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生体防御タンパク質に注目した、漢方薬の作用メカニズムの解明・有効成分の同定と新規治療薬の開発

平成 23 年度　総括研究報告書

研究代表者　水島　徹

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研究代表者　水島　徹　慶應義塾大学薬学部教授

研究要旨

HSP70、及びSOD誘導生薬のスクリーニングを行った。その結果、アルニカなど10種のHSP70誘導生薬、及びサルビアなど13種のSOD誘導生薬を発見した。これらのHSP70誘導生薬は、既存のHSP70誘導薬（テプレノン）よりも強力なHSP70誘導能、及び抗潰瘍作用（マウス）を示した。またサルビアは、PC-SODよりも強力な間質性肺炎、及び炎症性腸疾患抑制効果（マウス）を示した。

次に我々は、アルニカからHSP70誘導物質の単離、同定を試みた。オープンカラムで粗分けした後、分取用HPLCで分画し、誘導物質の単離・構造決定に成功した。この誘導物質はテプレノンよりも強力なHSP70誘導能を有しており、現在アルツハイマー病抑制効果などを検討している。

一方サルビア等からのSOD誘導物質の単離、同定も進めている。サルビアに関しては、比活性を30倍以上上げることに成功したので、近い内に構造決定できると考えている。

A. 研究目的

我々はこれまで、様々な疾患に対して HSP や SOD などの生体防御タンパク質が保護的に働くことを報告してきた。HSP は様々なストレスによって誘導され、細胞をストレスに耐性化する。また我々は、HSP が抗炎症作用やタンパク質の変性を抑制する作用を持つことを発見した。一方我々はテプレノン（胃薬）が HSP を誘導する（但し、誘導能はあまり高くない）ことを発見し、テプレノンはこの作用により胃潰瘍を抑制していることを証明した。さらに小腸潰瘍や炎症性腸疾患（炎症と細胞死が主な原因）、及びアルツハイマー病などの神経変性疾患（タンパク質の変性が原因）に対しても HSP が保護的に働くこと、及びテプレノンが有効であることを見出した（現在これらの疾患に対するテプレノンの臨床試験を行っている）。

活性酸素による組織傷害は間質性肺炎や炎症性腸疾患（いずれも難病）などの炎症性疾患の主要な原因である。そこで活性酸素を消去する SOD は古くから注目されてきたが、その血中安定性が低いために医薬品としての開発は成功しなかった。そこで我々は、SOD にリン脂質を結合させ安定化させた PC-SOD を開発し、間質性肺炎、及び炎症性腸疾患に対する第二相臨床試験でその有効性を示した。しかし生物製剤である PC-SOD は生産コスト、及び製剤としての安定性に問題があり、低分子の SOD 誘導薬が望まれている。

長年使われてきた漢方薬は、その安全性・有効性が確認されていることから、医薬品原料として注目されてきた。しかし現在まで、漢方薬由来の物質が新規医薬品として認可されたケースは少ない。我々はその原因として、そのような医薬品開発の多くが、受容体や酵素の阻害など西洋医薬品と同じ機構をターゲットとしており、漢方薬の特徴である緩やかな作用・副作用の少なさとマッチしていないこと、即ち漢方薬は西洋医薬品とは違う独自のターゲット（生体防御タンパク質の効果を高めるなど）を持っていることを考えている（研究計画で述べるように、この考えを支持する成果を最近あげた）。

そこで本研究で我々は、漢方薬（生薬）ライブラリーから HSP 誘導生薬、及び SOD 誘導生薬を検索し、誘導物質の同定、及び動物モデルでの評価を行い、疾患治療薬として開発する化合物を決定する。

ゲノム創薬などにより、21 世紀は新薬の開発ラッシュになると予想されていた。しかし現実には、発売される新薬の数は年々減少しており、製薬企業は医薬品開発戦略の変更を迫られている。この主な原因は臨床試験で発生する副作用であり、作用の強い医薬品より副作用の少ない医薬品を開発すべきであると考えられる。これまでの医薬品は受容体や酵素の阻害・活性化剤が主であり、生体内のバランスを大きく変えることにより副作用を導くと考えられる。そこで我々は、疾患というストレスに対して生体が自らを守るために誘導する生体防御タンパク質を増強させるタイプの医薬品が有用であると考えている。即ち、疾患に対する生体防御タンパク質の誘導が不十分であるために疾患が発症すると考え、医薬品によりその不足分を補うという考えである。生体が本来持っている反応を助けるだけであるので、副作用を起こしにくいと期待される。HSP 誘導薬や PC-SOD が間質性肺炎などの難病に有効であるという臨床結果は、このような医薬品は安全面で優れているだけでなく、従来型の医薬品では効果をあげられなかった疾患にも有効であることを示唆している。

本研究が成功すれば、種々の難病に対する治療薬が生まれるだけでなく、新しい医薬品開発戦略（生体防御タンパク質をターゲットとする医薬品を検索する材料として漢方薬を用いる）を製薬企業へ示すことになり、大きな波及効果が期待できる。

B. 研究方法

最近我々は、共同研究している中国企業（北京泰徳製薬）から得た漢方薬（生薬）ライブラリー（約 600 種）から HSP の誘導生薬をスクリーニングし、テプレノンよりも強力、かつ安全な数多くの HSP 誘導生薬を得た（特許出願済み）。我々はこの中からヤバツイを選択し、その HSP 誘導物質の同定に成功した（特許出願準備中）。この誘導物質

を小腸潰瘍、炎症性腸疾患、アルツハイマー病の動物モデルで評価したところ、テプレノンよりも強力な効果を示した。この結果は、漢方薬（生薬）ライブラリーからスクリーニングした HSP 誘導物質が医薬品として有用であることを示唆している。また最近我々は、HSP が間質性肺炎（有効な治療薬はなく、致死率は 80%を超える）、COPD（世界中で患者数が増大しており、有効な治療薬がない）、及び ALS やハンチントン舞踏症などの神経変性疾患の発症を抑制することを見出した。

そこで本研究で我々は、この漢方薬（生薬）ライブラリーをさらに充実させ、HSP 誘導生薬のスクリーニングを行い、有望な生薬を複数選択する。そして、誘導物質の同定、及び動物モデルでの評価を行い、種々の疾患治療薬として開発する HSP 誘導物質を決定する。

一方最近我々は、PC-SOD が間質性肺炎や炎症性腸疾患だけでなく、活性酸素による組織傷害がその主な原因となっている、腎炎、肝炎、膵炎、喘息、COPD、アトピー性皮膚炎の動物モデルにおいて有効性を示すことを見出した。そこで本研究で我々は、上述のライブラリーから SOD 誘導物質を検索・同定し、種々の疾患治療薬として開発する SOD 誘導物質を決定する。

（1） 漢方薬（生薬）ライブラリーの整備

上述の HSP 誘導生薬（ヤバツイ）は、化粧品として商品化が決定している。この成果を評価した北京泰徳製薬は中国政府から特別の許可を得て、2000 種以上の生薬を供与してくれることになった（最近では生薬を海外に出すことに中国政府は慎重になっており、このようなライブラリーを有する研究機関は国内にほとんどない）。そこでこの生薬の溶解法や投与法を確立し、スクリーニングの準備を行う。

（2） HSP、及び SOD 誘導生薬のスクリーニングと、誘導物質の同定

HSP、あるいは SOD 遺伝子プロモーターの下流にルシフェラーゼ遺伝子を挿入したプラスミドを導入した細胞を用いて一次スクリーニングを行い、イムノブロット法で二次スクリーニングを行う。毒性の少ない誘導薬を得たいので、三次スクリーニングではその生薬の細胞毒性を調べ、細胞毒性を示さない濃度で HSP、あるいは SOD を誘導するものを選択する。四次スクリーニングではその生薬をマウスに投与し、目的のタンパク質を誘導するかを検討する。

これらの結果から有望な生薬を選択し、その誘導物質の同定を行う。オープンカラムで粗分けした後、分取用 HPLC で分画し、誘導物質の構造を決定する。合成可能な物は合成し、難しいものは大量の生薬から精製する。

（3） HSP、及び SOD 誘導物質の疾患治療薬としての評価

それぞれの誘導物質の効果をまず試験管内で評価する。HSP 誘導物質に関しては、炎症抑制作用、細胞保護作用、及びタンパク質凝集抑制作用の程度を調べる。また SOD 誘導物質に関しては、活性酸素消去作用を調べる。次にその効果が HSP、あるいは SOD を介しているかを、siRNA を用いて検証する。

最終的には、種々の疾患動物モデルを用いて評価する。治療効果が見られた場合、その効果が HSP、あるいは SOD を介しているかを、そのタンパク質を誘導出来ないマウスを用いて判断する。有用な薬理効果が見られた場合には、他の臓器の状態を精査し副作用が表れていないかを調べる。尚、HSP 誘導物質の場合は GGA と、SOD 誘導物質の場合は PC-SOD と治療効果を比較する。結果を総合的に判断し、それぞれの疾患治療薬として開発する誘導物質を決定する。

C. 研究結果

本年一月我々は、自ら発見した HSP 誘導生薬（ヤバツイ）を化粧品として発売した。世界初の HSP 誘導化粧品として評価され、この分野のトップ商品になっている。この成果を評価した北京泰徳製薬は、中国政府から特別の許可を得て、2000 種以上の生薬を我々に提供してくれた。我々はこれら生薬の溶解法や投与法を確立し、2500 種以上の生薬からなる生薬ライブラリーを確立した（最近では生薬を海外に出すことに中国政府は慎重になっており、このようなライブラリーを有する研究機関は国内にほとんどない）。

そしてこのライブラリーを用いて、HSP70、及び SOD 誘導生薬のスクリーニングを行った。その結果、アルニカなど 10 種の HSP70

誘導生薬、及びサルビアなど13種のSOD誘導生薬を発見した。これらのHSP70誘導生薬は、既存のHSP70誘導薬（テプレノン）よりも強力なHSP70誘導能、及び抗潰瘍作用（マウス）を示した。またサルビアは、PC-SODよりも強力な間質性肺炎、及び炎症性腸疾患抑制効果（マウス）を示した。尚、アルニカは来年度発売する化粧品に配合されることが決定され、我々はアルニカの特許を化粧品会社へライセンスアウトし、現在共同で化粧品開発を行っている。

次に我々は、アルニカからHSP70誘導物質の単離、同定を試みた。オープンカラムで粗分けした後、分取用HPLCで分画し、誘導物質の単離・構造決定に成功した。この誘導物質はテプレノンよりも強力なHSP70誘導能を有しており、現在アルツハイマー病抑制効果などを検討している。

一方サルビア等からのSOD誘導物質の単離、同定も進めている。サルビアに関しては、比活性を30倍以上上げることに成功したので、近い内に構造決定できると考えている。

このように生体防御タンパク質誘導生薬のスクリーニングがうまくいっているので、研究計画を発展的に変更し、HSP47、及びHO-1（我々の研究から、医薬品や化粧品のターゲット分子として有望であることが示唆されている生体防御タンパク質）の誘導生薬のスクリーニングも開始することにした（研究計画・方法参照）。

D.考察

結果の欄に記載した

E.結論

このように平成22年度の我々の研究により、数多くの有望な生薬が発見された。

F.健康危険情報

該当なし

G.研究発表

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27 水島徹　アルツハイマー病と慢性炎症　東京大学医学部での招待講演　(2010)（東京）

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30 水島徹　トランスクリプトソーム解析による医薬品の副作用機構の解明と、その副作用感受性診断、及び創薬への応用　創薬バイオマーカー探索研究事業研究発表会での招待講演　(2011)（東京）

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H.知的財産権の出願・登録状況

1.特許取得

該当なし

2.実用新案登録

該当なし

3.その他

該当なし

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yamakawa, N., Suemasu, S., Kimoto, A., Arai, Y., Ishihara, T., Yokomizo, K., Okamoto, Y., Ohtsuka, M., Tanaka, K. and Mizushima, T.	Low direct cytotoxicity of loxoprofen on gastric mucosal cells.	***Biol. Pharm. Bull.***	33	398-403.	2010
Matsuda, M., Hoshino, T., Yamashita, Y., Tanaka, K., Maji, D., Sato, K., Adachi, H., Sobue, G., Ihn, H., Funasaka, Y. and Mizushima, T.	Prevention of ultraviolet B radiation-induced epidermal damage by expression of heat shock protein 70.	***J. Biol. Chem.***	285	5848-5858.	2010
Tanaka, K., Ishihara, T., Azuma, A., Kudoh, S., Ebina, M., Nukiwa, T., Sugiyama, Y., Tasaka, Y., Namba, T., Ishihara, T., Sato, K., Mizushima, Y. and Mizushima, T.	Therapeutic effect of lecithinized superoxide dismutase (PC-SOD) on bleomycin-induced pulmonary fibrosis.	***Am. J. Pathol.***	298	L348-L360.	2010
Ishihara, T., Tanaka, K., Tashiro, S., Yoshida, K. and Mizushima, T.	Protective effect of rebamipide against celecoxib-induced gastric mucosal cell apoptosis.	***Biochem. Pharmacol.***	79	1622-1633.	2010
Hoshino, T., Matsuda, M., Yamashita, Y., Takehara, M., Fukuya, M., Mineda, K., Maji, D., Ihn, H., Adachi, H., Sobue, G., Funasaka, Y. and Mizushima, T.	Suppression of melanin production by expression of HSP70.	***J. Biol. Chem.***	285	13254-13263.	2010
Namba, T., Tanaka, K., Ito, Y., Hoshino, T., Matoyama, M., Yamakawa, N., Isohama, Y., Azuma, A. and Mizushima, T.	Induction of EMT-like phenotypes by an active metabolite of leflunomide and its contribution to pulmonary fibrosis.	***Cell Death Differ.***	17	1882-1895.	2010
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Namba, T., Hoshino, T., Suemasu, S., Takarada-Iemata, M., Hori, O., Nakagata, N., Yanaka, A. and Mizushima, T.	Suppression of expression of endoplasmic reticulum chaperones by *Helicobacter pylori* and its role in exacerbation of NSAID-induced gastric lesions.	***J. Biol. Chem.***	285	37302-37313	2010

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Synthesis and biological evaluation of loxoprofen derivatives

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) achieve their anti-inflammatory actions through an inhibitory effect on cyclooxygenase (COX). Two COX subtypes, COX-1 and COX-2, are responsible for the majority of COX activity at the gastrointestinal mucosa and in tissues with inflammation, respectively. We previously suggested that both gastric mucosal cell death due to the membrane permeabilization activity of NSAIDs and COX-inhibition at the gastric mucosa are involved in NSAID-induced gastric lesions. We have also reported that loxoprofen has the lowest membrane permeabilization activity among the NSAIDs we tested. In this study, we synthesized a series of loxoprofen derivatives and examined their membrane permeabilization activities and inhibitory effects on COX-1 and COX-2. Among these derivatives, 2-{4′-hydroxy-5-[(2-oxocyclopentyl)methyl]biphenyl-2-yl}propanoate **31** has a specificity for COX-2 over COX-1. Compared to loxoprofen, oral administration of **31** to rats produced fewer gastric lesions but showed an equivalent anti-inflammatory effect. These results suggest that **31** is likely to be a therapeutically beneficial and safer NSAID.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) comprise one of the most frequently used classes of medicines in the world and account for nearly 5% of all prescribed medications.[1] NSAIDs are inhibitors of cyclooxygenase (COX), a protein essential for the synthesis of prostaglandins (PGs), which have a strong capacity to induce inflammation. However, NSAID administration is associated with gastrointestinal complications, such as gastric ulcers and bleeding. In the United States, about 16,500 people per year die as a result of NSAID-associated gastrointestinal complications.[2] Inhibition of COX by NSAIDs was thought to be fully responsible for their gastrointestinal side effects, because PGs have a strong protective effect on the gastrointestinal mucosa. In 1991, two subtypes of COX, COX-1 and COX-2, which are responsible for the majority of COX activity at the gastrointestinal mucosa and tissues with inflammation, respectively, were identified.[3,4] Thus, it is reasonable to speculate that selective COX-2 inhibitors maintain anti-inflammatory activity without gastrointestinal side effects. In fact, a greatly reduced incidence of gastroduodenal lesions has been reported for selective COX-2 inhibitors (such as celecoxib and rofecoxib).[5–7] Thus, increasing the specificity for COX-2 over COX-1 is one of the strategies that could be employed to develop safer NSAIDs. However, a recently raised issue concerning the use of selective COX-2 inhibitors is their potential risk for cardiovascular thrombotic events (see Section 3).[8,9] Because of this concern, rofecoxib and valdecoxib were withdrawn from the worldwide market.[8,10]

It is now believed that the inhibition of COX by NSAIDs is not the sole explanation for the gastrointestinal side effects of NSAIDs.[11] We previously demonstrated that NSAIDs induce necrosis and apoptosis in cultured gastric mucosal cells and in the gastric mucosa in a manner independent of COX inhibition.[12–16] We clearly showed that the primary target of NSAIDs for the induction of necrosis and apoptosis is the cytoplasmic membranes.[12,14] The following pathway has been proposed to describe the molecular mechanism governing this apoptosis.[12,17,18] Permeabilization of cytoplasmic membranes stimulates Ca^{2+} influx and increases intracellular Ca^{2+} levels, which in turn induces the endoplasmic reticulum (ER) stress response. In this response, an apoptosis-inducing transcription factor, C/EBP homologous transcription factor (CHOP), is induced, resulting in mitochondrial dysfunction and apoptosis.[13,19] Furthermore, we have suggested that both COX inhibition (decrease in the gastric level of PGE_2) and gastric mucosal cell death are required for the formation of NSAID-induced gastric lesions in vivo.[16,20] Thus, decreasing the membrane permeabilization activity of NSAIDs is another strategy that could be followed to develop safer compounds that provide the clinical effects sought.

Loxoprofen sodium (**1**, Fig. 1) has been used clinically for many years as a standard NSAID in Japan, and clinical studies have suggested that it is safer than other NSAIDs, such as

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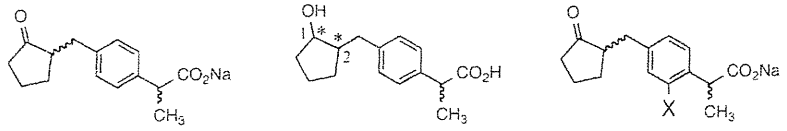


Figure 1. Structure of loxoprofen sodium and its derivatives.

indomethacin.[21,22] Loxoprofen is a pro-drug, which is converted to its active metabolite (the *trans*-alcohol form, **2**, Fig. 1) by aromatic aldehyde–ketone reductase only after absorption in the gastrointestinal tract.[23] We recently reported that loxoprofen has lower membrane permeabilization activity than other NSAIDs.[24] Therefore, synthetic modification of loxoprofen to either increase specificity for COX-2 or decrease membrane permeabilization activity is a valuable strategy to obtain safer NSAIDs.

We recently reported that the loxoprofen derivatives 2-fluoroloxoprofen and 2-bromoloxoprofen (**4a** and **4b**, respectively, Fig. 1) have lower membrane permeabilization activity and their oral administration to rats produced fewer gastric lesions. Nevertheless, these compounds had equivalent anti-inflammatory effects compared to loxoprofen.[25] In the present study, we synthesized a series of loxoprofen derivatives and examined their membrane permeabilization activities and inhibitory effects on COX-1 and COX-2. Among these derivatives, 2-{4′-hydroxy-5-[(2-oxocyclopentyl)methyl]biphenyl-2-yl}propanoate (**31**, Scheme 3) has a specificity for COX-2 and its oral administration produced fewer gastric lesions but showed an equivalent anti-inflammatory effect, compared to loxoprofen. These results suggest that this compound could be a valuable candidate for use as a safer NSAID.

2. Chemistry

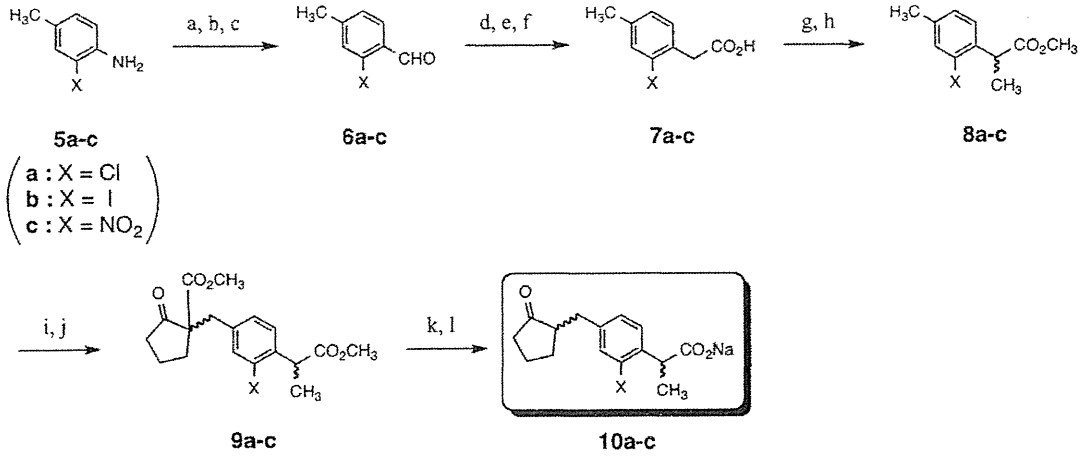
Loxoprofen derivatives with modification at the 2-position of the phenyl ring by halogens and the nitro group **10a–c** were obtained by the method described previously[25] (Scheme 1).

Loxoprofen derivatives with modification at the 3- or 2-position of the phenyl ring by a para-substituted aryl group were synthesized via the Suzuki–Miyaura cross-coupling reaction[26,27] between aryl bromide derivatives **14** or **4b** and a variety of commercially available boronic acids (Schemes 2 and 3).

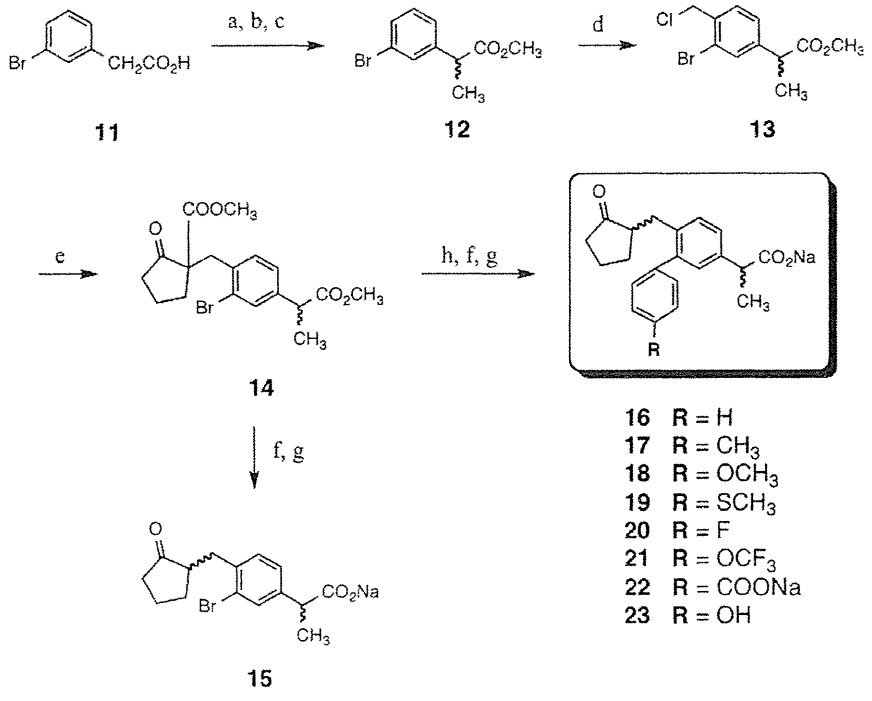
The synthetic route for target compounds **16–23** is outlined in Scheme 2. The commercially available (3-bromophenyl)acetic acid **11** was converted to the methyl 2-(3-bromophenyl)propanoate **12** by methyl esterification and α-methylation. Friedel–Crafts chloromethylation of **12** under Lewis acid conditions gave the methyl 2-[3-bromo-4-(chloromethyl)phenyl]propanoate **13**, having an active methylene group. The hetero-nuclear multiple-bond connectivity (HMBC) nuclear magnetic resonance (NMR) spectrum of **13** revealed correlations between the methylene carbon and the 5-position proton on the phenyl ring or the methylene carbon and the 2- and 6-position protons on the phenyl ring (data not shown).

Treatment of compound **13** with methyl 2-oxocyclopentanecarboxylate provided the key intermediate **14**. Compound **15** (3-bromoloxoprofen) was obtained by decarboxylation, hydrolysis and treatment with NaOH of **14**. The cross-coupling reaction between **14** and a variety of boronic acids afforded the precursors of target compounds **16–23**. Finally, the carboxylic acid group was transformed into the sodium salt by treatment with NaOH to yield target compounds **16–23**.

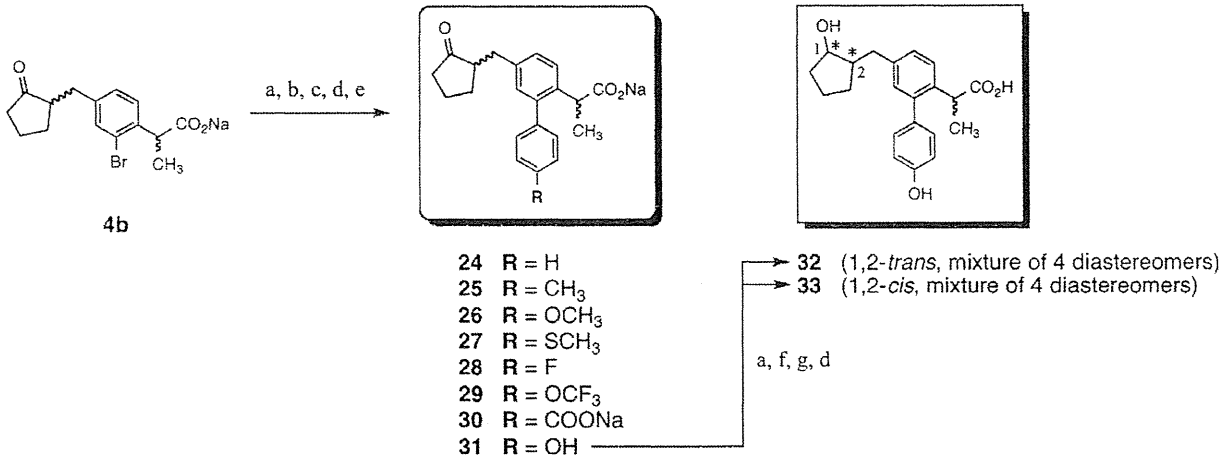
The synthetic route for target compounds **24–31** is outlined in Scheme 3. A key intermediate **4b** was prepared, as described previously.[25] The methyl ester of **4b** was prepared by treatment with methanol in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*,*N*-dimethyl-4-aminopyridine (DMAP). After the cross-coupling reaction between the compound **4b** and a variety of boronic acids, the ester group was converted to a carboxylic acid group by alkaline hydrolysis,



Scheme 1. Synthesis of loxoprofen derivatives with modification at the 2-position of the phenyl ring by Cl (**10a**), I (**10b**) and NO_2 (**10c**). Reagents and conditions: (a) 3 M HCl aq, $NaNO_2$, $CuSO_4$, Na_2SO_3, AcONa, H_2O, 0 °C; (b) NH_2OH·HCl, $(HCHO)_n$, AcONa, H_2O; (c) concd HCl, reflux; (d) Me $OCH_2P(OH_3)Cl$, $C_6H_{18}KNSi_2$, toulene; (e) 3 M HCl aq, acetone, reflux; (f) PFC (2.0 mol %), H_5IO_6, acetonitrile; (g) concd HCl, CH_3OH, reflux; (h) 2.0 M LDA, CH_3I, dry THF, −70 to −40 °C; (i) NBS, AIBN, CCl_4, reflux; (j) dry Na_2CO_3, methyl 2-oxocyclopentanecarboxylate, dry acetone, reflux; (k) concd HCl, reflux; (l) 1 M NaOH aq, C_2H_5OH, reflux.



Scheme 2. Synthesis of loxoprofen derivatives with modification at the 3-position of the phenyl ring by Br (**15**) and a *para*-substituted aryl group (**16–23**). Reagents and conditions: (a) MeOH, HCl, reflux; (b) LDA, THF, −78 °C; (c) CH_3I, −78 to −50 °C; (d) $AlCl_3$, $SnCl_4$, 1,3-dioxolane, CH_3OCH_2Cl, 0 °C to rt; (e) methyl 2-oxyocyclopentanecarboxylate, K_2CO_3, acetone, reflux; (f) AcOH, HCL, reflux; (g) 1 M NaOH aq, C_2H_5OH, reflux; (h) R-⟨⟩-$B(OH)_2$, $PD(PPh_3)_4$, Na_2CO_3, THF, reflux.



Scheme 3. Synthesis of loxoprofen derivatives with modification at the 2-position of the phenyl ring by a *para*-substituted aryl group (**24–33**). Reagents and conditions: (a) 6 M HCL aq, CH_2Cl_2; (c) R-⟨⟩-$B(OH)_2$, $PD(PPh_3)_4$, Na_2CO_3, THF, reflux; (d) KOH, C_2H_5OH, H_2O, reflux; (e) 1 M NaOH, C_2H_5OH, reflux; (f) 4-DMAP, EDC, CH_2OH; (g) $NaBH_4$, C_2H_5OH.

followed by acidification. Finally, the carboxylic acid group was transformed into the sodium salt by treatment with NaOH to yield target compounds **24–31**.

The reduction products of **31**, *trans*-alcohol **32** and *cis*-alcohol **33** were prepared by treatment of the methyl ester intermediate of **31** with sodium borohydride ($NaBH_4$) followed by alkaline hydrolysis. The structures of **32** and **33** were identified based on the characteristic NMR signal of the proton on the asymmetric carbon attached to the hydroxyl group.

All target compounds were pure and stable. The final compounds were characterized by ^1H NMR, ^{13}C NMR, infrared spectroscopy (IR), high resolution mass spectra (HR-MS) and elemental analysis.

3. Results and discussion

We have employed loxoprofen sodium **1** (Fig. 1) as a lead compound to obtain NSAIDs with lower membrane permeabilization activity or higher COX-2 specificity. On this basis we synthesized a series of derivatives of **1** by modification of the phenyl ring with electron withdrawing groups such as halogens or modified phenyl

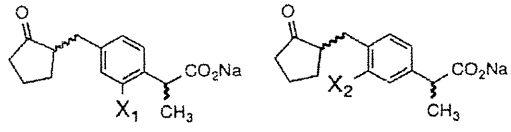
rings. We previously reported that two of the compounds, 2-fluoroloxoprofen **4a** and 2-bromoloxoprofen **4b** (Fig. 1), have lower membrane permeabilization activity than **1**.[25] In this study, we examined the membrane permeabilization activities and inhibitory effects on COX-1 and COX-2 of other derivatives to find other valuable compounds, such as those with COX-2 specificity.

We previously established an assay system for assessing the membrane permeabilization activity of NSAIDs, using calcein-loaded liposomes. Calcein fluorescence is very weak at high concentrations due to self-quenching, so the addition of membrane-permeabilizing drugs to a medium containing calcein-loaded liposomes causes an increase in fluorescence by diluting the calcein.[14] In this study, we used the EC_{50} index, defined as the concentration of each compound required for 50% release of calcein.

Table 1 shows the membrane permeabilization activities and inhibitory effects on COX-1 and COX-2 of loxoprofen derivatives with modification at the 3- or 2-position of the phenyl ring by halogens and the nitro group. The inhibitory effect on COX-1 and COX-2 is shown as the IC_{50} index, defined as the concentration of each compound required for 50% inhibition of each form of COX. Compared to **1**, 4a and **4b**, 2-chloroloxoprofen **10a** and 2-iodoloxoprofen **10b** showed higher membrane permeabilization activity, thus demonstrating that the species of halogen introduced to **1** is an important determinant of the membrane permeabilization activity. We also found that 3-bromoloxoprofen **15** has much higher membrane permeabilization activity than **4b** (Table 1), showing that the modification position on the phenyl ring is also important. Furthermore, we found that 2-nitroloxoprofen **10c** has lower membrane permeabilization activity and a lower inhibitory effect on COX-1 and COX-2 than **1** (Table 1).

The orientation of the active metabolite of **1** and interaction between the compound and amino acid residues in the active site of COX-1 or COX-2 were examined by molecular modeling and docking studies. As shown in Fig. 2, the cyclopentanone ring interacts with Y385 and S530, whereas propanoic acid interacts with R120 and Y355. All of these amino acids were reported to be important for the interaction between COXs and NSAIDs.[28–31] It is also well known that COX-2 has a side pocket[28,32] (Fig. 2). Thus, it could be predicted that introduction of a bulky functional group into the 3- or 2-position of the phenyl ring of **1** results in an increase in its specificity for COX-2 over COX-1. Therefore, we synthesized loxoprofen derivatives with modification at the 3- or 2-position of the phenyl ring by para-substituted aryl groups.

Table 1
In vitro membrane permeabilization assay and human whole blood assay for inhibition of COX-1- and COX-2-derived PG biosynthesis; loxoprofen derivatives with modification at the 3- or 2-position of the phenyl ring by halogens and the nitro group



Compounds	X_1 or X_2	EC_{50} (mM)	IC_{50} (μM)		COX-1/COX-2
		Calcein release	COX-1	COX-2	
1		800[a]	24[a]	10[a]	2.5[a]
4a	X_1 = F	>1000[a]	24[a]	14[a]	0.2[a]
4b	X_1 = Br	>1000[a]	30[a]	65[a]	0.1[a]
10a	X_1 = Cl	100	4	2	1.8
10b	X_1 = I	150	270	540	0.5
10c	X_1 = NO_2	>1000	93	49	1.9
15	X_2 = Br	<100	49	23	2.1

Calcein-loaded liposomes were incubated with each compound. The release of calcein from the liposomes was determined by measuring fluorescence intensity. Triton X-100 (10 μM) was used to establish the 100% level of membrane permeabilization. EC_{50} value (concentration of each compound required for 50% release of calcein) is shown.

The inhibitory effect of each compound on COX-1- and COX-2-derived PG biosynthesis was measured and the IC_{50} value (concentration of each compound required for 50% inhibition) and the COX-1/COX-2 ratio of IC_{50} value are shown. The values of IC_{50} were estimated from the sigmoid-like dose–response curve (4-parameter logistic curve model) drawn by the logistic-curve fitting software (ImageJ 1.43u; National Institutes of Health, USA). Mean values are presented (n = 3).

[a] Data from our previous report.[25]

Table 2 shows the membrane permeabilization activities and inhibitory effects on COX-1 and COX-2 of these derivatives, indicating the importance of the modification position of the phenyl ring (3- or 2-position) for determining membrane permeabilization activity and inhibitory effect on COX-1 and COX-2. For example, the membrane permeabilization activity and inhibitory effects on COX-1 and COX-2 of **31** were much higher than those of **23** (Table 2) and we have no clear explanation for this difference. All derivatives except **23** showed higher membrane permeabilization activity than **1**. On the other hand, none of these derivatives showed a more potent inhibitory activity on COX-1 and COX-2 than **1**. Among these derivatives, 2-{4′-hydroxy-5-[(2-oxocyclopentyl)methyl]biphenyl-2-yl}propanoate 31 showed the most potent inhibitory effect on COX-2 and the highest specificity for COX-2 over COX-1; the extent of this specificity is similar to that of celecoxib (Table 2). The combined results show that **31** is a loxoprofen derivative with higher membrane permeabilization activity, a similar inhibitory effect on COX-2, and a higher specificity for COX-2, compared to **1**. On this basis we selected this compound for further investigation (see below).

As described above, **1** is a pro-drug and the *trans*-alcohol derivative is the active metabolite. In order to test whether or not **31** maintains this characteristic, we examined the COX-inhibitory activity of the *trans*- and *cis*-alcohol forms of **31** (**32** and **33**, respectively). The *trans*-alcohol derivative of **1** (**2**, Fig. 1) showed a more potent inhibitory effect on both COX-1 and COX-2 than **1** or its *cis*-alcohol derivative (**3**, Fig. 1) (Table 2). In contrast to the case of **1**, the inhibitory effect on COX-2 was similar between **31**, 32 and **33** (Table 2). Furthermore, the inhibitory effect of **32** on COX-1 was less than that of **33** (Table 1). These results suggest that **31** does not retain the pro-drug characteristic of **1**.

We then evaluated the activity of **31** in vivo. Compound **1** (40 or 50 mg/kg) and equivalent molar amounts of **31** were orally administered to rats and the lesion index was calculated (see Section 5.5). Administration of **1** produced gastric lesions in a dose-dependent manner (Fig. 3), as described previously.[21,22] In contrast, production of gastric lesions was not detected after oral administration of **31** (Fig. 3). We also measured the gastric level of PGE_2 by enzyme immunoassay (EIA) after oral administration of these compounds. As shown in Fig. 3B, the administration of **31** decreased the level of PGE_2, albeit to an extent less than that seen with **1**. Considering our hypothesis that both a decrease in the gastric level of PGE_2 and an increase in gastric mucosal damage due to membrane permeabilization activity of NSAIDs are involved in the production of NSAID-induced gastric lesions, the lower lesion-producing activity of **31** seems to be due to its selectivity for COX-2, resulting in less activity for decreasing the gastric level of PGE_2.

Finally, we compared the anti-inflammatory effects of **31** to **1** by employing a rat carrageenan-induced footpad edema assay. As shown in Fig. 4A, the volume of edema was significantly decreased after oral administration of **1**, confirming it's previously described anti-inflammatory activity.[23,33] The effects of **31** were mostly the same as that of **1** (Fig. 4A). We also found that the level of PGE_2 associated with the footpad edema decreased after oral administration of **31** and the extent was similar to that seen with **1** (Fig. 4B). These results show that **31** has an anti-inflammatory activity equivalent to **1**. This finding may be related to the

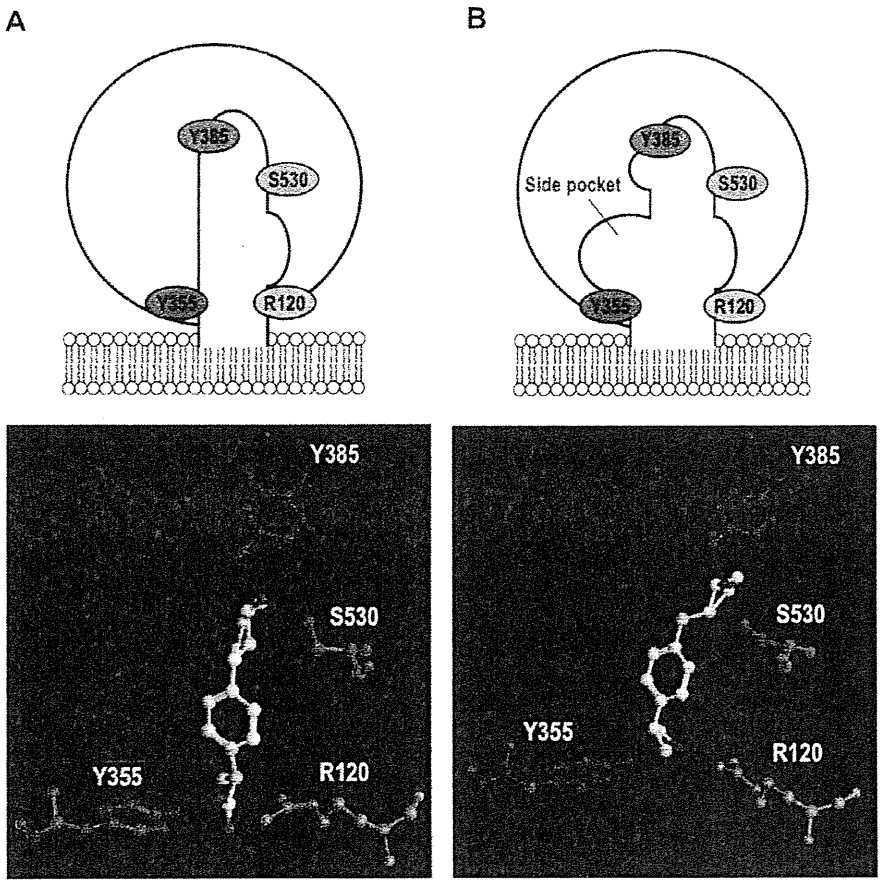


Figure 2. Potential binding mode of (*S*)-2-{4-[((1*R*, 2*S*)-2-hydroxycyclopentyl)methyl]phenyl}propanoic acid to the active site of sheep COX-1 (A) or murine COX-2 (B). Hydrogen atoms of the amino acid residues and the ligand have been removed.

in vitro observation that the inhibitory effect of **1** on COX-2 was indistinguishable from that of **31** (Table 2).

The inhibitory activity of **31** on COX-2 was much higher than that of **23** (Table 2), indicating the importance of the modification position of the phenyl ring (3- or 2-position) for determining the inhibitory effect on COX-2. Thus, we compared the interaction with COX-2 between **23** and **31** by molecular modeling and docking studies. The interaction between the cyclopentanone ring with Y385 and S530 and propanoic acid with R120 and Y355 was similar between **31** (Fig. 5B) and the active metabolite of **1** (Fig. 2B). Furthermore, the introduced phenyl ring of **31** interacts with some amino acids (H90, R513, F518 and V523) (Fig. 5B), which are reported to be located in the side pocket of COX-2.[34,35] On the other hand, molecular modeling and docking studies suggested that the interaction between the cyclopentanone ring with Y385 and S530 and propanoic acid with R120 and Y355 was not possible for **23** (Fig. 5A). As a result, lowest U_{total} index is calculated to be 59.2 and 29.5 kcal/mol for **23** and **31**, respectively; the lower lowest U_{total} index means the higher interaction of two molecules.[36]

A recently raised issue concerning the use of selective COX-2 inhibitors is their potential risk for cardiovascular thrombotic events.[8,9] This may be due to the fact that prostacyclin, a potent anti-aggregator of platelets and a vasodilator, is mainly produced by COX-2 in vascular endothelial cells, while thromboxane A_2, a potent aggregator of platelets and a vasoconstrictor, is mainly produced by COX-1 in platelets.[37–39] Because of this concern, rofecoxib and valdecoxib were withdrawn from the worldwide market.[8,10] On the other hand, it is not clear whether or not celecoxib use is a potential risk factor for cardiovascular thrombotic events. It was proposed that the weaker COX-2 specificity of celecoxib compared to rofecoxib and valdecoxib (COX-1/COX-2 ratios of IC_{50} index of celecoxib, rofecoxib and valdecoxib are 37, 141 and 270, respectively) is responsible for the relative safety of celecoxib in relation to cardiovascular thrombotic events.[40–42] From this point of view, **31** may be safer for use with respect to possible cardiovascular thrombotic events compared to rofecoxib and valdecoxib.

4. Conclusion

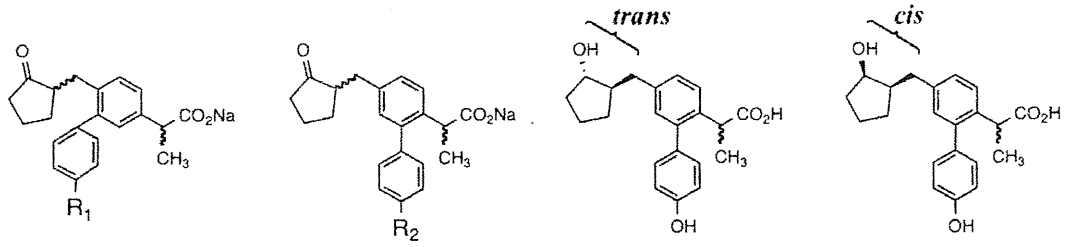
We have found that a loxoprofen derivative, **31**, administered orally to rats, produced fewer gastric lesions but provided similar anti-inflammatory effects compared to **1**. This may be due to its selectivity for COX-2, resulting in a lower propensity for the gastric level of PGE_2 to be reduced. Although **31** exhibits higher membrane permeabilization activity and does not maintain the pro-drug characteristic of **1**, we consider that it is likely to be therapeutically beneficial as a safer NSAID.

5. Experimental

5.1. Molecular modeling studies

Docking studies were performed with MOE (The Molecular Operating Environment) Version 2009.10 software (Chemical Computing Group Inc., Montreal, Canada).

Table 2
In vitro membrane permeabilization assay and human whole blood assay for inhibition of COX-1- and COX-2-derived PG biosynthesis: loxoprofen derivatives with modification at the 3-position (**16–23**) and the 2-position (**24–31**) of the phenyl ring by a para-substituted aryl group



32 **33**

Compounds	R_1 or R_2	EC_{50} (mM)	IC_{50} (μM)		COX-1/COX-2
		Calcein release	COX-1	COX-2	
1		800[a]	24[a]	10[a]	2.5[a]
2			1.3[a]	2.4[a]	0.6[a]
3			6.3[a]	12.2[a]	0.6
16	R_1 = H	<100	54	290	0.2
17	R_1 = CH_3	<100	56	420	0.1
18	R_1 = OCH_3	<100	800	>1000	–
19	R_1 = SCH_3	<10	758	>1000	–
20	R_1 = F	<100	174	36	1.0
21	R_1 = OCF_3	<10	460	72	6.4
22	R_1 = CO_2Na	200	>1000	>1000	–
23	R_1 = OH	>1000	>1000	–	–
24	R_2 = H	<100	310	70	4.4
25	R_2 = CH_3	<100	470	540	0.9
26	R_2 = OCH_3	<100	74	430	0.2
27	R_2 = SCH_3	<100	575	150	3.8
28	R_2 = F	20	174	36	4.8
29	R_2 = OCF_3	6	515	>1000	–
30	R_2 = CO_2Na	<10	>1000	76	–
31	R_2 = OH	25	326	11	31
32			650	20	33
33			47	17	2.8
Celecoxib		0.09[a]	7[b]	0.19[b]	37[b]

Experiments and data analysis were performed as described in the legend of Table 1.
[a] Data from our previous report.[25]
[b] Data from a reference.[41]

5.1.1. Construction of the ligand molecule

The ligand molecule of (*S*)-2-{4-[((1*R*,2*S*)-2-hydroxycyclopentyl)methyl]phenyl}propanoic acid was constructed using the Builder module. The geometric stereochemistry was constrained, and all carboxylic acid groups were modeled in their ionized forms.

5.1.2. Construction of the receptor protein

The crystal structures of sheep COX-1 complexed with aspirin (1PTH)[30] and murine COX-2 complexed with indomethacin (4COX)[28] were obtained from the Protein Data Bank. After removal of the ligand and water, the structure of each receptor protein was optimized with the addition of hydrogen atoms and charge to acidic amino acid residues.

5.1.3. Molecular docking of the ligand with COX-1 and COX-2

Modeling calculations were performed only for each active site of COX-1 and COX-2 using the automatic docking program (ASE Dock 2005), which includes energy minimization applied to the ligand. The ligand–receptor complexes were subjected to energy minimization to convergence using the standard conditions at MMFF94 force fields. All amino acid residues within a 4.5 Å radius around the ligand were minimized, and the best conformation of ligand corresponding to the minimum docking energy of each ligand–receptor complex was adopted.

5.2. Chemistry

All solvents and reagents were purchased from Tokyo Kasei Chemical Co. (Tokyo, Japan) and Wako Pure Chemical Industries (Tokyo, Japan), and used without further purification. Fourier transform IR spectra were recorded on a JASCO FT/IR-410 spectrophotometer using potassium bromide (KBr) pellets. ^1H NMR and ^{13}C NMR spectra were recorded on a JNM AL-300 spectrometer (JEOL Ltd., Tokyo, Japan) operating at 300 MHz, in a ca. 2% solution of $CDCl_3$ or CD_3OD. Coupling constant (*J*) values are estimated in hertz (Hz) and spin multiples are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Mass spectra were detected with a fast atom bombardment (FAB) mass spectrometer (JMS-700, JEOL Ltd, Tokyo, Japan). The progress of all reactions was monitored by thin-layer chromatography (TLC) with silica gel glass plates (60 F_{254}) (Merck Ltd, Tokyo, Japan), and spots were visualized with ultraviolet (UV) light (254 nm) and stained in 5% ethanolic phosphomolybdic acid. Column chromatography was performed using Silica Gel 60 N (Kanto Chemical Co., Tokyo, Japan). Elemental analysis was performed for C and H (Instrumental Analysis Center, Kumamoto University) and was within ±0.4% of the theoretical values. Loxoprofen sodium (**1**), loxoprofen-OH (**2**, **3**), and compound **4b** were synthesized as reported previously.[25]

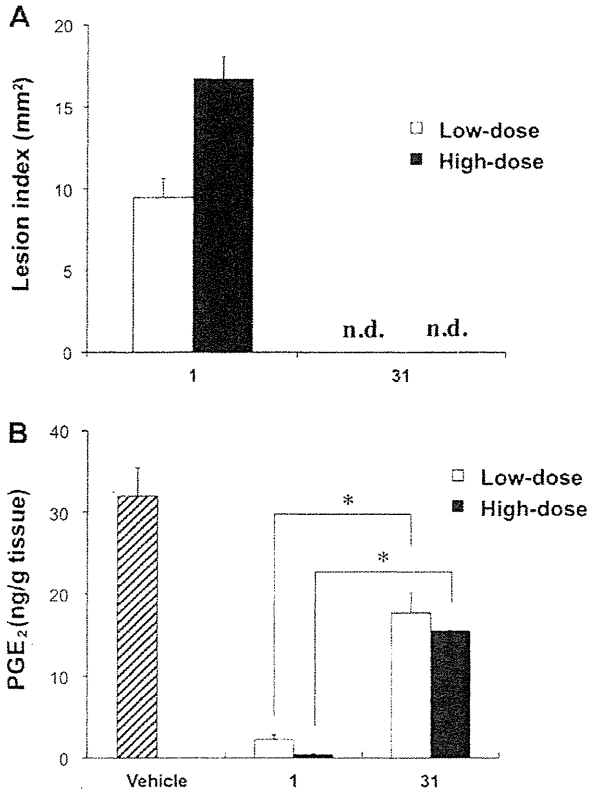


Figure 3. Production of gastric lesions and gastric PGE_2 levels in the presence of loxoprofen sodium and its derivative. Rats were orally administered a low (40 or 54 mg/kg) or high (50 or 67 mg/kg) dose of **1** or **31**, respectively, or vehicle and their stomachs were removed after 8 h. Stomachs were scored for hemorrhagic damage (A). Gastric PGE_2 level was determined by EIA (B). Values are mean ± SEM (n = 3–6). * P <0.05; n.d., not detected.

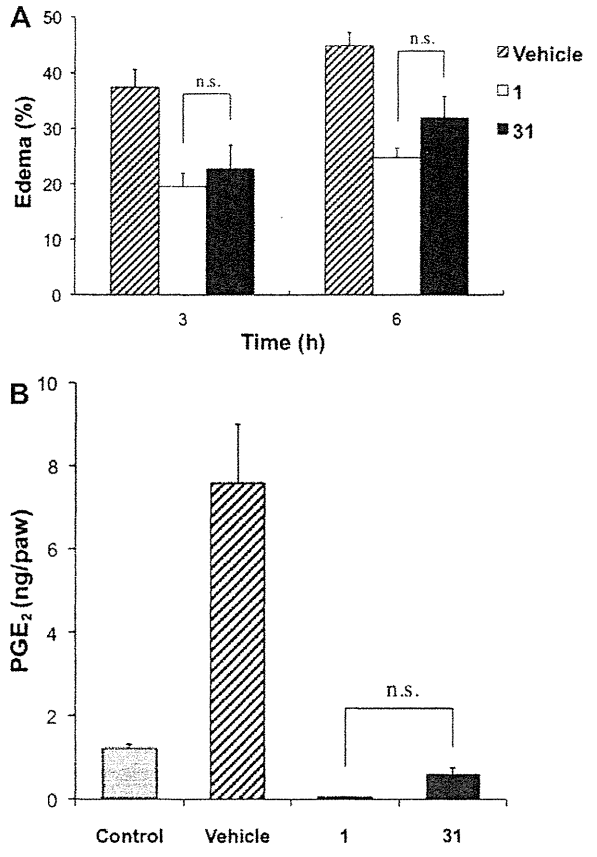


Figure 4. Anti-inflammatory activities of loxoprofen sodium and its derivative. Rats were orally administered 10 or 13 mg/kg of **1** or **31**, respectively, or vehicle and 1 h later received an intradermal injection of carrageenan (1%) into the left hindpaw. Footpad edema was measured 3 h and 6 h after the administration of carrageenan (A). The level of PGE_2 in the footpad was determined by EIA. Control rats were not treated with carrageenan (B). Values are mean ± SEM (n = 3–6). n.s., not significant.

5.2.1. Synthesis of 2-{2-halogeno (or nitro)-4-[(2-oxocyclopentyl)methyl]phenyl}propanoic acid (10a–c)

Compounds **10a–c** were synthesized from the corresponding starting materials **5a–c** by the method described previously.[25]

5.2.1.1. 2-Chloro-4-methylbenzaldehyde (6a). Yellow liquid (yield 52.0%), ^{1}H NMR ($CDCl_3$) δ: 2.34 (3H, s, Ar-CH_3), 7.66 (1H, d, J = 7.5, Ar-H5), 8.14 (1H, d, J = 7.5 Hz, Ar-H6), 8.90 (1H, s, Ar-H3), 10.34 (1H, br s, CHO). EI-MS (m/z): 154.07 (M^+).

5.2.1.2. 2-Iodo-4-methylbenzaldehyde (6b). Red-brown solid (yield 40.1%), ^{1}H NMR ($CDCl_3$) δ: 2.35 (3H, s, Ar-CH_3), 7.26 (1H, d, J = 8.1, Ar-H5), 7.89 (1H, d, J = 8.1 Hz, Ar-H6), 7.90 (1H, s, Ar-H3), 10.34 (1H, br s, CHO). EI-MS (m/z): 245.99 (M^+).

5.2.1.3. 4-Methyl-2-nitrobenzaldehyde (6c). Yellow liquid (yield 36.3%), ^{1}H NMR ($CDCl_3$) δ: 2.54 (3H, s, Ar-CH_3), 7.59 (1H, d, J = 8.4 Hz, Ar-H5), 7.87 (1H, d, J = 7.7 Hz, Ar-H6), 7.89 (1H, s, Ar-H3), 10.36 (1H, s, CHO). EI-MS (m/z): 164.99 (M^+).

5.2.1.4. 2-Chloro-4-methylphenylacetic acid (7a). White solid (yield 59.9%), ^{1}H NMR ($CDCl_3$) δ: 2.34 (3H, s, Ar-CH_3), 3.56 (2H, s, CH_2), 7.06 (1H, dd, J = 7.7, 1.8 Hz, Ar-H5), 7.17 (1H, d, J = 7.7 Hz, Ar-H6), 7.27 (1H, s, Ar-H3), 10.54 (1H, s, CO_2H). FAB-MS (m/z): 184.59 (M^+).

5.2.1.5. 2-Iodo-4-methylphenylacetic acid (7b). White solid (yield 61.3%), ^{1}H NMR ($CDCl_3$) δ: 2.29 (3H, s, Ar-CH_3), 3.76 (2H, s, CH_2), 7.21–7.70 (2H, m, Ar-H5, Ar-H6), 7.68 (1H, s, Ar-H3), 10.56 (1H, s, CO_2H). FAB-MS (m/z): 275.69 (M^+).

5.2.1.6. 4-Methyl-2-nitrophenylacetic acid (7c). White solid (yield 60.0%), ^{1}H NMR ($CDCl_3$) δ: 2.44 (3H, s, Ar-CH_3), 4.01 (2H, s, CH_2), 7.23 (1H, d, J = 7.7 Hz, Ar-H5), 7.41 (1H, d, J = 8.1 Hz), 7.95 (1H, s, Ar-H3), 10.66 (1H, s, CO_2H). FAB-MS (m/z): 196.21 (M^++H).

5.2.1.7. Methyl 2-(2-chloro-4-methylphenyl)propanoate (8a). Slightly-yellow liquid (yield: 71.4%), ^{1}H NMR ($CDCl_3$) δ: 1.48 (3H, d, J = 7.0 Hz, α-CH_3), 2.34 (3H, s, Ar-CH_3), 3.66 (3H, s, OCH_3), 3.66 (1H, q, J = 7.2 Hz, CH), 7.08 (1H, dd, J = 8.1, 1.8 Hz, Ar-H5), 7.17 (1H, d, J = 7.7 Hz, Ar-H6), 7.28 (1H, d, J = 1.8 Hz, Ar-H3). FAB-MS (m/z): 213.20 (M^++H).

5.2.1.8. Methyl 2-(2-iodo-4-methylphenyl)propanoate (8b). Colorless liquid (yield: 65.3%), ^{1}H NMR ($CDCl_3$) ^{1}H NMR ($CDCl_3$) δ: 1.44 (3H, d, J = 7.0 Hz, α-CH_3), 2.27 (3H, s, Ar-CH_3), 3.67 (3H, s, OCH_3), 4.07 (1H, q, J = 7.2 Hz, CH), 7.20–7.13 (2H, m, Ar-H5, Ar-H6), 7.69 (1H, s, Ar-H3). FAB-MS (m/z): 305.13 (M^++H).

5.2.1.9. Methyl 2-(4-methyl-2-nitrophenyl)propanoate (8c). Yellow liquid (yield: 54.3%), ^{1}H NMR ($CDCl_3$) δ: 1.58 (3H, d, J = 7.0 Hz, α-CH_3), 2.42 (3H, s, Ar-CH_3), 3.66 (3H, s, OCH_3), 4.27 (1H, q, J = 7.1 Hz, CH), 7.36–7.39 (2H, m, Ar-H5, Ar-H6), 7.74 (1H, s, Ar-H3). FAB-MS (m/z): 224.28 (M^++H).

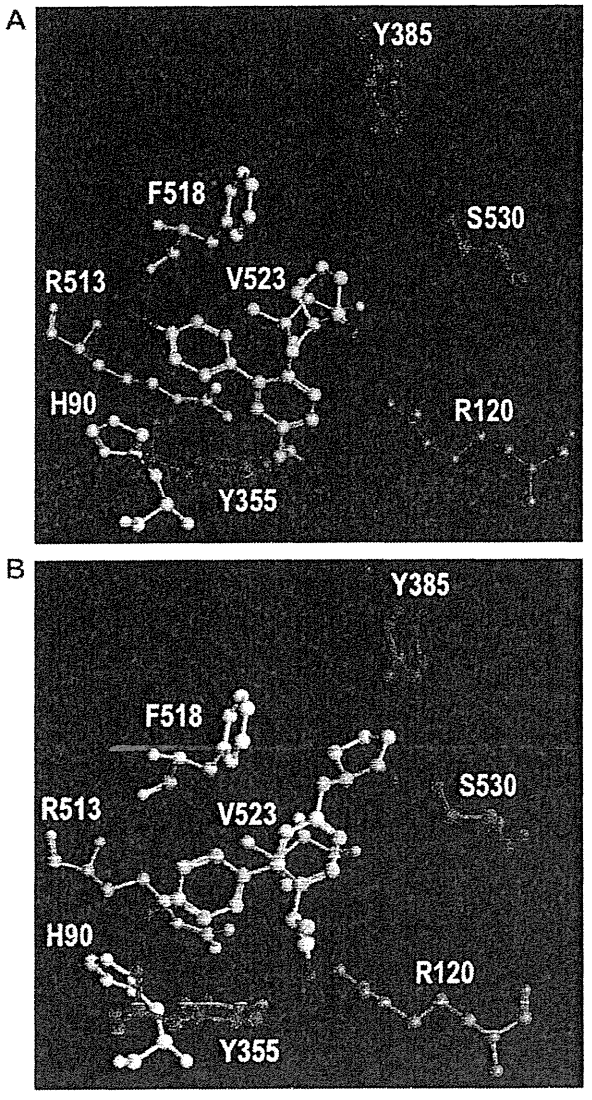


Figure 5. Potential binding mode of **23** (A) or **31** (B) to the active site of murine COX-2. Hydrogen atoms of the amino acid residues and the ligand have been removed.

5.2.1.10. Methyl 1-[3-chloro-4-(1-methoxy-1-oxopropan-2-yl)-benzyl]-2-oxocyclopentanecarboxylate (9a). Colorless liquid (yield: 54.0%), ^{1}H NMR (CD_3Cl_3) δ: 1.47 (3H, d, J = 7.3 Hz, α-CH_3), 1.69–2.14 (4H, m, H3′, H4′), 2.35–2.50 (2H, m, H5′), 3.22 (1H, d, J = 14.3 Hz, CH_2), 3.49 (1H, d, J = 14.3 Hz, CH_2), 3.66 (1H, q, J = 7.1 Hz, CH), 3.67 (3H, s, CO_2CH_3), 3.74 (3H, s, CO_2CH_3), 7.08 (1H, d, J = 8.1 Hz, Ar-H5), 7.14 (1H, d, J = 8.1 Hz, Ar-H6), 7.30 (1H, s, Ar-H3). FAB-MS (*m/z*): 353.21 (M^++H).

5.2.1.11. Methyl 1-[3-iodo-4-(1-methoxy-1-oxopropan-2-yl)-benzyl]-2-oxocyclopentanecarboxylate (9b). Colorless liquid (yield: 53.3%), ^{1}H NMR (CD_3Cl_3) δ: 1.43 (3H, d, J = 7.3 Hz, α-CH_3), 1.70–2.17 (4H, m, H3′, H4′), 2.36–2.47 (2H, m, H5′), 2.95 (1H, d, J = 13.9 Hz, CH_2), 3.17 (1H, d, J = 13.9 Hz, CH_2), 3.68 (3H, s, CO_2CH_3), 3.73 (3H, s, CO_2CH_3), 4.06 (1H, q, J = 7.1 Hz, CH), 7.10 (1H, d, J = 8.8 Hz, Ar-H5), 7.17 (1H, d, J = 8.1 Hz, Ar-H6), 7.64 (1H, s, Ar-H3). FAB-MS (*m/z*): 445.11 (M^++H).

5.2.1.12. Methyl 1-[4-(1-methoxy-1-oxopropan-2-yl)-3-nitrobenzyl]-2-oxocyclopentanecarboxylate (9c). Yellow liquid (yield: 38.6%), ^{1}H NMR (CD_3Cl_3) δ: 1.58 (3H, d, J = 7.3 Hz, α-CH_3), 1.75–2.22 (4H, m, H3′, H4′), 2.40–2.51 (2H, m, H5′), 3.05 (1H, d, J = 14.1 Hz, CH_2), 3.32 (1H, d, J = 13.9 Hz, CH_2), 3.67 (3H, s, CO_2CH_3), 3.74 (3H, s, CO_2CH_3), 4.28 (1H, q, J = 7.2 Hz, CH), 7.39 (2H, br s, Ar-H5, Ar-H6), 7.73 (1H, br s, Ar-H3). FAB-MS (*m/z*): 364.31 (M^++H).

5.2.1.13. Sodium 2-{2-chloro-4-[(2-oxocyclopentyl)methyl]-phenyl}propanoate (10a). White solid (yield: 96.0%), IR (KBr) ν: 1736 (CO_2^-), 1713 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.38 (3H, d, J = 7.1 Hz, α-CH_3), 1.53–2.03 (4H, m, H3′, H4′), 2.06–2.58 (4H, m, H1′, H5′, CH_2), 3.23 (1H, dd, J = 12.7, 3.2 Hz, CH_2), 3.52 (1H, q, J = 7.1 Hz, CH), 7.15 (1H, d, J = 7.9 Hz, Ar-H5), 7.21 (1H, d, J = 7.9 Hz, Ar-H6), 7.38 (1H, s, Ar-H3). ^{13}C NMR (CD_3OD) δ: 19.80 (α-CH_3), 21.44 (C5′), 30.17 (C4′), 33.69 (CH_2), 38.80 (C3′), 49.68 (CH), 50.71 (C1′), 127.43 (Ar-C5), 129.53 (Ar-C3), 131.81 (Ar-C1), 134.56 (Ar-C6), 136.34 (Ar-C2), 145.69 (Ar-C4), 182.40 (CO_2Na), 222.45 (C=O). HR-FAB-MS (*m/z*): 325.0580 (M^++Na, calcd for $C_{15}H_{16}ClNa_2O_3$: 325.0583). Anal. Calcd for $C_{15}H_{16}ClNa_2O_3 \cdot H_2O$: C, 56.17; H, 5.66. Found: C, 56.25, H, 5.75.

5.2.1.14. Sodium 2-{2-iodo-4-[(2-oxocyclopentyl)methyl]phenyl}propanoate (10b). White solid (yield: 94.1%), IR (KBr) ν: 1733 (CO_2^-), 1715 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.34 (3H, d, J = 7.0 Hz, α-CH_3), 1.48–2.14 (4H, m, H3′, H4′), 2.01–2.38 (3H, m, H1′, H5′), 2.46 (1H, dd, J = 13.4, 9.0 Hz, CH_2), 2.98 (1H, dd, J = 13.0, 3.5 Hz, CH_2), 3.85 (1H, q, J = 7.1 Hz, CH), 7.12 (1H, d, J = 8.1 Hz, Ar-H5), 7.34 (1H, d, J = 8.1 Hz, Ar-H6), 7.64 (1H, s, Ar-H3). ^{13}C NMR (CD_3OD) δ: 20.04 (α-CH_3), 21.42 (C5′), 29.97 (C4′), 35.27 (CH_2), 38.95 (C3′), 51.93 (CH), 54.07 (C1′), 102.35 (Ar-C2), 128.70 (Ar-C5), 130.13 (Ar-C6), 140.61 (Ar-C4), 141.10 (Ar-C3), 146.39 (Ar-C1), 182.03 (CO_2Na), 222.54 (C=O). HR-FAB-MS (*m/z*): 416.9935 (M^++Na, calcd for $C_{15}H_{16}INa_2O_3$: 416.9940). Anal. Calcd for $C_{21}H_{21}NaO_3 \cdot H_2O$: C, 43.71; H, 4.40. Found: C, 43.64, H, 4.22.

5.2.1.15. Sodium 2-{2-nitro-4-[(2-oxocyclopentyl)methyl]phenyl}propanoate (10c). Yellow solid (yield: 69.4%), IR (KBr) ν: 1738 (CO_2^-), 1711 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.39 (3H, d, J = 7.3 Hz, α-CH_3), 1.53–2.07 (4H, m, H3′, H4′), 1.94–2.38 (3H, m, H1′, H5′), 2.52 (1H, dd, J = 7.1, 3.5 Hz, CH_2), 3.02 (1H, dd, J = 13.9, 5.1 Hz, CH_2), 3.92 (1H, q, J = 7.1 Hz, CH), 7.32 (1H, d, J = 8.1 Hz, Ar-H5), 7.45 (1H, d, J = 8.1 Hz, Ar-H6), 7.55 (1H, s, Ar-H3). ^{13}C NMR (CD_3OD) δ: 19.82 (α-CH_3), 21.21 (C5′), 29.85 (C4′), 35.38 (CH_2), 38.86 (C3′), 45.17 (CH), 51.71 (C1′), 125.18 (Ar-C3), 130.85 (Ar-C1), 134.31 (Ar-C6), 137.74 (Ar-C5), 140.78 (Ar-C4), 151.04 (Ar-C2), 181.20 (CO_2Na), 222.26 (C=O). HR-FAB-MS (*m/z*): 336.0814 (M^++Na, calcd for $C_{15}H_{16}NNa_2O_5$: 336.0824). Anal. Calcd for $C_{15}H_{16}NNaO_5 \cdot H_2O$: C, 54.38; H, 5.48; N, 4.23. Found: C, 54.36, H, 5.45, N, 4.09.

5.2.2. Synthesis of loxoprofen derivatives with modification at the 3-position of the phenyl ring (15–23)

5.2.2.1. Methyl 2-(3-bromophenyl)propanoate (12). (3-Bromophenyl)acetic acid **11** (5.0 g, 23.3 mmol) and methanol (50 mL) were refluxed for 3 h in the presence of 0.2 mL of concentrated hydrochloric acid (HCl) to give the methyl (3-bromophenyl)acetate. After neutralization with saturated $NaHCO_3$ and washing with brine, a pure product was obtained from the diethyl ether extract. This methyl acetate (4.9 g, 21.4 mmol) in dry THF (35 mL) was added dropwise to a stirred solution of 2.0 mol/L lithium diisopropylamide (LDA) (12.9 mL, 25.8 mmol) in THF/ethylbenzene/heptane at −78 °C under argon (Ar), and after 30 min, iodomethane (CH_3I) (2.0 mL, 32.2 mmol) was added slowly. The resulting solution was stirred for 5 h with the temperature changed from −78 to −40 °C, then evaporated to dryness, and extracted with CH_2Cl_2 (50 mL). Evaporation of the solvent and purification of the residue

by silica gel chromatography (*n*-hexane/AcOEt, 20:1) yielded the title compound as a colorless liquid (77.2%). ^{1}H NMR ($CDCl_3$) δ: 1.49 (3H, d, J = 7.1 Hz, α-CH_3), 3.67 (3H, s, CO_2CH_3), 3.68 (1H, q, J = 7.1 Hz, CH), 7.18 (1H, t, J = 7.5 Hz, Ar-H5), 7.23 (1H, dt, J = 7.8, 1.7 Hz, Ar-H6), 7.38 (1H, dt, J = 7.3, 1.8 Hz, Ar-H4), 7.44 (1H, st, J = 1.7 Hz, Ar-H2). FAB-MS (*m/z*): 243.02 (M^++H, calcd for $C_{10}H_{12}{}^{79}BrO_2$: 243.00).

5.2.2.2. Methyl 2-[3-bromo-4-(chloromethyl)phenyl]propanoate (13). To a suspension of aluminium(III) chloride ($AlCl_3$) (1.52 g, 11.4 mmol) in CH_2Cl_2 (10 mL), 1,3-dioxolane (1.21 mL, 17.5 mmol) was added and the mixture was stirred at 0 °C for 30 min. Tin (IV) chloride ($SnCl_4$) (2.68 mL, 14.6 mmol), **5** (1.78 g, 7.31 mmol) in CH_2Cl_2 (5 mL) and chloromethylmethyl ether (5.50 mL, 73.1 mmol) were added to the reaction mixture. After stirring at room temperature for 20 h, the mixture was poured into dilute HCl solution, and the product was extracted with CH_2Cl_2. Evaporation of the solvent and purification of the residue by silica gel chromatography (*n*-hexane/AcOEt, 10:1) yielded the title compound as a colorless liquid (50.3%). ^{1}H NMR ($CDCl_3$) δ: 1.49 (3H, d, J = 7.3 Hz, α-CH_3), 3.67 (3H, s, CO_2CH_3), 3.70 (1H, q, J = 7.1 Hz, CH), 4.67 (2H, s, CH_2), 7.26 (1H, dd, J = 7.9, 1.8 Hz, Ar-H6), 7.43 (1H, d, J = 7.9 Hz, Ar-H5), 7.53 (1H, sd, J = 1.8 Hz, Ar-H2). FAB-MS (*m/z*): 291.12 (M^++H, calcd for $C_{11}H_{13}{}^{79}BrClO_2$: 290.98).

5.2.2.3. Methyl 1-[2-bromo-4-(1-methoxy-1-oxopropan-2-yl)-benzyl]-2-oxocyclopentanecarboxylate (14). To a suspension of potassium carbonate (K_2CO_3) (1.26 g, 9.1 mmol) in acetone (20 mL), methyl 2-oxocyclopentanecarboxylate (0.64 mL, 5.1 mmol) was added and the mixture was stirred at room temperature for 30 min. A solution of **13** (1.47 g, 5.1 mmol) in acetone (5 mL) was added and the resulting mixture was refluxed for 12 h. The reaction mixture was cooled to room temperature, filtered through paper, and the filtrate was evaporated to dryness. The resulting residue was purified on silica gel chromatography (*n*-hexane/AcOEt, 7:2) to yield the title compound as a colorless oil (78.0%). ^{1}H NMR (CD_3Cl_3) δ: 1.47 (3H, d, J = 7.3 Hz, α-CH_3), 1.71–2.13 (4H, m, H3′, H4′), 2.36–2.55 (2H, m, H5′), 3.28 (1H, d, J = 14.3 Hz, CH_2), 3.51 (1H, d, J = 14.3 Hz, CH_2), 3.66 (1H, q, J = 7.3 Hz, CH), 3.67 (3H, s, CO_2CH_3), 3.74 (3H, s, CO_2CH_3), 7.12 (2H, d, J = 0.7 Hz, Ar-H5, Ar-H6), 7.49 (1H, s, Ar-H2). FAB-MS (*m/z*): 396.22 (M^++H, calcd for $C_{18}H_{21}{}^{79}BrO_5$: 396.06).

5.2.2.4. General procedure for the decarboxylation and hydrolysis by acid. To the bis-methylester intermediate **14** (ca. 5 mmol) in acetic acid (AcOH) (40 mL), concentrated HCl (80 mL) was added and the mixture was refluxed for 12 h. After cooling to room temperature, the reaction mixture was evaporated to dryness. The resulting residue was diluted with CH_2Cl_2 (50 mL), followed by addition of saturated NaHCO3 solution (50 mL). After removal of organic layer, CH_2Cl_2 (30 mL) was added, and the aqueous layer was adjusted to acidity (pH 1) with 6 M HCl. The organic layer was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, and evaporated to dryness. The resulting precipitate was collected to yield the carboxylic acid (precursor of **15**) (92%).

5.2.2.5. General procedure for preparation of the sodium salts of compounds. To a solution of the carboxylic acid (precursor of **15**) in EtOH (30 mL), 1 M NaOH solution (1.0 equiv, ca. 2.2 mmol) was added and refluxed for 2 h. After cooling to room temperature, the resulting mixture was evaporated to dryness. The precipitated product was collected, and recrystallized with ethanol/ether to yield title compounds **15**.

*5.2.2.5.1. Sodium 2-{3-bromo-4-[(2-oxocyclopentyl)methyl]phenyl}propanoate (**15**).* ^{1}H NMR (CD_3OD) δ: 1.38 (3H, d, J = 7.3 Hz, α-CH_3), 1.53–2.02 (4H, m, H3′, H4′), 2.07–2.50 (3H, m, H1′, H5′), 2.56 (1H, dd, J = 13.9, 9.3 Hz, CH_2), 3.22 (1H, dd, J = 13.6, 4.8 Hz, CH_2), 3.52 (1H, q, J = 7.2 Hz, CH), 7.15 (1H, d, J = 7.7 Hz, Ar-H5), 7.26 (1H, d, J = 7.7 Hz, Ar-H6), 7.56 (1H, s, Ar-H3). ^{13}C NMR (CD_3OD) δ: 19.83 (α-CH_3), 21.44 (C5′), 30.15 (C4′), 36.19 (CH_2), 38.80 (C3′), 49.62 (CH), 50.75 (C1′), 125.00 (Ar-C3), 128.04 (Ar-C6), 131.78 (Ar-C5), 132.88 (Ar-C2), 138.05 (Ar-C1), 145.91 (Ar-C4), 182.38 (CO_2Na), 222.37 (C=O). HR-FAB-MS (*m/z*): 369.0089 (M^++Na, calcd for $C_{15}H_{16}{}^{79}BrNa_2O_3$: 369.0078). Anal. Calcd for $C_{15}H_{16}{}^{79}BrNaO_3{\cdot}H_2O$: C, 49.33; H, 4.97. Found: C, 49.42, H, 5.05.

5.2.2.6. General procedure for the Suzuki–Miyaura cross-coupling reaction. The intermediate **14** (1.0 equiv, ca. 0.9 mmol) and each arylboronic acid (R-PhB(OH)$_2$) (1.5 equiv) were dissolved in THF (16 mL), followed by addition of 2 M Na_2CO_3 in water (3 mL) and $Pd(PPh_3)_4$ (0.03 equiv). After refluxing overnight, the reaction mixture was cooled to room temperature, and diluted with water. The mixture was extracted with AcOEt, dried over anhydrous sodium sulfate (Na_2SO_4), and filtered. The filtrate was evaporated to dryness, and the residue was purified on silica gel chromatography (*n*-hexane/AcOEt, 7:2) to yield the biphenyl compound (bis-methylester intermediate, the precursor of **16–23**) as a yellow oil (52–85%). Decarboxylation, hydrolysis by acid and sodium salt preparation of the bis-methylester intermediate (the precursor of **16–23**) was done as described above to yield **16–23**.

*5.2.2.6.1. Sodium 2-{6-[(2-oxocyclopentyl)methyl]biphenyl-3-yl}-propanoate (**16**).* Yield: 69%, three steps. IR (KBr) ν: 1423, 1712 (CO_2^-), 1730 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.32 (3H, d, J = 7.3 Hz, α-CH_3), 1.42–2.35 (6H, m, H3′, H4′, H5′), 2.38–2.50 (1H, m, H1′), 3.05 (1H, dd, J = 14.1, 3.0 Hz, CH_2), 3.14 (1H, d, J = 12.4, 3.0 Hz, CH_2), 3.48 (1H, q, J = 7.1 Hz, CH), 7.05–7.10 (3H, s, Ar-H5, Ar-H6), 7.23 (3H, m, Ar-H2′, Ar-H4′), 7.25–7.31 (2H, m, Ar-H3′), 7.47 (1H, s, Ar-H2). ^{13}C NMR (CD_3OD) δ: 19.88 (α-CH_3), 21.39 (C5′), 30.23 (C4′), 33.32 (CH_2), 36.20 (C3′), 38.79 (CH), 51.65 (C1′), 127.80 (Ar-C5), 128.07 (Ar-C4′), 129.13 (Ar-C2′), 130.44 (Ar-C6), 130.48 (Ar-C3′), 131.78 (Ar-C2), 132.93 (Ar-C1, Ar-C3), 136.08 (Ar-C4), 138.05 (Ar-C4), 143.52 (Ar-C1′), 183.38 (CO_2Na), 222.83 (C=O). HR-FAB-MS (*m/z*): 367.1289 (M^++Na, calcd for $C_{21}H_{21}Na_2O_3$: 367.1286). Anal. Calcd for $C_{21}H_{21}NaO_3{\cdot}H_2O$: C, 76.11; H, 7.00. Found: C, 76.24, H, 7.05.

*5.2.2.6.2. Sodium 2-{4′-methyl-6-[(2-oxocyclopentyl)methyl]biphenyl-3-yl}propanoate (**17**).* Yield: 70%, three steps. IR (KBr) ν: 1420, 1711 (CO_2^-), 1733 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.30–2.16 (6H, m, H3′, H4′, H5′), 1.41 (3H, d, J = 7.3 Hz, α-CH_3), 2.31 (3H, s, Ar-CH_3), 2.34–2.24 (1H, m, H1′), 2.48 (1H, dd, J = 20.5, 12.8 Hz, CH_2), 3.16 (1H, dd, J = 24.4, 13.7 Hz, CH_2), 3.64 (1H, q, J = 7.1 Hz, CH), 7.10 (1H, d, Ar-H6), 7.16–7.18 (5H, m, Ar-H5, Ar-H2′, Ar-H3′), 7.49 (1H, s, Ar-H2). ^{13}C NMR (CD_3OD) δ: 19.22 (α-CH_3), 21.33 (C5′), 30.14 (C4′), 33.30 (Ar-CH_3), 36.32 (CH_2), 38.82 (C3′), 45.79 (CH), 50.40 (C1′), 125.23 (Ar-C5), 127.84 (Ar-C6), 129.84 (Ar-C2′), 130.15 (Ar-C3′), 132.18 (Ar-C3), 132.81 (Ar-C4′), 137.42 (Ar-C2), 140.08 (Ar-C4), 142.54 (Ar-C1), 143.55 (Ar-C1′), 178.38 (CO_2Na), 222.18 (C=O). HR-FAB-MS (*m/z*): 381.1447 (M^++Na, calcd for $C_{22}H_{23}Na_2O_3$: 381.1443). Anal. Calcd for $C_{22}H_{23}NaO_3{\cdot}H_2O$: C, 76.11; H, 7.00. Found: C, 76.24, H, 7.05.

*5.2.2.6.3. Sodium 2-{4′-methoxy-6-[(2-oxocyclopentyl)methyl]-biphenyl-3-yl}propanoate (**18**).* Yield: 75%, three steps. IR (KBr) ν: 1416, 1713 (CO_2^-), 1729 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.21–2.04 (6H, m, H3′, H4′, H5′), 1.41 (3H, d, J = 7.0 Hz, α-CH_3), 2.07–2.22 (1H, m, H1′), 2.39 (1H, dd, J = 14.5, 3.1 Hz, CH_2), 3.15 (1H, dd, J = 14.1, 5.3 Hz, CH_2), 3.57 (1H, q, J = 7.1 Hz, CH), 2.80 (3H, s, Ar-OCH_3), 6.93 (2H, d, J = 7.1 Hz, Ar-H3′), 7.15 (1H, d, J = 7.7 Hz, Ar-H6), 7.17 (1H, s, Ar-H2), 7.19 (2H, d, J = 6.2 Hz, Ar-H2′), 7.27 (1H, dd, J = 8.1, 1.8 Hz, Ar-H5). ^{13}C NMR (CD_3OD) δ: 18.96 (α-CH_3), 21.46 (C5′), 30.27 (C4′), 33.69 (CH_2), 38.56 (C3′), 46.19 (CH), 51.45 (C1′), 55.79 (Ar-OCH_3), 114.85 (Ar-C3′), 115.44 (Ar-C5), 127.34 (Ar-C6), 130.53 (Ar-C2′), 131.37 (Ar-C1′), 137.68

(Ar-C2), 140.20 (Ar-C1), 143.44 (Ar-C3), 157.64 (Ar-C4), 160.28 (Ar-C4′), 178.51 (CO_2Na), 222.83 (C=O). HR-FAB-MS (*m/z*): 397.1389 (M^++Na, calcd for $C_{22}H_{23}Na_2O_4$: 397.1392). Anal. Calcd for $C_{22}H_{23}NaO_4 \cdot 0.5H_2O$: C, 68.92; H, 6.31. Found: C, 68.88, H, 6.25.

*5.2.2.6.4. Sodium 2-{4′-(methylthio)-6-[(2-oxocyclopentyl)met]-hylbiphenyl-3-yl}propanoate (**19**).* Yield: 74%, three steps. IR (KBr) *ν*: 1417, 1711 (CO_2^-), 1731 (C=O), cm $^{-1}$. ^{1}H NMR (CD_3OD) *δ*: 1.24–2.22 (7H, m, H1′, H3′, H4′, H5′), 1.41 (3H, d, *J* = 7.0 Hz, α-CH_3), 2.40 (1H, dd, *J* = 13.9, 10.3 Hz, CH_2), 2.49 (3H, s, Ar-SCH_3), 3.15 (1H, dd, *J* = 13.9, 4.4 Hz, CH_2), 3.57 (1H, q, *J* = 7.1 Hz, CH), 7.15–7.23 (4H, m, Ar-H5, Ar-H6, Ar-H3′), 7.27–7.30 (3H, m, Ar-H2, Ar-H2′). ^{13}C NMR (CD_3OD) *δ*: 15.74 (Ar-SCH_3), 19.96 (α-CH_3), 21.31 (C5′), 30.33 (C4′), 33.37 (CH_2), 38.71 (C3′), 49.91 (CH), 51.66 (C1′), 127.32 (Ar-C3′), 127.78 (Ar-C5), 130.46 (Ar-C1), 130.54 (Ar-C6), 130.94 (Ar-C2′), 136.18 (Ar-C3), 138.58 (Ar-C2), 140.23 (Ar-C1′), 142.54 (Ar-C4), 143.41 (Ar-C4′), 183.03 (CO_2Na), 222.83 (C=O). HR-FAB-MS (*m/z*): 413.1169 (M^++Na, calcd for $C_{22}H_{23}Na_2SO_3$: 413.1163). Anal. Calcd for $C_{22}H_{23}NaSO_3 \cdot 0.5H_2O$: C, 66.15; H, 6.06. Found: C, 66.28, H, 6.05.

*5.2.2.6.5. Sodium 2-{4′-fluoro-6-[(2-oxocyclopentyl)methyl]biphenyl-3-yl}propanoate (**20**).* Yield: 68%, three steps. IR (KBr) *ν*: 1203 (Ar-F), 1410, 1709 (CO_2^-), 1730 (C=O), cm $^{-1}$. ^{1}H NMR (CD_3OD) *δ*: 1.23–2.23 (7H, m, H1′, H3′, H4′, H5′), 1.41 (3H, d, *J* = 7.3 Hz, α-CH_3), 2.39 (1H, dd, *J* = 14.1, 10.1 Hz, CH_2), 3.13 (1H, dd, *J* = 14.1, 4.2 Hz, CH_2), 3.58 (1H, q, *J* = 7.2 Hz, CH), 7.07–7.19 (4H, m, Ar-H5, Ar-H6, Ar-H3′), 7.25–7.31 (3H, m, Ar-H2, Ar-H2′). ^{13}C NMR (CD_3OD) *δ*: 19.95 (α-CH_3), 21.31 (C5′), 30.32 (C4′), 33.32 (CH_2), 38.68 (C3′), 49.91 (CH), 51.66 (C1′), 115.83 (d, J_{C-F} = 21.1 Hz, Ar-C3′), 127.97 (Ar-C5), 130.54 (d, J_{C-F} = 3.7 Hz, Ar-C2′), 132.19 (Ar-C6), 132.29 (Ar-C2), 132.29 (Ar-C1), 139.60 (d, J_{C-F} = 3.1 Hz, Ar-C1′), 142.06 (Ar-C3), 143.46 (Ar-C4), 164.98 (Ar-C4′), 183.02 (CO_2Na), 222.67 (C=O). HR-FAB-MS (*m/z*): 385.1199 (M^++Na, calcd for $C_{21}H_{20}FNa_2O_3$: 385.1192). Anal. Calcd for $C_{21}H_{20}FNaO_3 \cdot H_2O$: C, 66.31; H, 5.83. Found: C, 66.28, H, 5.99.

*5.2.2.6.6. Sodium 2-{6-[(2-oxocyclopentyl)methyl]-4′-(trifluoromethoxy)biphenyl-3-yl}propanoate (**21**).* Yield: 54%, three steps. IR (KBr) *ν*: 1422, 1709 (CO_2^-), 1731 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) *δ*: 1.35–2.23 (7H, m, H1′, H3′, H4′, H5′), 1.41 (3H, d, *J* = 7.3 Hz, α-CH_3), 2.41 (1H, dd, *J* = 7.1, 3.5 Hz, CH_2), 3.14 (1H, dd, *J* = 14.1, 5.7 Hz, CH_2), 3.58 (1H, q, *J* = 7.1 Hz, CH), 7.19–7.40 (7H, m, Ar-H2, Ar-H5, Ar-H6, Ar-H2′, Ar-H3′). ^{13}C NMR (CD_3OD) *δ*: 19.95 (α-CH_3), 21.30 (C5′), 30.34 (C4′), 33.24 (CH_2), 38.66 (C3′), 49.91 (CH), 51.69 (C1′), 121.70 (d, *J* = 1.2 Hz, Ar-C3′), 128.24 (Ar-C5), 129.27 (Ar-OCF_3), 130.43 (Ar-C6), 130.61 (Ar-C1′), 132.19 (Ar-C2′), 136.16 (Ar-C2), 141.64 (Ar-C1), 142.63 (Ar-C3), 143.61 (Ar-C4), 149.48 (d, *J* = 1.2 Hz, Ar-C4′), 183.02 (CO_2Na), 222.57 (C=O). HR-FAB-MS (*m/z*): 451.1112 (M^++Na, calcd for $C_{22}H_{20}F_3Na_2O_4$: 451.1109). Anal. Calcd for $C_{22}H_{20}F_3NaO_4 \cdot H_2O$: C, 59.19; H, 4.97. Found: C, 59.22, H, 5.00.

*5.2.2.6.7. Sodium 5′-(1-carboxylatoethyl)-2′-[(2-oxocyclopentyl)]-methyl]biphenyl-4-carboxylate (**22**).* Yield: 81%, three steps. IR (KBr) *ν*: 1424, 1690, 1720 (CO_2^-), 1728 (C=O), cm $^{-1}$. ^{1}H NMR (CD_3OD) *δ*: 1.18–2.17 (7H, m, H1′, H3′, H4′, H5′), 1.38 (3H, d, *J* = 7.1 Hz, α-CH_3), 2.38 (1H, dd, *J* = 14.5, 10.1 Hz, CH_2), 3.11 (1H, dd, *J* = 14.1, 5.1 Hz, CH_2), 3.55 (1H, q, *J* = 7.0 Hz, CH), 7.15–7.28 (5H, m, Ar-H2, Ar-H5, Ar-H6, Ar-H2′), 7.95 (2H, dd, *J* = 6.5, 1.9 Hz, Ar-H3′). ^{13}C NMR (CD_3OD) *δ*: 19.02 (α-CH_3), 21.30 (C5′), 30.39 (C4′), 33.32 (CH_2), 38.58 (C3′), 46.16 (CH), 51.61 (C1′), 128.17 (Ar-C5), 130.06 (Ar-C6), 130.59 (Ar-C2′), 130.62 (Ar-C3′), 130.75 (Ar-C2), 131.19 (Ar-C1), 137.46 (Ar-C3), 140.53 (Ar-C4), 142.70 (Ar-C4′), 147.85 (Ar-C1′), 169.66 (Ar-CO_2Na), 178.18 (CO_2Na), 222.35 (C=O). HR-FAB-MS (*m/z*): 433.1002 (M^++Na, calcd for $C_{22}H_{20}Na_3O_5$: 433.1004). Anal. Calcd for $C_{22}H_{20}Na_2O_5 \cdot 2H_2O$: C, 59.19; H, 5.42. Found: C, 59.31, H, 5.27.

*5.2.2.6.8. Sodium 2-{4′-hydroxy-6-[(2-oxocyclopentyl)methyl]]-biphenyl-3-yl}propanoate (**23**).* Yield: 47%, three steps. IR (KBr) *ν*: 1316 (Ar-OH), 1422, 1714 (CO_2^-), 1733 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) *δ*: 1.36–2.23 (7H, m, H1′, H3′, H4′, H5′), 1.40 (3H, d, *J* = 6.6 Hz, α-CH_3), 2.40 (1H, dd, *J* = 13.9, 10.3 Hz, CH_2), 3.15 (1H, dd, *J* = 13.9, 4.4 Hz, CH_2), 3.56 (1H, q, *J* = 6.8 Hz, CH), 6.80 (2H, dd, *J* = 6.6, 2.2 Hz, Ar-H3′), 7.17–7.07 (4H, m, Ar-H2, Ar-H6, Ar-H2′), 7.24 (1H, dd, *J* = 7.7, 1.8 Hz, Ar-H5). ^{13}C NMR (CD_3OD) *δ*: 19.97 (α-CH_3), 21.30 (C5′), 30.27 (C4′), 33.45 (CH_2), 38.77 (C3′), 50.00 (CH), 51.61 (C1′), 115.90 (Ar-C3′), 127.34 (Ar-C5), 130.43 (Ar-C6), 130.66 (Ar-C1′), 131.45 (Ar-C2′), 134.68 (Ar-C2), 136.29 (Ar-C1), 143.09 (Ar-C3), 143.28 (Ar-C4), 157.41 (Ar-C4′), 183.24 (CO_2Na), 223.09 (C=O). HR-FAB-MS (*m/z*): 360.1332 (M^++Na, calcd for $C_{21}H_{21}Na_2O_4$: 360.1338). Anal. Calcd for $C_{21}H_{21}NaO_4 \cdot H_2O$: C, 66.66; H, 6.13. Found: C, 66.58, H, 6.11.

5.2.3. Synthesis of loxoprofen derivatives with modification at the 2-position of the phenyl ring by para-substituted aryl group (24–31)

A carboxy group of 2-{2-bromo-4-[(2-oxocyclopentyl)methyl]phenyl}propanoic acid was methyl esterificated to give methyl 2-{2-bromo-4-[(2-oxocyclopentyl)methyl]phenyl}propanoate (see below), which was then reacted with corresponding arylboronic acid under the conditions of Suzuki–Miyaura coupling reaction, as described above. The resulting biphenyl compounds were hydrolyzed by base (see below), and converted to the sodium salt by the same procedure described above.

5.2.3.1. Methyl ester protection of the carboxy group of 2-{2-bromo-4-[(2-oxocyclopentyl)methyl]phenyl}propanoic acid. To 2-{2-bromo-4-[(2-oxocyclopentyl)methyl]phenyl}propanoic acid (1.5 equiv, ca. 3.3 mmol) in CH_2Cl_2 (30 mL) and methanol (2 equiv, ca. 4.4 mmol), DMAP (1 equiv, ca. 2.2 mmol) and EDC (2 equiv, ca. 4.4 mmol) were added, followed by stirring for 15 min at room temperature. The reaction mixture was poured into cold water, and the resulting solution was extracted with CH_2Cl_2. Evaporation of the solvent and purification of the residue by silica gel chromatography (*n*-hexane/AcOEt, 3:1) yielded methyl 2-{2-bromo-4-[(2-oxocyclopentyl)methyl]phenyl}propanoate as a colorless oil (92%).

5.2.3.2. General procedure for alkaline hydrolysis. To the methylester intermediate (biphenyl compound from **4b**) (ca. 5 mmol) in ethanol (20 mL), 0.063 mM aqueous solution of KOH (5 mL) was added and refluxed for 2 h. After cooling to room temperature, the reaction mixture was evaporated to dryness. The resulting residue was diluted with CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ solution (50 mL) was added. The organic layer was removed, CH_2Cl_2 (30 mL) was added, and the aqueous layer was adjusted to acidity (pH 1) with 6 M HCl. The organic layer was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, and evaporated to dryness. The resulting precipitate was collected to yield the precursor of **24–31** (90–94%).

5.2.3.3. Sodium 2-{5-[(2-oxocyclopentyl)methyl]biphenyl-2-yl}propanoate (24). Yield: 74%, three steps. IR (KBr) *ν*: 1422, 1713 (CO_2^-), 1731 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) *δ*: 1.23 (3H, dd, *J* = 7.3, 1.5 Hz, α-CH_3), 1.56–2.44 (7H, m, H1′, H3′, H4′, H5′), 2.53 (1H, dd, *J* = 13.6, 9.2 Hz, CH_2), 3.05 (1H, d, *J* = 13.7, 4.2 Hz, CH_2), 3.71 (1H, q, *J* = 7.2 Hz, CH), 6.94 (1H, s, Ar-H3), 7.10 (1H, d, *J* = 8.1 Hz, Ar-H5), 7.32–7.39 (5H, m, Ar-H2′, Ar-H3′, Ar-H4′), 7.46 (1H, d, *J* = 8.1 Hz, Ar-H6). ^{13}C NMR (CD_3OD) *δ*: 21.28 (α-CH_3), 21.47 (C5′), 30.10 (C4′), 36.08 (CH_2), 39.10 (C3′), 45.71 (CH), 52.13 (C1′), 127.72 (Ar-C1), 128.67 (Ar-C4′), 128.99 (Ar-C2′), 129.04 (Ar-C3′), 130.67 (Ar-C6), 131.29 (Ar-C3), 138.38 (Ar-C2), 141.56 (Ar-C4), 143.12 (Ar-C4), 143.49 (Ar-C1′), 183.50 (CO_2Na), 223.14 (C=O). HR-FAB-MS (*m/z*): 367.1291 (M^++Na, calcd for