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Synthesis and anti-inflammatory activity of the major metabolites of imrecoxib

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ABSTRACT

We have developed a novel and moderately selective COX-2 inhibitor, imrecoxib, as a new anti-inflammatory drug. We describe herein the preparation of the major metabolites M2 and M4 of imrecoxib, as well as the in vitro and in vivo activities of the two compounds. The results showed that both M2 and M4 are potential COXs inhibitors with a moderate COX-1/COX-2 selectivity, and their anti-inflammatory activity in vivo was equal to or slightly higher than the clinical celecoxib.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most common medications for the treatment of pain, inflammation and fever, and their anti-inflammatory effects are exhibited by inhibiting cyclooxygenase (COX) which catalyzes the bioconversion of arachidonic acid to prostaglandins.[1] Nowadays, it is well established that there are at least two COX isozymes, COX-1 and COX-2.[2,3] The isozyme COX-1 is constitutive and responsible for the physiological production of prostaglandins, and is critical for protection of gastric mucosa, platelet aggregation and renal blood flow; whereas the COX-2 isozyme is inducible and responsible for the elevated production of prostaglandins during inflammation.[4,5] Traditional NSAIDs, such as naproxen, ibuprofen, diclofenac, flurbiprofen, indomethacin and aspirin, inhibit both isoforms of COX, furthermore COX-1 more strongly than COX-2, but harmful side effects in particular gastrointestinal (GI) irritation, ulcerogenicity and renal toxicity, often limit their chronic use.[6] Thus, several new inhibitors were developed recently which selectively inhibit the COX-2 enzyme without interfering with COX-1 enzymatic activity. These molecules include celecoxib,[7] rofecoxib[8] and valdecoxib,[9] with fewer gastrointestinal side effects to traditional NSAIDs. But an increased risk of myocardial infarction and cardiovascular thrombotic events associated with the use of highly selective COX-2 inhibitors were subsequently observed, which resulted in the recent withdrawal of Rofecoxib and Valdecoxib from the market.[10,11] Hence an appropriately selective inhibition of COX-2 over COX-1 may be useful for the treatment of inflammation and inflammation-associated disorders with reduced gastrointestinal toxicities when compared with traditional NSAIDs and less cardiovascular thrombotic events than highly selective COX-2 inhibitors.

Imrecoxib, 4-(4-methylsulfonyl-phenyl)-1-propyl-3-(*p*-tolyl)-3-pyrrolin-2-one, developed in our laboratory, is a novel and moderately selective COX-2 inhibitor. It inhibits COX-1 and COX-2 with IC_{50} values of 115 ± 28 nmol/L and 18 ± 4 nmol/L, respectively.[12] Imrecoxib has been in the phase III of clinical trials in China for the treatment of acute and chromic inflammatory diseases.

In a preclinical study, it was found that the half-life $t_{1/2}$ of imrecoxib is short, however, the time period of its anti-inflammatory activity lasted longer. This observation is probably due to the presence of active metabolites of imrecoxib. Further metabolic study showed that imrecoxib was metabolized after iv administration with less than 2% of the dose recovered in urine and feces as parent drug;[13] seven metabolites (Scheme 1) of imrecoxib were identified in vivo, including the 4′-hydroxymethyl (M4), 4′-carboxy-(M2), 4′-hydroxymethyl-5-hydroxyl (M3), 4′-hydroxymethyl-5-carbonyl (M5) metabolites, the glucuronide conjugate of M4 (M1), the glucuronide conjugate of M2 (M6), and the glucuronide conjugate of M3 (M7); approximately 75% of the dose was excreted as the 4′-carboxy metabolite (M2), and mainly in feces, and ca. 15% of the dose as the 4′-hydroxymethyl metabolite (M4), mainly in urine; The major metabolic pathway was 4′-methyl group hydroxylation to form the 4′-hydroxymethyl metabolite (M4) which was further oxidized to the 4′-carboxy metabolite (M2).[14] The parent compound was primarily excreted as the carboxylic acid metabolite in feces after dosing. In order to further confirm the structure and activity of the main metabolites M2 and M4, we performed the synthesis and the evaluation for anti-inflammatory activity of the two metabolites.

It was found that the preparation of M2 and M4 by direct chemical oxidation of Imrecoxib failed because of intricate products, for example, the C5-methylene oxidation. A scheme of de novo synthesis using 4-acetoxymethyl phenylacetic acid and 4-ethoxycar-

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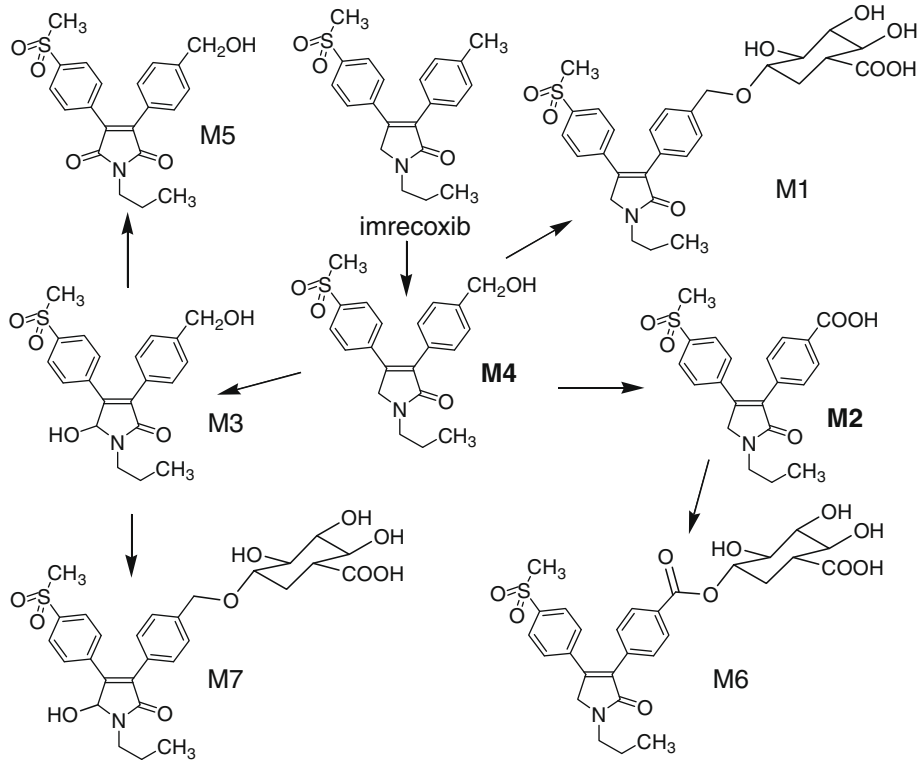
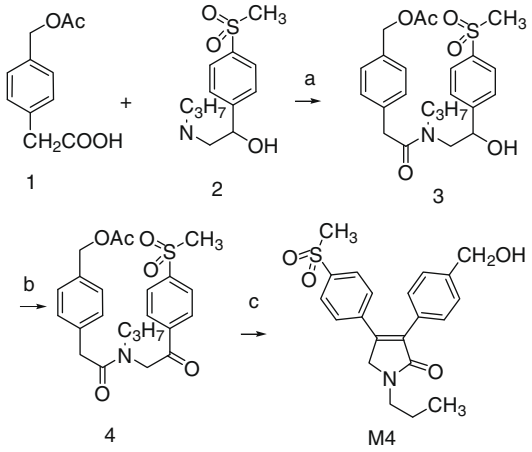


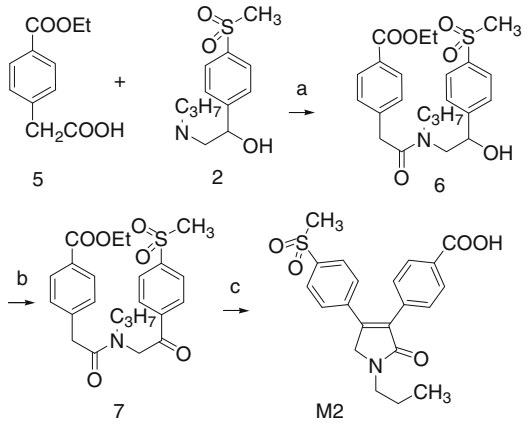
Scheme 1. The metabolites and metabolic pathway of imrecoxib.

bonyl phenylacetic acid, respectively, instead of 4-methyl phenylacetic acid based on the synthetic route of imrecoxib[15] was performed (Schemes 2 and 3).

4-(Bromomethyl)phenylacetic acid, prepared by the reported method,[16] was mixed with excessive sodium acetate and acetic acid, and heated for 12 h to give 4-(acetoxymethyl)phenylacetic acid **1** as one intermediate of M4. The commercially available 4-(methylsulfonyl)acetophenone was brominated at the position of ketone carbonyl to produce bromoacetopenone, that was reduced by sodium boronhydride and followed by cyclized to yield 4-(methylsulfonyl)styrene oxide, then reacted with *n*-propyl amine to give another intermediate *n*-propylamino alcohol **2**, which in turn was acylated by the acyl chloride of **1** to afford acylamide **3**. The key intermediate **4** was obtained from the oxidation of **3** with DMSO. Finally by the intramolecular condensation, **4** was cyclized to provide the target compound M4.



Scheme 2. The synthesis of metabolite M4. Reagents and condition: a: (1) $SOCl_2$, reflux; (2) **2**/pyridine, rt b: DMSO/Ac_2O; c: potassium t-butoxide/t-butanol.



Scheme 3. The synthesis of metabolite M2. Reagents and condition: a: $SOCl_2$, reflux, **2**/pyridine; b: DMSO/Ac_2O; c: LiOH /t-butanol.

The metabolite M2 was prepared using a similar sequence of reactions illustrated Scheme 2. The ethyl 4-ethoxycarbonyl phenylacetate (di-ester) was produced by the alcoholysis of cyanic

Table 1
The inhibitory effect of M2 and M4 on activity of COX-1 and COX-2

Compounds	IC_{50}[a] (10^{-7} M)		COX-2 SI[b]
	COX-1	COX-2	
M2	27.8	4.10	6.8
M4	0.87	0.14	6.2
Imrecoib	1.15	0.18	6.4

[a] Concentration required for 50% inhibition.
[b] In vitro COX-2 selectivity index (IC_{50}·COX-1/IC_{50}·COX-2).

Table 2
The effect of metabolites M4 and M2 on rat carrageenan-induced paw edema

Group	Dose (mg/kg)	The degree of the paw swelling (%) X ± SD			
		1 h	2 h	3 h	4 h
Control	–	34.1 ± 5.3*	54.7 ± 6.9*	55.0 ± 8.5*	53.3 ± 6.6*
M4	10	25.05 ± 10.4** (26.5)	29.52 ± 12.8** (46.0)	24.14 ± 12.3** (56.1)	19.23 ± 13.9** (63.9)
M2	10	18.6 ± 10.0** (45.4)	26.3 ± 15.4** (51.9)	24.5 ± 10.1** (55.5)	18.2 ± 5.6** (65.9)
Imrecoxib	10	23.4 ± 7.8** (31.3)	33.1 ± 10.8** (39.5)	27.3 ± 4.1** (50.4)	26.4 ± 9.6** (50.4)
Celecoxib	10	26.1 ± 7.2** (23.5)	34.7 ± 16.9* (36.6)	30.4 ± 5.2* (44.7)	27.0 ± 1.8* (49.3)

*$p < 0.05$, **$p < 0.01$ versus control. The data in parentheses represent inhibitory rate (%).

groups from commercially available 4-cyano phenylacetonitrile. The aliphatic acid ester in di-ester was selectively hydrolyzed by sodium carbonate to afford 4-ethoxycarbonyl phenylacetic acid **5**. Then **5** was treated with $SOCl_2$ to give the acyl chloride, that reacted with *n*-propylamino alcohol **2** to yield an amide **6**, followed by the treatment of oxidant to give the key intermediate **7**. The metabolite M2 was obtained from the intramolecular cyclization of **7**, when treated with LiOH.

The structures of synthetic M4 and M2 were confirmed with the authentic metabolic M4 and M2, which were identified by the analysis from LC–MS, NMR spectra.

Then, compounds M4 and M2 synthesized were evaluated for their ability to inhibit COX-2 and COX-1 by cellular assay, using freshly harvested mouse peritoneal macrophages as described in the literature.[17] The results showed that both M4 and M2 exhibited potent inhibition against COX-2 and COX-1 as listed in Table 1. The inhibitory effect of M2 on calcimycin-induced COX-1 activity was dose dependent at the concentrations of 10–10,000 nM, with IC_{50} value of 2.78×10^{-6} M, which was evidently lower than the parent compound; Nevertheless the inhibitory effect of M4, with IC_{50} value of 8.7×10^{-8} M, was slightly higher than the parent compound. In addition, the inhibitory effect of M2 on LPS-induced COX-2 activity was dose dependent at the concentrations of 10–1000 nM, with IC_{50} value of 4.1×10^{-7} M, that was evidently lower than the parent compound; However the inhibitory effect of M4, with IC_{50} value of 1.4×10^{-8} M, was slightly higher than the parent compound. The selective ratio ($IC_{50\cdot COX\text{-}1}/IC_{50\cdot COX\text{-}2}$) of inhibition by M2 and M4 were close to that of inhibition in the parent compound. Hence both M2 and M4 have a moderate COX-1/COX-2 selectivity, and may be also selective COX-2 inhibitors with fewer gastrointestinal side effects to traditional NSAIDs, and fewer cardiovascular side effects to highly selective COX-2 inhibitors (see Table 2).

The oral anti-inflammatory activities of M2 and M4 were evaluated at a 10 mg/kg single dose using the carrageenan-induced paw edema method in rats, and compared with imrecoxib and celecoxib as reference compounds in this model. The results indicated that the inhibitory effect of M2 at investigated time points on paw edema was slightly higher than M4 and the control compounds. In addition, the solubility of M2 in water is higher compared to that of M4 and imrecoxib, owing to the carboxylic acid group in M2 structure. Moreover, M2 is metabolic stable because it is major metabolite of imrecoxib, which may avoid some side effects from the metabolism of M2 as lead compound. Hence, the good anti-inflammation of M2 in vivo may result from the high bioavailability and highly stable metabolism of this structure.

In summary, the structures of M2 and M4, as major metabolites of imrecoxib, were further confirmed by the preparation of two authoritative samples 3-(4′-carboxy-phenyl)-4-(4′-methylsulfonyl-phenyl)-1-propyl-3-pyrrolin-2-one and 3-(4′-hydroxymethyl-phenyl)-4-(4′-methylsulfonyl-phenyl)-1-propyl-3-pyrrolin-2-one. The potential anti-inflammatory activity of M2 and M4 were demonstrated by in vitro and in vivo assay for the two compounds, which showed that both M2 and M4 were moderately selective COX-2 inhibitors. This not only explained the long action period of the parent drug, but may afford new potent candidates with improved metabolic profile and bioavailability for the development of anti-inflammatory drug. It is matter-of-course that the above-described data are preliminary and not enough to develop M2 or M4 as drug candidates. More in vitro and in vivo experiments such as human whole blood assay, blood bleeding/clotting tests, and preliminary pharmacokinetic experiments have to be conducted before considering M2 and M4 as new candidates.

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