline was measured.$^{27}$ The log $P_{o/w}$ values were 1.98—3.94 (Table 2). Lipophilicity increased with increasing length of the side chain as seen in [14$^C$]2 and [14$^C$]3, and replacement of the methylene of the side chain with oxygen as in [14$^C$]5 and [14$^C$]6 decreased log $P_{o/w}$ values due to its increased polarity. The measured values are somewhat in the outside range of those generally considered optimal for good blood brain barrier (BBB) penetration of drugs, although the well-known parabolic relationship between the lipophilicity of a molecule and its uptake by the brain is not universally applicable.$^{28,29}$ It should be noted that [14$^C$]3 features a relatively low lipophilicity (log $P_{o/w}$=2.40) while retaining high COX-2 inhibitory potency and high selectivity, as shown in Table 1. In this study there was no correlation between the measured $P_{o/w}$ and log $P_{o/w}$ values for the compounds.

**Biodistribution** To explore the in vivo characteristics of the five 11$^C$-labeled compounds, biodistribution studies were performed in normal male ddY mice. The radioactivity concentrations in various tissues as a function of time following intravenous injection of the radiotracers are represented in Table 3. Four 11$^C$-radiotracers except for 11$^C$1 exhibited a relatively fast clearance of radioactivity in the blood within 60 min. The half-lives of the blood clearance were 12.3 min,

![Image](image-url)
1.3 min, 2.9 min, 4.2 min, and 3.8 min for [11C]2, [11C]3, [11C]5, and [11C]6, respectively. The highest initial concentrations of radioactivity were observed in the liver for all tracers, followed by a gradual increase and/or relatively slow clearance of radioactivity. In addition, the peak concentration of radioactivity observed in the small intestine may reflect hepatobiliary clearance of the parent tracers and/or their radioactive metabolites. [11C]1 displayed very high levels of radioactivity in the blood and most organs such as the liver, lungs and kidneys in contrast to the other four radiotracers.

Whole brain uptake for all tracers was low, with values ranging from 0.18% injected dose (ID)/g for [11C]2 to 1.84% ID/g for [11C]6 at 1 min after administration which, with exception of [11C]2, then decreased slowly over 60 min. In the case of [11C]2 which showed the lowest brain uptake, a slight increase of radioactivity in the brain was observed at later time points. The brain-to-blood ratios of all tracers were consequently much less than 1.0 unity (0.4—0.77) throughout the observation period. These results clearly demonstrate that all tracers investigated have low BBB permeability. Notably, the lipophilicity of the radiotracers inversely correlated well ($r^2 = 0.90, p = 0.01$) with brain uptake at 1 min post injection (Fig. 1), which is most likely to be reflective of the initial extraction of the radiotracers from the blood. This relationship may be due to binding to plasma proteins which is expected to increase with larger log $P_{ow}$ values of the indomethacin analogs. These radiotracers likely bind to the plasma proteins similar to other NSAIDs, including indomethacin binding with high affinity to serum albumin. Non-specific binding to albumin and other plasma proteins is known to correlate positively and linearly in vitro with increasing lipophilicity. However, it remains controversial the extent to which non-specific binding to plasma proteins inhibits BBB penetration. This theory cannot explain why some radiotracer...
ers exhibiting high (>90%) plasma protein binding may enter the brain nearly as well as those that exhibit much lower plasma protein binding. Certainly, some tracers including $[^{11}C]$palmitic acid$^{33}$ and $[^{11}C]$arachidonic acid$^{34,35}$ with calculated log $P > 7$ have been used successfully in PET experiments although their maximal brain uptakes are lower than those of commonly used neuroreceptor radioligands. Therefore, lipophilicity is, in any case, an imperfect proxy for BBB permeability. Currently, there is no general understanding of the relationship between lipophilicity and the distribution characteristics for radiotracers of COX-2 inhibitors. Unfortunately, high lipophilicity is inherent in currently available COX inhibitors because of hydrophobic interactions with the COX binding sites in both isozymes.$^{36}$

**Metabolism in Plasma and Brain** The metabolic behavior of each radiotracer in vivo was studied in mice at 30 min after injection. As summarized in Table 4, each radiotracer was rapidly metabolized. HPLC analysis of plasma for $[^{11}C]$I showed no unchanged form and one polar peak (polar 1) of which the retention time corresponds to that of indomethacin. In the other four tracers, 14—32% of the radioactivity was recovered as unchanged parent compounds, and a few radiolabeled metabolites including $[^{11}C]$indomethacin (polar 1) were found. The findings seemingly indicated hydrolysis of the ester or amide moiety of the tracers by esterase in the blood and peripheral organs. Unidentified metabolites (polar 3) were predominant for both $[^{11}C]$J and $[^{11}C]$S. Remmel et al. have shown for indomethacin phenethylamide in rodents that the initial step in the metabolism is the oxidative pathway on its amide side chain, resulting in the formation of hydroxylated and O-demethylated metabolites in addition to their glucuronides.$^{37}$

If this route of metabolism is also the case for the radiotracers studied here, then more polar metabolites than the parent compounds would be produced.

In the brain samples only 9—26% of the radioactivity was detected as intact forms and the presence of two radiolabeled metabolites, $[^{11}C]$indomethacin (polar 1) and an unidentified fraction (polar 2), was found. A polar 3 metabolite observed in plasma extracts was absent in the brain samples. The metabolism probably occurred in the brain because plasma metabolites are expected to poorly penetrate the BBB. However, this consideration requires further investigation. Thus, one of the reasons for the low accumulation of radioactivity in the brain has been found in the marked metabolism of the studied $^{11}$C-labeled radiotracers, which occurred in the plasma and brain tissue. However, a low initial uptake of each radiotracer was not explained by the rapid metabolism.

**Specific Binding** To determine the specific binding of radiotracers to COX-2 enzymes, blocking experiments were carried out using several COX-2 selective inhibitors with BBB permeability, celecoxib and $N$-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulphonamide (NS-398), a nonselective COX inhibitor, indomethacin, and carrier-loading. None of any COX inhibitors and each unlabeled compound caused significant inhibition in the brain uptake and brain-to-blood ratio of $[^{11}C]$J, $[^{11}C]$J, $[^{11}C]$J or $[^{11}C]$J as summarized in Table 5 which involves the representative blocking results with celecoxib and NS-398, suggesting the absence of COX-2-specific and saturable binding. On the other hand, in the case of $[^{11}C]$J, a small but statistically significant inhibition (ca. 15% reduction) was observed in the brain uptake of radioactivity with celecoxib or NS-398 (Table 5). This finding seems to indicate a small fraction of displaceable binding in the brain, but additional evidence is necessary. The rapid degradation of all $^{11}$C-COX inhibitors and the presence of radiometabolites in the brain as described above can also contribute to the lack of specific binding for COX-2 enzyme in vivo blocking.

**Effect of Cyclosporine A** The BBB expresses various

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**Table 4.** $^{11}$C-Labeled Metabolites in Plasma and Brain at 30 min after Intravenous Injection of $^{11}$C-Labeled Indomethacin Esters and Amides into Mice

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Parent</th>
<th>Polar 1 (indomethacin)</th>
<th>Polar 2</th>
<th>Polar 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[^{11}C]$J</td>
<td>Retention time&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0 min</td>
<td>2.9 min</td>
<td>2.1 min</td>
</tr>
<tr>
<td>Plasma</td>
<td>ND</td>
<td>100</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>26±6</td>
<td>52±3</td>
<td>22±9</td>
<td></td>
</tr>
<tr>
<td>$[^{11}C]$J</td>
<td>Retention time&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.2 min</td>
<td>5.2 min</td>
<td>4.1 min</td>
</tr>
<tr>
<td>Plasma</td>
<td>15±4</td>
<td>72±3</td>
<td>13±1</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>22±4</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>$[^{11}C]$J</td>
<td>Retention time&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.7 min</td>
<td>4.8 min</td>
<td>3.3 min</td>
</tr>
<tr>
<td>Plasma</td>
<td>32±5</td>
<td>11±2</td>
<td>14±5</td>
<td>43±7</td>
</tr>
<tr>
<td>Brain</td>
<td>21±7</td>
<td>6±4</td>
<td>ND</td>
<td>73±7</td>
</tr>
<tr>
<td>$[^{11}C]$J</td>
<td>Retention time&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.7 min</td>
<td>8.1 min</td>
<td>5.3 min</td>
</tr>
<tr>
<td>Plasma</td>
<td>17±4</td>
<td>28±8</td>
<td>9±5</td>
<td>46±9</td>
</tr>
<tr>
<td>Brain</td>
<td>9±2</td>
<td>7±4</td>
<td>ND</td>
<td>84±5</td>
</tr>
<tr>
<td>$[^{11}C]$J</td>
<td>Retention time&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.0 min</td>
<td>8.4 min</td>
<td>6.6 min</td>
</tr>
<tr>
<td>Plasma</td>
<td>14±5</td>
<td>41±6</td>
<td>20±8</td>
<td>25±17</td>
</tr>
<tr>
<td>Brain</td>
<td>9±2</td>
<td>7±1</td>
<td>ND</td>
<td>84±3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean±S.D. (n=3), ND=not detected.  
<sup>b</sup> Retention time on HPLC analysis.  
<sup>c</sup> Besides a parent peak, 78% of the radioactivity was eluted with the broadening of peaks including polar 1 and polar 2 and metabolite profiles could not be accurately determined.

**Table 5.** Radioactivity of $^{11}$C-COX Inhibitors in the Brain and the Blood of Mice Co-injected with COX-2 Inhibitor, Celecoxib and NS-398<sup>a</sup>

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Brain uptake (%ID/g)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Brain-to-blood ratio&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Celecoxib</td>
<td>NS-398</td>
</tr>
<tr>
<td>$[^{11}C]$J</td>
<td>0.54±0.13</td>
<td>0.46±0.07</td>
</tr>
<tr>
<td>$[^{11}C]$J</td>
<td>0.40±0.04</td>
<td>0.34±0.09</td>
</tr>
<tr>
<td>$[^{11}C]$J</td>
<td>0.68±0.08</td>
<td>0.58±0.09</td>
</tr>
<tr>
<td>$[^{11}C]$J</td>
<td>0.73±0.12</td>
<td>0.72±0.09</td>
</tr>
<tr>
<td>$[^{11}C]$J</td>
<td>0.80±0.06</td>
<td>0.76±0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mice received the following blocker solution (1 mg/kg per animal, 0.1 ml), celecoxib, and NS-398 dissolved in DMSO co-injected with each radiotracer. In the control mice the same amount of DMSO was co-injected. The radioactivity in the tissue was measured at 30 min for $[^{11}C]$J and $[^{11}C]$J, and at 15 min for $[^{11}C]$J and $[^{11}C]$J after each $^{11}$C-COX inhibitor injection.  
<sup>b</sup> Mean±S.D. (n=5).  
<sup>*</sup> Significant decrease (p<0.05) compared to the control (Student’s t-test).
transporters such as P-glycoprotein (P-gp) (also called as ABCB1) and multidrug resistance-associated proteins (MRPs). Recently, the modulation of several PET-radioligands by P-gp in vivo has been evaluated in rodents, showing that P-gp plays an important role in restricting brain uptake of radioligands. Low brain uptakes of four radiotracers may attribute to the P-gp. Zrieki et al. reported that the hept ester derivative of indomethacin is neither a substrate nor a competitive inhibitor of P-gp in Caco-2 cells. On the other hand, in a previous study, we found that 2-(4-aminosulfonylphenyl)-3-(8-methoxyphenyl)-indole with high COX-2 inhibiting activity was found to be a substrate for the P-gp in in vitro monolayer efflux assays, but its 11C-labeled analog was not sensitive to P-gp modulation with cyclosporine (Cys A) in vivo. In this study, we investigated P-gp modulation of four radiotracers, although they were not applied to the in vitro monolayer efflux assays. As shown in Table 6, pretreatment with Cys A (50 mg/kg) seemed to slightly enhanced brain uptake and/or the brain-to-blood ratio for [11C]2, [11C]3, [11C]5 and [11C]6 with and without statistical significance, thus seemingly indicating a possibility of P-gp-mediated transport of these [11C]-tracers. Cys A has several advantages, thus seemingly indicating a possible contribution of P-gp-mediated efflux of the parent radiotracer it-self, rather than a P-gp-mediated efflux of the parent radiotracer itself, although the small number of radiotracers (n=5), suggesting that the lipophilicity could be used to predict the BBB permeability for derivatives of indomethacin and related compounds. In this study, we found that one compound [11C]3 features relatively low lipophilicity (log P7.4=2.40) while retaining high COX-2 inhibitory potency and high selectivity. This might be good news for guiding the design of COX-2 selective radiotracers. Further structural refinement of the radiotracers is necessary to achieve better enhancement of brain uptake and sufficient metabolic stability.

**Table 6. Effect of P-Glycoprotein Modulation with Cyclosporine A on the Brain Uptake of Radioactivity after Intravenous Injection of [11C]-COX Inhibitors into Mice**

<table>
<thead>
<tr>
<th>Radiotracer</th>
<th>Control</th>
<th>CysA</th>
<th>CysA/Control</th>
<th>Control</th>
<th>CysA</th>
<th>CysA/Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>[11C]2</td>
<td>0.40±0.04</td>
<td>1.55±1.04*</td>
<td>3.88</td>
<td>0.46±0.02</td>
<td>1.27±0.85*</td>
<td>2.76</td>
</tr>
<tr>
<td>[11C]3</td>
<td>0.68±0.08</td>
<td>0.99±0.09**</td>
<td>1.46</td>
<td>0.86±0.20</td>
<td>0.90±0.05</td>
<td>1.05</td>
</tr>
<tr>
<td>[11C]5</td>
<td>0.73±0.12</td>
<td>1.11</td>
<td>1.52</td>
<td>0.66±0.08</td>
<td>0.92</td>
<td>1.39</td>
</tr>
<tr>
<td>[11C]6</td>
<td>0.80±0.06</td>
<td>1.71±0.25**</td>
<td>2.13</td>
<td>0.62±0.03</td>
<td>1.20±0.25**</td>
<td>1.94</td>
</tr>
</tbody>
</table>

a) Mice were pretreated with cyclosporine A (CysA) (50 mg/kg, i.v.) 30 min before injection of the tracers, and the radioactivity in the tissue was measured at 30 min for [11C]2 and [11C]3, and at 15 min for [11C]5 and [11C]6 after each [11C]-COX inhibitor injection. b) Mean±S.D. (CysA: n=4 for [11C]2 and [11C]5, n=5 for [11C]3, n=2 for [11C]5, control, n=5 for each group). *p<0.05 and **p<0.01, compared to the control (Student’s t-test).

**Conclusion**

This paper describes the effect of altering the structure of the alkyl side chain of indomethacin esters and amides in regard to COX-2 inhibitory potency and in vivo brain uptake in order to find a suitable radiotracer candidate for imaging brain COX-2. The in vivo behavior of all radiotracers in mice is characterized by low brain uptake and fast metabolic susceptibility. No specific binding of the radiotracers toward the COX-2 enzyme in the brain was clearly revealed, although [11C]3 seemed to show a small specific signal. It should be noted that there was an inverse correlation between the brain uptake of radioactivity at 1 min postinjection and the lipophilicity values (log P7.4) of the [11C]-tracers investigated here.
treated in vacuo. The residue was purified by chromatography on silica gel with hexane:EtOAc=3:1 to provide 3 (843 mg, 70.6%) as a pale yellow solid: mp 137—138 °C; 1H-NMR (CDCl3) δ: 7.66 (2H, d, J = 8.4 Hz), 7.49 (2H, d, J = 8.4 Hz), 6.87 (2H, m), 6.70 (1H, dd, J = 2.4, 9.2 Hz), 5.56 (1H, s), 3.82 (3H, s), 3.63 (2H, s), 3.20 (2H, m), 2.38 (3H, s), 1.39 (2H, m), 1.21 (4H, m), 0.83 (3H, t, J = 7.2 Hz); Fourier transform (FT-IR) (KBr) cm⁻¹: 3379, 1670; FAB-MS m/z: 427.25 (M+H)². HR-MS: Calcd for C₁₅H₁₅ClNO₂ (M+H)²: 427.2483; found: 427.2511.

Butyl-(1-p-chlorobenzyl)-5-hydroxy-2-methylindole-3-acetamide (11) Compound 11 was obtained in a similar manner as 7 by demethylation of 4 using BBr₃. The crude product was purified by chromatography on silica gel with hexane:EtOAc=3:1 to give 10 (106 mg, 73.3%) as a white powder: mp 91—93 °C; 1H-NMR (CDCl3) δ: 7.65 (2H, d, J = 8.4 Hz), 7.47 (2H, d, J = 8.4 Hz), 7.00 (1H, d, J = 8.8 Hz), 6.60 (1H, dd, J = 2.4, 8.8 Hz), 5.19 (1H, s), 4.77 (1H, s), 4.42 (2H, d, J = 5.2 Hz), 4.12 (2H, m), 2.40 (3H, s), 1.38 (2H, m), 1.26 (2H, m), 0.92 (3H, m); FT-IR (neat) cm⁻¹: 3237, 1686; FAB-MS m/z: 415.2 (M+H)².

1-P-chlorobenzyl-5-hydroxy-2-methylindole-3-acetic Acid (12) Compound 12 was obtained in a similar manner to the synthesis of 3 from 11 using 2-ethoxyethanol. The crude product was purified by chromatography on silica gel with CHCl₃:acetone=2:1 to give 11 (543 mg, 56.5%) as a white powder: mp 223—225 °C; 1H-NMR (DMSO-d₆) δ: 9.16 (1H, s), 7.65 (4H, q, J = 6.0, 8.8 Hz), 6.86 (1H, d, J = 8.4 Hz), 6.82 (1H, d, J = 2.0 Hz), 6.55 (1H, dd, J = 2.4, 8.8 Hz), 5.37 (2H, s), 2.19 (3H, s); FAB-MS m/z: 434.2 (M+H)². HR-MS: Calcd for C₁₂H₁₀ClNO₂ (M+H)²: 434.0684; found: 434.0671.

Induction of COX Activity Inhibition of COX activity was assayed by using the Colorimetric COX Inhibitor Screening Assay kit (Cayman Chemical, No. 761011, lot: 194562, 040471, 0408012, Michigan, U.S.A.). This assay measures the heme-catalyzed hydroperoxide activity of ovine COX by monitoring the appearance of oxidized N,N,N’,N’-tetramethylp-phenylenediamine (TMPD)²⁸. Dimethylsulfoxide (DMSO) (10 μl, control) or a solution of the studied compound in DMSO (10⁻³—10⁻⁴ M) was added to a 96-well plate with 0.1 μl Tris–HCl assay buffer (pH 8.0) (150 μl). 4.4% solution of heme in DMSO (10 μl) and a solution of ovine COX-1 or COX-2 in 80 μl Tris–HCl (pH 8.0) containing 0.1% Tween 20 and 300 nm diethylidithiocarbamate. After 5 min of preincubation at room temperature, a solution of TROX (20 μl) and arachidonic acid (20 μl) dissolved in 1.1 μl ethanol containing 5 μl KOH was added to the mixture. The mixture was incubated for a further 5 min and the absorbance was measured on a plate reader at 595 nm. Celecoxib and indomethacin were used as the reference compounds. The results are shown in Table 2.

[^25]: ¹⁹F NMR of the deuterated compounds: ¹⁹F NMR (CDCl₃, 282.4 MHz) for compound 11: δ = -10.03 (1F, m) for F-CH₂ of 11. For compound 12: δ = -10.03 (1F, m) for F-CH₂ of 12.

[^26]: In a 96-well plate with 0.1 μl Tris–HCl assay buffer (pH 8.0) (150 μl). 4.4% solution of heme in DMSO (10 μl) and a solution of ovine COX-1 or COX-2 in 80 μl Tris–HCl (pH 8.0) containing 0.1% Tween 20 and 300 nm diethylidithiocarbamate. After 5 min of preincubation at room temperature, a solution of TROX (20 μl) and arachidonic acid (20 μl) dissolved in 1.1 μl ethanol containing 5 μl KOH was added to the mixture. The mixture was incubated for a further 5 min and the absorbance was measured on a plate reader at 595 nm. Celecoxib and indomethacin were used as the reference compounds. The results are shown in Table 2.
3.3 min for a solution of L-ascorbic acid (0.1 ml, 100 mg/ml) in water and evaporated to dryness. The residue was dissolved in physiological saline containing 0.125% Tween 80. The labeled compound was analyzed by HPLC: TSKgel Prep Nova-pak 60A equipped with an RCM 8 µm Prep Nova-pak 60A. No decomposition of the radiotracers under the conditions treated was confirmed.

Tissue Distribution of Radiotracers in Mice

Male ddY mice (8—9 weeks old, body weight: 34—41 g) were used for the following experiments: (A) tissue distribution, (B) a blocking study to determine specific uptake and (C) cyclosporine A (CysA) treatment, and all mice were injected with each radiotracer (2.0—2.8 MBq/8—97 pmol) in a physiological saline (0.2 ml) or with an equivalent volume of phosphate buffered saline (PBS; 1/15 M, pH 7.4) was vortexed at 20 s 3 times and then centrifuged (1500rpm for 1 min). An aliquot was taken from the organic phase (0.2 ml) and the aqueous phase (0.2 ml for [11C] and [12C]) 0.4 ml for [13C] 1 ml for [14C] and [15C]6 and the [11C] radioactivity was measured in an auto-gamma-counter. The tissue distribution was performed on mice 1, 5, 15, 30 and 60 min after radiotracer injection. Group B mice received the following blocker solution: 10% trichloroacetic acid (TCA) in MeCN or an ice-cold 1 : 1 mixture of MeCN and 50 mM CH3COOH–CH3COONa (50 : 50). No decomposition of the radiotracers under the conditions treated was confirmed.

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References


