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トランスクリプトーム解析を利用した医薬品の副作用発症機構の解明と、それに基づいた副作用予測システム、副作用治療法、及び副作用の少ない新薬の開発戦略の確立

平成 23 年度　総括研究報告書

研究代表者　水島　徹

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トランスクリプトーム解析を利用した医薬品の副作用発症機構の解明と、それに基づいた副作用予測システム、副作用治療法、及び副作用の少ない新薬の開発戦略の確立

研究代表者　水島　徹　慶應義塾大学薬学部教授

研究要旨

平成23年度我々はゲフィチニブによるHSP70減少機構を解析し、ゲフィチニブがある種のmiRNAの発現を誘導し、HSP70の翻訳を抑制するというメカニズムを明らかにした。またHSP70を増やすことが知られている胃薬・ゲラニルゲラニルアセトン（GGA）をマウスに投与したところ、ゲフィチニブ依存の肺線維化、及びHSP70の発現抑制が見られなくなることを見出した。

一方我々は、保有する既存薬ライブラリーからHSP70の発現を抑制するものを検索し、それらがマウスで肺線維化を起こすかを検討した。その結果、10数種の既存薬がHSP70の発現を抑制すると同時に肺線維化を起こすことを見出した。

A. 研究目的

既存薬による副作用発症機構が理解されていないため、基礎研究段階で新薬候補品の副作用を予測出来ずに（臨床試験で初めて副作用が発見され）、臨床試験が失敗している。そこで本研究で我々は、トランスクリプトーム解析を基に医薬品の副作用発症機構を解明し、新薬候補品の副作用を予測するシステムを確立する。また、副作用の少ない新薬の開発や副作用治療法の確立も目指す。以下に、我々のこれまでの研究成果を述べる。

アスピリンを代表とするNSAIDは優れた抗炎症薬として世界中でよく使用されているが、その胃潰瘍副作用（NSAID潰瘍）が臨床現場で大きな問題になっている（米国では年間16500人が亡くなっている）。我々はNSAIDが誘導する遺伝子を網羅的に解析し（トランスクリプトーム解析）、NSAIDが膜傷害性を持つこと、及びこれがNSAID潰瘍の原因であることを見出した。この成果を受けて製薬企業は、膜傷害性を指標に新薬候補品の胃潰瘍副作用を予測するスクリーニングを開始している。また我々は、膜傷害性の少ないNSAIDの合成に世界で初めて成功し、それらが充分な抗炎症作用を示すにも関わらず、ほとんど胃潰瘍を起こさないことを見いだした（現在、製薬企業で開発中）。

また我々は、間質性肺炎副作用が問題になっているレフルノミドに関しても、トランスクリプトーム解析を行った。その結果、レフルノミドが上皮間葉転換（EMT）を起こすこと、及びこれが間質性肺炎副作用の原因であることを明らかにした。一部の製薬企業では、EMT誘導を指標に新薬候補品の間質性肺炎副作用を予測するスクリーニングを開始している。また我々は、このEMT誘導を抑制する薬剤の肺内投与が、レフルノミド依存のマウス間質性肺炎を抑制することを見出した（副作用治療法の確立に繋がる成果）。

以上の成果を受けて本研究で我々は、他の薬剤による間質性肺炎副作用（平成23-24年度実施）、及び薬疹など他の副作用（平成24-25年度実施）に関して、トランスクリプトーム解析を用いて副作用発症機構を解明し、新薬候補品の副作用を予測するシステムを確立すると共に、副作用の少ない新薬の開発、及び副作用治療法の確立も目指す。

B. 研究方法

（1）ゲフィチニブの間質性肺炎副作用に関する研究

ゲフィチニブ（イレッサ）の間質性肺炎（肺繊維症）副作用による死亡者は多く、社会問題になっている。一方、ある種の肺癌治療にはこの医薬品が必要不可欠であり、その治療法の確立、及び副作用の少ないゲフィチニブ誘導体（改良薬）の開発が急務になっている。

最近我々は、ゲフィチニブによる遺伝子発現変化の網羅的解析から、ゲフィチニブが熱ショックタンパク質（HSP）70（強力な細胞保護作用と抗炎症作用を持つ）の発現を強く抑制することを発見した。また我々はマウスを用いて、ゲフィチニブ依存に肺繊維化を起こす系（薬剤性間質性肺炎の動物モデル）を確立し、このモデルにおいてゲフィチニブ依存にHSP70の発現が抑制されること、及びHSP70過剰発現マウス（ゲフィチニブによるHSP70発現抑制が起こらないマウス）では、ゲフィチニブ依存の肺繊維化も見られないことを見出した。以上の結果は、ゲフィチニブはHSP70の発現を抑制することにより、間質性肺炎（肺繊維症）を起こすことを示唆している。そこで以下に述べる研究を行う。

①HSP70に着目した新薬候補品の間質性肺炎副作用予測システムの確立（平成23年度実施）

我々が保有する既存薬ライブラリーからHSP70の発現を抑制するものを検索し、それらがマウスで肺線維化を起こすかを検討した。肺線維化を起こした既存薬（10数種）に関して、症例報告や副作用データベースを用いて、間質性肺炎副作用の有無を調べた。その結果、複数の既存薬に関して間質性肺炎副作用報告があり、HSP70発現抑制作用を調べることが間質性肺炎副作用の予測システムとして有用であることを示唆した。今後、製薬企業にこのシステムの導入を促す（目標：少なくとも3社）。

②HSP70誘導薬による、ゲフィチニブ依存性間質性肺炎治療法の確立（平成23年度実施）

上述の結果は、HSP70 誘導薬がゲフィチニブ依存の間質性肺炎治療に有効であることを示唆している。我々は日本で最もよく使われている胃薬・ゲラニルゲラニルアセトン（GGA、商品名セルベックス）が HSP70 を誘導することを報告している（***JBC***, 2007, 2009, 2010 など）。そこで我々が確立した動物モデルを用いて、ゲフィチニブ依存性間質性肺炎治療薬としての GGA の有効性を検討したところ、GGA 投与によりゲフィチニブ依存の肺繊維化、及び HSP70 の発現抑制が見られなくなることを見出した。今後臨床研究へ繋げるための準備を行う。(GGA は既に臨床で使われているので、すぐに臨床研究を行うことが出来る)。(目標：25 年度中の臨床研究開始)

③間質性肺炎副作用の少ないゲフィチニブ誘導体の発見（平成 24 年度実施予定）

数多くのゲフィチニブ誘導体を合成しその中から、試験管内で HSP70 発現抑制効果がなく、かつゲフィチニブと同程度の癌細胞増殖抑制効果を有するものを選択する。次に動物実験を行い、ゲフィチニブと同程度の抗癌作用を持ち、かつ肺繊維化を起こさないものを選択する。特許を取得したのち、間質性肺炎副作用の少ないゲフィチニブ改良薬としての開発を製薬企業へ提案する。（目標：25 年度中の特許出願）。

（２）他の薬剤性間質性肺炎に関する研究（平成 24 年度実施予定）。

レフルノミドやゲフィチニブ以外にも、抗癌剤（イマチニブなど）、抗リウマチ薬（メトトレキサートなど）、漢方薬（小紫胡湯など）が間質性肺炎を起こすことが知られているが、その発症機構は分かっていない。そこで、これらの薬剤による遺伝子発現変化の網羅的解析（トキシコゲノミックス・データベース等を利用する）からその副作用発症機構を解明する。

また上述のゲフィチニブの場合と同様の方法で、新薬候補品の副作用を予測するシステムの確立、副作用治療法の確立、副作用の少ない誘導体の発見を目指す。（目標：少なくとも２薬剤の副作用機構の解明）

（３）他の副作用に関する研究（平成 25 年度実施予定）

抗脂血症薬による横紋筋融解症、抗てんかん薬による薬剤性過敏症（薬疹）、抗生物質によるスティーブンス・ジョンソン症候群、糖尿病薬による肝障害などに関しても、副作用発症機構を解明し、新薬候補品の副作用を予測するシステムを確立すると共に、副作用治療法の開発、及び副作用の少ない誘導体の発見を目指す。（目標：少なくとも２薬剤の副作用機構の解明）

C. 研究結果

本研究の一年目（平成 23 年度）我々は、ゲフィチニブ（イレッサ）の間質性肺炎（肺線維症）副作用に関する研究を主に行った。我々は、ゲフィチニブによる遺伝子発現変化の網羅的解析から、ゲフィチニブが熱ショックタンパク質（HSP）70 の発現を強く抑制することを発見した。またマウスを用いてゲフィチニブ依存に肺線維化を起こす系（薬剤性間質性肺炎の動物モデル）を確立し、このモデルにおいてゲフィチニブ依存に HSP70 の発現が抑制されること、及び HSP70 過剰発現マウスでは、ゲフィチニブ依存の肺繊維化も見られないことを見出していた。平成 23 年度我々はこのゲフィチニブによる HSP70 減少機構を解析し、ゲフィチニブがある種の miRNA の発現を誘導し、HSP70 の翻訳を抑制するというメカニズムを明らかにした。また HSP70 を増やすことが知られている胃薬・ゲラニルゲラニルアセトン（GGA）をマウスに投与したところ、ゲフィチニブ依存の肺線維化、及び HSP70 の発現抑制が見られなくなることを見出した（***PLoS ONE*** 2011）。以上の結果はこれまで未解明であったゲフィチニブによる薬剤性間質性肺炎の発症機構を明らかにしただけでなく、その治療法を示唆して点において重要である。GGA は既に臨床で使われており安全性が担保されている。そこで、比較的容易に臨床研究を行うことが出来るので、今後その準備を行う。尚、この研究は NHK のニュースや朝日新聞、日経新聞などの新聞で全国報道され、大きな反響を呼んだ。特に、患者さんからの電話での問い合わせは 100 件を超え、臨床研究への移

行に対する患者さんの高い期待を感じた。

一方我々は、保有する既存薬ライブラリーからHSP70の発現を抑制するものを検索し、それらがマウスで肺線維化を起こすかを検討した。その結果、10数種の既存薬がHSP70の発現を抑制すると同時に肺線維化を起こすことを見出した。またその内複数の既存薬に関しては、間質性肺炎副作用の臨床報告があった。以上の結果から、HSP70発現抑制作用を調べることが間質性肺炎副作用の予測システムとして有用であると考え、現在そのような提案を製薬企業に対して行っている。

平成23年度は上述の研究以外にも、抗癌剤（イマチニブなど）、抗リウマチ薬（メトトレキサートなど）、漢方薬（小柴胡湯など）などの間質性肺炎発症機構を解明するために、これらの薬剤が誘導する遺伝子の網羅的な解析を行い（トランスクリプトーム解析）、興味深い知見を数多く得たので次年度の研究に繋げたい。

D.考察

結果の欄に記載した。

E.結論

結果の欄に記載した。

F.健康危険情報

該当なし

G.研究発表

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32 水島徹　NSAID 潰瘍発症機構とその対策　日本消化器病学会招待講演　(2012)（東京）

33 Tohru Mizushima PC-SOD, as a drug for IPF. Invited lecture in Asan Medical Center (2012) (Seoul)

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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

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Tanaka, K., Tanaka, Y., Suzuki, T. and Mizushima, T.	Protective Effect of *b*-(1,3-1,6)-D-glucan against irritant-induced gastric lesions.	***Br. J. Nutr.***	106	475-485.	2011
Hoshino, T., Murao, N., Namba, T., Takehara, M., Adachi, H., Katsuno, M., Sobue, G., Matsushima, T., Suzuki, T. and Mizushima, T.	Suppression of Alzheimer's disease-related phenotypes by expression of heat shock protein 70 in mice.	***J. Neurosci.***	31	5225-5234.	2011
Takehara, M., Hoshino, T., Namba, T., Yamakawa, N. and Mizushima, T.	Acetaminophen-induced differentiation of human breast cancer stem cells and inhibition of tumor xenograft growth in mice	***Biochem. Pharmacol.***	81	1124-1135.	2011
Mizushima, T.	Drug discovery and development focusing on existing medicines: Drug re-profiling strategy.	***J.Biochem.***	49	499-505.	2011
Tanaka, K., Tanaka, Y., Miyazaki, Y., Namba, T., Sato, K., Aoshiba, K., Azuma, A. and Mizushima, T.	Therapeutic effect of lecithinized superoxide dismutase on pulmonary emphysema.	***J. Pharmacol. Exp. Ther.***	338	810-818.	2011
Ishihara, T., Suemasu, S., Asano, T., Tanaka, K. and Mizushima, T.	Stimulation of gastric ulcer healing by heat shock protein 70.	***Biochem. Pharmacol***	82	728-736.	2011
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Hoshino, T., Namba, T., Takehara, M., Murao, N., Sugimoto, Y., Narumiya, S., Matsushima, T., Suzuki, T. and Mizushima, T.	Improvement of cognitive function in Alzheimer's disease model mice by genetic and pharmacological inhibition of the EP_4 receptor.	***J. Neurochem.***	120	795-805.	2012
Yamashita, Y., Ikeda, T., Matsuda, M., Maji, D., Hoshino, T. and Mizushima, T.	purification and characterization of hsp-inducers from *eupatorium lindleyanum.*	***Biochem. Pharmacol..***	82	909-922.	2012

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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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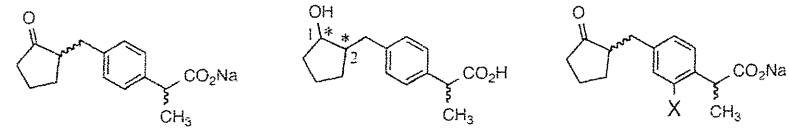


Figure 1. Structure of loxoprofen sodium and its derivatives.

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

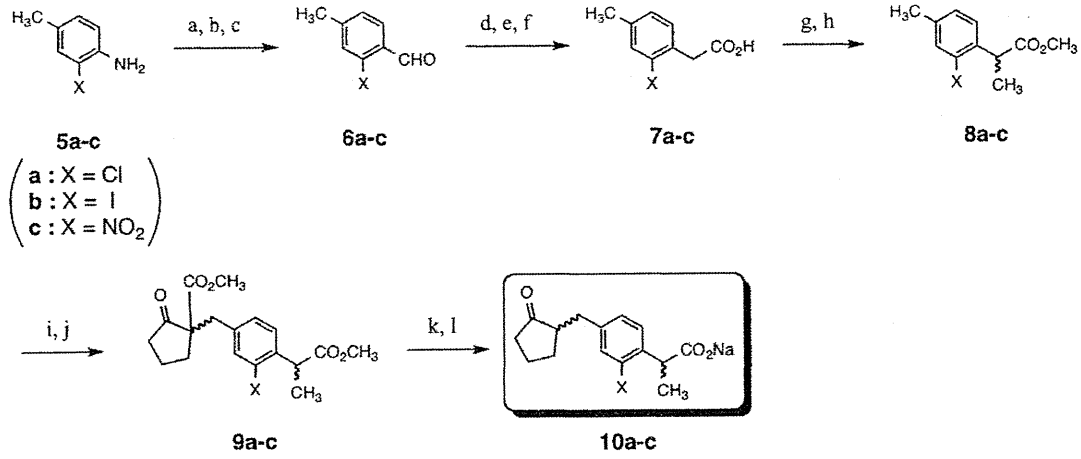
Loxoprofen derivatives with modification at the 2-position of the phenyl ring by halogens and the nitro group **10a–c** were obtained by the method described previously[25] (Scheme 1).

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).

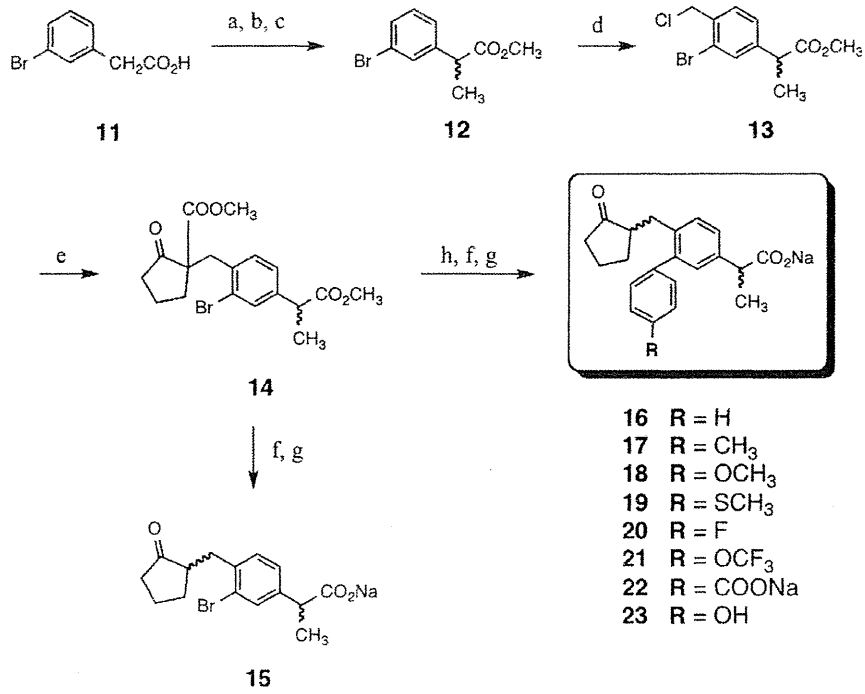
The synthetic route for target compounds **16–23** is outlined in Scheme 2. The commercially available (3-bromophenyl)acetic acid **11** was converted to the methyl 2-(3-bromophenyl)propanoate **12** by methyl esterification and α-methylation. Friedel–Crafts chloromethylation of **12** under Lewis acid 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**.

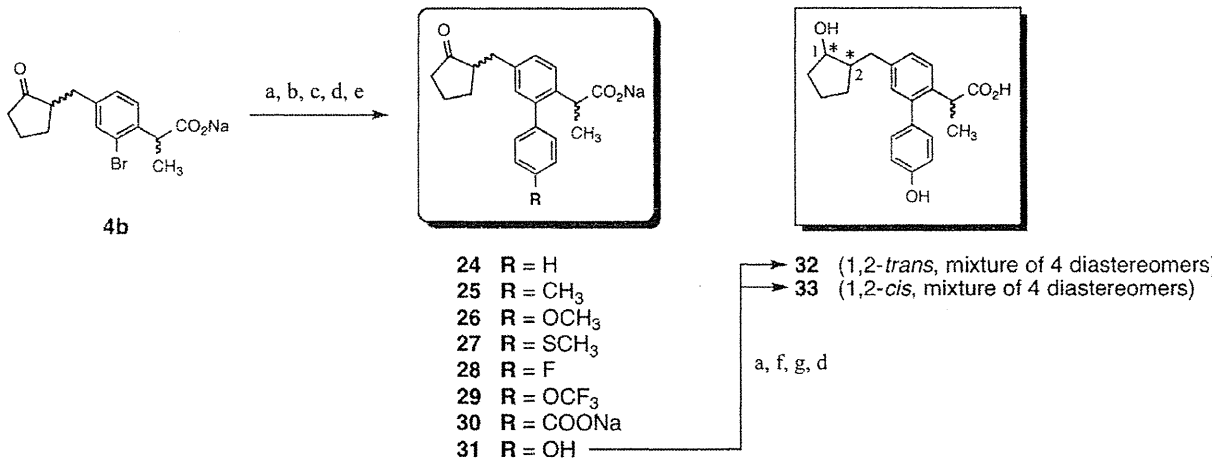
The synthetic route for target compounds **24–31** is outlined in Scheme 3. A key intermediate **4b** was prepared, as described previously.[25] The methyl ester of **4b** was prepared by treatment with methanol in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *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-C6H4-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-C6H4-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

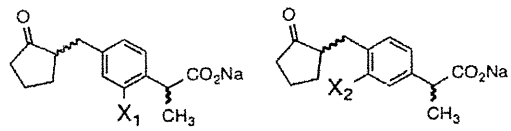
rings. We previously reported that two of the compounds, 2-fluoroloxoprofen **4a** and 2-bromoloxoprofen **4b** (Fig. 1), have lower membrane permeabilization activity than **1**.[25] In this study, we examined the membrane permeabilization activities and inhibitory effects on COX-1 and COX-2 of other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

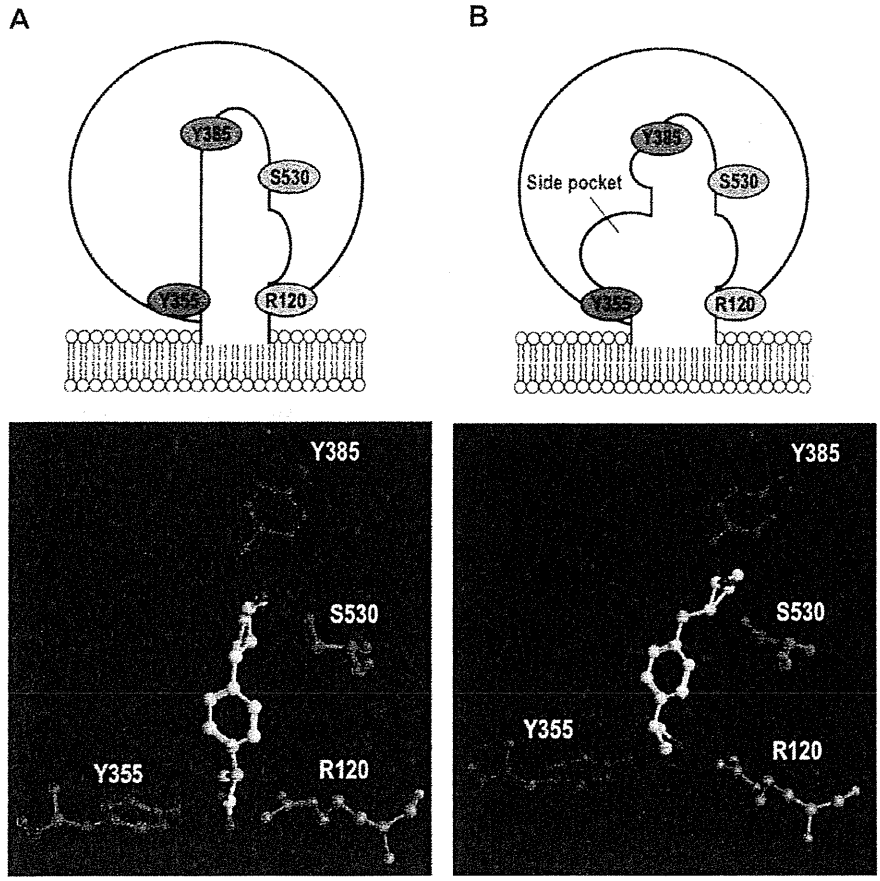


Figure 2. Potential binding mode of (*S*)-2-{4-[((1*R*, 2*S*)-2-hydroxycyclopentyl)methyl]phenyl}propanoic acid to the active site of sheep COX-1 (A) or murine COX-2 (B). Hydrogen atoms of the amino acid residues and the ligand have been removed.

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

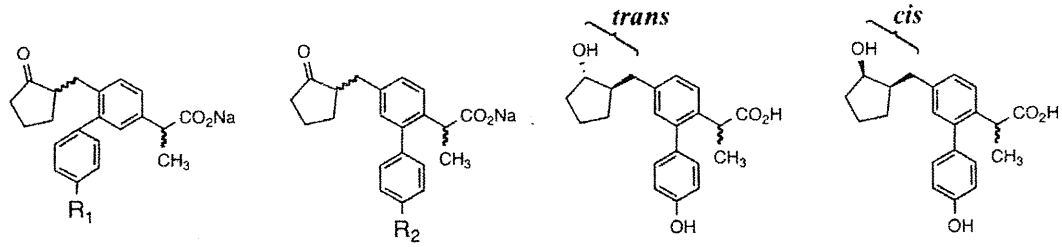
We have found that a loxoprofen derivative, **31**, administered orally to rats, produced fewer gastric lesions but provided similar anti-inflammatory effects compared to **1**. This may be due to its selectivity for COX-2, resulting in a lower propensity for the gastric level of PGE_2 to be reduced. 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]

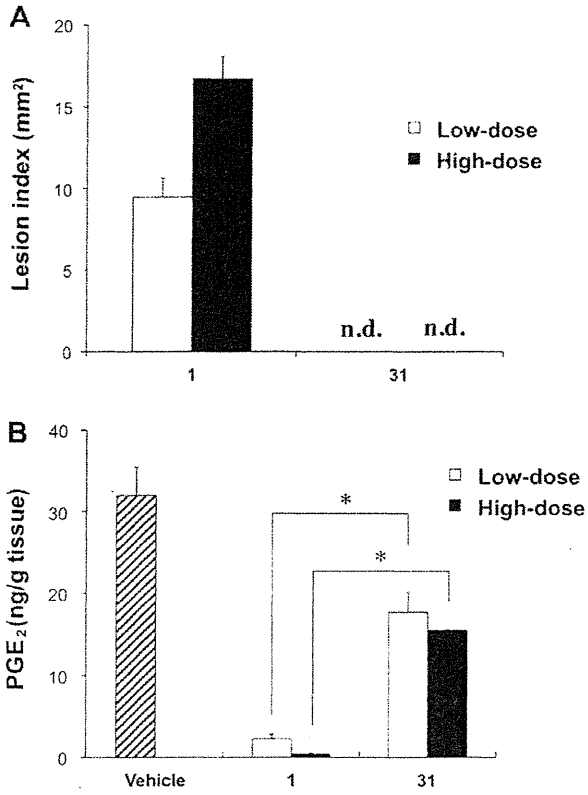


Figure 3. Production of gastric lesions and gastric PGE_2 levels in the presence of loxoprofen sodium and its derivative. Rats were orally administered a low (40 or 54 mg/kg) or high (50 or 67 mg/kg) dose of **1** or **31**, respectively, or vehicle and their stomachs were removed after 8 h. Stomachs 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.

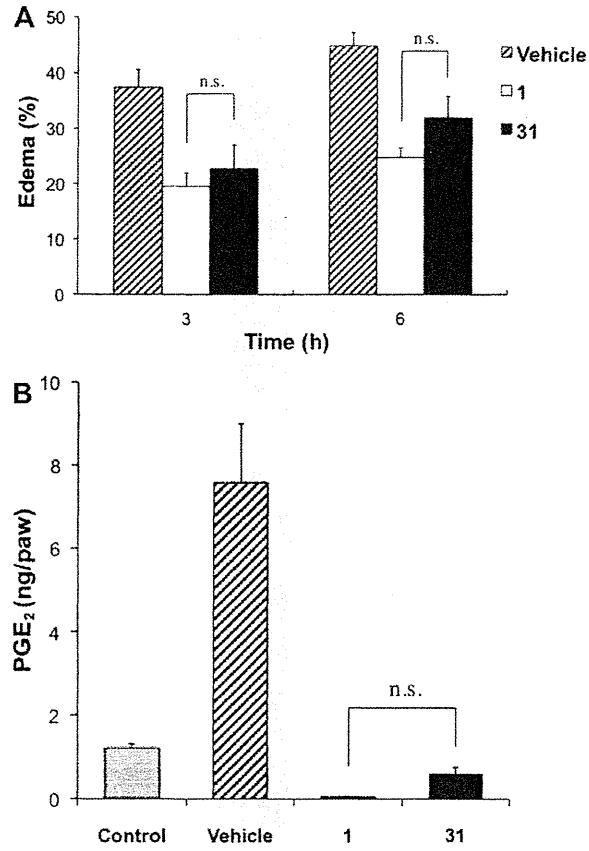


Figure 4. Anti-inflammatory activities of loxoprofen sodium and its derivative. Rats were orally administered 10 or 13 mg/kg of **1** or **31**, respectively, or vehicle and 1 h later received an intradermal injection of carrageenan (1%) into the left hindpaw. Footpad edema was measured 3 h and 6 h after 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).

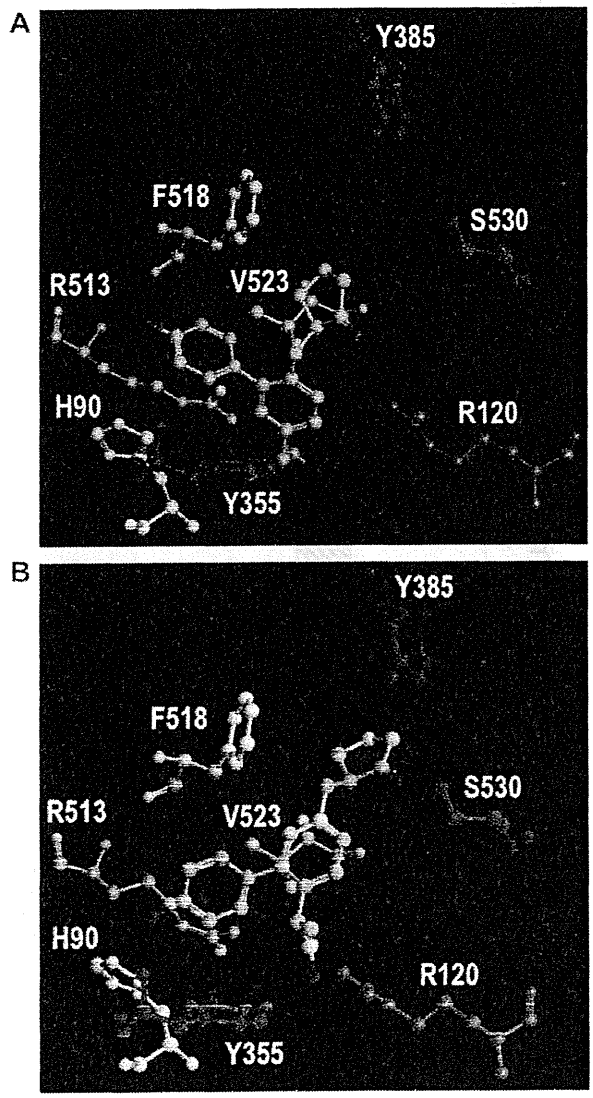


Figure 5. Potential binding mode of **23** (A) or **31** (B) to the active site of murine COX-2. Hydrogen atoms of the amino acid residues and the ligand have been removed.

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}. ^1H 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}. ^1H 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}. ^1H 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}. ^1H 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}. ^1H 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}. ^1H 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

$C_{21}H_{21}Na_2O_3$: 367.1286). Anal. Calcd for $C_{21}H_{21}NaO_3 \cdot 0.5H_2O$: C, 71.22; H, 6.36. Found: C, 71.37, H, 6.27.

5.2.3.4. Sodium 2-{4′-methyl-5-[(2-oxocyclopentyl)methyl]]biphenyl-2-yl}propanoate (25). Yield: 77%, three steps. IR (KBr) *v*: 1420, 1712 (CO_2^-), 1733 (C=O), cm $^{-1}$. ^{1}H NMR (CD_3OD) *δ*: 1.21 (3H, dd, *J* = 7.3, 1.5 Hz, α-CH_3), 1.54–2.44 (7H, m, H1′, H3′, H4′, H5′), 2.36 (3H, s, Ar-CH_3), 2.51 (1H, dd, *J* = 13.4, 9.3 Hz, CH_2), 3.04 (1H, dd, *J* = 13.7, 9.3 Hz, CH_2), 3.72 (1H, q, *J* = 7.1 Hz, CH), 6.92 (1H, t, *J* = 1.8 Hz, Ar-H3), 7.08 (1H, dt, *J* = 8.1, 1.8 Hz, Ar-H5), 7.18 (2H, d, *J* = 7.7 Hz, Ar-H3′), 7.26 (2H, d, *J* = 7.7 Hz, Ar-H2′), 7.44 (1H, d, *J* = 8.1 Hz, Ar-H6). ^{13}C NMR (CD_3OD) *δ*: 21.19 (α-CH_3), 21.47 (C5′), 21.44 (C4′), 30.08 (Ar-CH_3), 36.07 (CH_2), 39.08 (C3′), 45.70 (CH), 52.12 (C1′), 128.61 (Ar-C1), 128.80 (Ar-C5), 129.58 (Ar-C2′), 130.53 (Ar-C3′), 131.31 (Ar-C6), 137.33 (Ar-C3), 138.29 (Ar-C4′), 140.52 (Ar-C2), 141.64 (Ar-C1′), 143.09 (Ar-C4), 183.58 (CO_2Na), 223.09 (C=O). HR-FAB-MS (*m/z*): 381.1439 (M^++Na, calcd for $C_{22}H_{23}Na_2O_3$: 381.1443). Anal. Calcd for $C_{22}H_{23}NaO_3 \cdot 0.5H_2O$: C, 72.03; H, 6.66. Found: C, 71.92, H, 6.58.

5.2.3.5. Sodium 2-{4′-methoxy-5-[(2-oxocyclopentyl)methyl]biphenyl-2-yl}propanoate (26). Yield: 70%, three steps. IR (KBr) *v*: 1416, 1711 (CO_2^-), 1732 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) *δ*: 1.22 (3H, dd, *J* = 7.0, 1.5 Hz, α-CH_3), 1.53–2.40 (7H, m, H1′, H3′, H4′, H5′), 2.51 (1H, dd, *J* = 13.6, 9.5 Hz, CH_2), 3.04 (1H, dd, *J* = 13.4, 3.8 Hz, CH_2), 3.73 (1H, q, *J* = 7.0 Hz, CH), 3.81 (3H, s, Ar-OCH_3), 6.92–6.95 (3H, m, Ar-H3, Ar-H3′), 7.07 (1H, d, *J* = 8.1 Hz, Ar-H5), 7.31 (2H, d, *J* = 8.4 Hz, Ar-H2′), 7.43 (1H, d, *J* = 8.1 Hz, Ar-H6). ^{13}C NMR (CD_3OD) *δ*: 21.23 (α-CH_3), 21.45 (C5′), 30.08 (C4′), 36.08 (CH_2), 39.09 (C3′), 45.70 (CH), 52.03 (C1′), 55.72 (Ar-OCH_3), 114.43 (Ar-C2′), 128.60 (Ar-C1), 128.72 (Ar-C5), 131.44 (Ar-C6), 131.70 (Ar-C3′), 135.81 (Ar-C3), 138.29 (Ar-C4′), 141.73 (Ar-C2), 142.78 (Ar-C1′), 160.07 (Ar-C4), 183.60 (CO_2Na), 223.10 (C=O). HR-FAB-MS (*m/z*): 397.1399 (M^++Na, calcd for $C_{22}H_{23}Na_2O_4$: 397.1392). Anal. Calcd for $C_{22}H_{23}NaO_4 \cdot H_2O$: C, 67.22; H, 6.38. Found: C, 67.33, H, 6.42.

5.2.3.6. Sodium 2-(4′-(methylthio)-5-((2-oxocyclopentyl)methyl)biphenyl-2-yl)propanoate (27). Yield: 60%, three steps. IR (KBr) *v*: 1417, 1712 (CO_2^-), 1730 (C=O), cm $^{-1}$. ^{1}H NMR (CD_3OD) *δ*: 1.23 (3H, dd, *J* = 7.1, 1.3 Hz, α-CH_3), 1.55–2.45 (7H, m, H1′, H3′, H4′, H5′), 2.49 (3H, s, Ar-SCH_3), 2.52 (1H, dd, *J* = 13.9, 9.2 Hz, CH_2), 3.05 (1H, dd, *J* = 13.6, 4.0 Hz, CH_2), 3.70 (1H, q, *J* = 7.2 Hz, CH), 6.94 (1H, t, *J* = 1.8 Hz, Ar-H3), 7.10 (1H, dt, *J* = 8.1, 2.2 Hz, Ar-H5), 7.31 (4H, dd, *J* = 14.5, 8.6 Hz, Ar-H2′, Ar-H3′), 7.45 (1H, d, *J* = 8.1 Hz, Ar-H6). ^{13}C NMR (CD_3OD) *δ*: 15.91 (Ar-SCH_3), 21.21 (α-CH_3), 21.45 (C5′), 30.09 (C4′), 36.06 (CH_2), 39.06 (C3′), 45.73 (CH), 52.12 (C1′), 127.36 (Ar-C3′), 128.70 (Ar-C1), 128.98 (Ar-C5), 129.05 (Ar-C6), 131.17 (Ar-C2′), 138.33 (Ar-C3), 138.44 (Ar-C2), 140.32 (Ar-C1′), 141.61 (Ar-C4), 142.49 (Ar-C4′), 183.42 (CO_2Na), 223.01 (C=O). HR-FAB-MS (*m/z*): 413.1165 (M^++Na, calcd for $C_{22}H_{23}Na_2SO_3$: 413.1163). Anal. Calcd for $C_{22}H_{23}NaSO_3 \cdot H_2O$: C, 64.54; H, 6.10. Found: C, 64.69, H, 6.17.

5.2.3.7. Sodium 2-{4′-fluoro-5-[(2-oxocyclopentyl)methyl]biphenyl-2-yl}propanoate (28). Yield: 64%, three steps. IR (KBr) *v*: 1204 (Ar-F), 1414, 1710 (CO_2^-), 1730 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) *δ*: 1.22 (3H, dd, *J* = 7.3, 1.1 Hz, α-CH_3), 1.55–2.41 (7H, m, H1′, H3′, H4′, H5′), 2.52 (1H, dd, *J* = 13.6, 9.2 Hz, CH_2), 3.05 (1H, dd, *J* = 13.6, 4.0 Hz, CH_2), 3.64 (1H, q, *J* = 7.2 Hz, CH), 6.93 (1H, t, *J* = 1.8 Hz, Ar-H3), 7.13–7.07 (3H, m, Ar-H5, Ar-H3′), 7.38–7.46 (3H, m, Ar-H6, Ar-H2′). ^{13}C NMR (CD_3OD) *δ*: 21.08 (α-CH_3), 21.45 (C5′), 30.09 (C4′), 36.04 (CH_2), 39.06 (C3′), 45.73 (CH), 52.09 (C1′), 115.60 (d, J_{C-F} = 21.1 Hz, Ar-C3′), 128.68 (Ar-C1), 129.19 (Ar-C5), 131.32 (Ar-C6), 132.46 (d, J_{C-F} = 8.1 Hz, Ar-C2′), 138.50 (Ar-C3), 139.57 (d, J_{C-F} = 3.7 Hz, Ar-C1′), 141.64 (Ar-C2), 142.02 (Ar-C4), 142.49 (d, J_{C-F} = 1.9 Hz, Ar-C4′), 183.28 (CO_2Na), 222.98 (C=O). HR-FAB-MS (*m/z*): 385.1188 (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, 66.44, H, 5.76.

5.2.3.8. Sodium 2-{5-[(2-oxocyclopentyl)methyl]-4′-(trifluoromethoxy)biphenyl-2-yl}propanoate (29). Yield: 56%, three steps. IR (KBr) *v*: 1421, 1709 (CO_2^-), 1731 (C=O), cm $^{-1}$. ^{1}H NMR (CD_3OD) *δ*: 1.25 (3H, dd, *J* = 7.0, 1.1 Hz, α-CH_3), 1.51–2.45 (7H, m, H1′, H3′, H4′, H5′), 2.53 (1H, dd, *J* = 13.6, 9.5 Hz, CH_2), 3.05 (1H, dd, *J* = 13.6, 4.0 Hz, CH_2), 3.62 (1H, q, *J* = 7.2 Hz, CH), 6.95 (1H, t, *J* = 2.2 Hz, Ar-H3), 7.13 (1H, dt, *J* = 8.1, 2.2 Hz, Ar-H3′), 7.28 (2H, dd, *J* = 8.8, 0.7 Hz, Ar-H5,), 7.46–7.51 (3H, m, Ar-H6, Ar-H2′). ^{13}C NMR (CD_3OD) *δ*: 21.12 (α-CH_3), 21.44 (C5′), 30.08 (C4′), 35.99 (CH_2), 39.04 (C3′), 47.75 (CH), 52.04 (C1′), 121.51 (Ar-C3′), 128.77 (Ar-C1), 129.46 (Ar-C5), 131.17 (Ar-OCF_3), 131.23 (Ar-C6), 132.37 (Ar-C2′), 138.62 (Ar-C1′), 141.52 (Ar-C3), 141.57 (Ar-C2), 142.58 (Ar-C4),, 149.45 (Ar-C4′), 183.28 (CO_2Na), 222.98 (C=O). HR-FAB-MS (*m/z*): 451.1107 (M^++Na, calcd for $C_{22}H_{20}F_3Na_2O_4$: 451.1109). Anal. Calcd for $C_{22}H_{20}F_3NaO_4 \cdot 0.5H_2O$: C, 60.41; H, 4.84. Found: C, 60.34, H, 4.98.

5.2.3.9. Sodium 2′-(1-carboxylatoethyl)-5′-[(2-oxocyclopentyl)-methyl]biphenyl-4-carboxylate (30). Yield: 74%, three steps. IR (KBr) *v*: 1420, 1689, 1712 (CO_2^-), 1727 (C=O), cm $^{-1}$. ^{1}H NMR (CD_3OD) *δ*: 1.22 (3H, dd, *J* = 7.1, 1.6 Hz, α-CH_3), 1.33–2.42 (7H, m, H1′, H3′, H4′, H5′), 2.53 (1H, dd, *J* = 13.6, 9.2 Hz, CH_2), 3.06 (1H, dd, *J* = 13.6, 4.0 Hz, CH_2), 3.73 (1H, q, *J* = 7.1 Hz, CH), 6.96 (1H, st, *J* = 1.6 Hz, Ar-H3), 7.11 (1H, dt, *J* = 8.1, 1.8 Hz, Ar-H5), 7.40 (2H, d, *J* = 8.4 Hz, Ar-H2′), 7.44 (1H, d, *J* = 8.1 Hz, Ar-H6), 7.99 (2H, d, *J* = 8.4 Hz, Ar-H3′). ^{13}C NMR (CD_3OD) *δ*: 20.00 (α-CH_3), 21.45 (C5′), 30.11 (C4′), 36.06 (CH_2), 39.05 (C3′), 45.55 (CH), 52.11 (C1′), 128.64 (Ar-C1), 129.18 (Ar-C5′), 130.01 (Ar-C2′), 130.10 (Ar-C3′), 131.23 (Ar-C6), 137.37 (Ar-C3), 138.52 (Ar-C2), 141.47 (Ar-C4′), 142.81 (Ar-C4), 145.50 (Ar-C1′), 175.36 (Ar-CO_2Na), 183.17 (CO_2Na), 223.02 (C=O). HR-FAB-MS (*m/z*): 433.1001 (M^++Na, calcd for $C_{22}H_{20}Na_3O_5$: 433.1004). Anal. Calcd for $C_{22}H_{20}Na_2O_5 \cdot H_2O$: C, 61.68; H, 5.18. Found: C, 61.54, H, 5.06.

5.2.3.10. Sodium 2-{4′-hydroxy-5-[(2-oxocyclopentyl)methyl]-biphenyl-2-yl}propanoate (31). Yield: 50%, three steps. IR (KBr) *v*: 1318 (Ar-OH), 1421, 1710 (CO_2^-), 1731 (C=O), cm^{-1}. ^{1}H NMR (CD_3OD) *δ*: 1.22 (3H, dd, *J* = 7.3, 1.5 Hz, α-CH_3), 1.52–2.42 (7H, m, H1′, H3′, H4′, H5′), 2.50 (1H, d, *J* = 13.9 Hz, CH_2), 3.02 (1H, d, *J* = 13.6 Hz, CH_2), 3.75 (1H, q, *J* = 7.2 Hz, CH), 6.80 (2H, d, *J* = 8.4 Hz, Ar-H3′), 6.92 (1H, s, Ar-H3), 7.05 (1H, d, *J* = 8.1 Hz, Ar-H5), 7.21 (2H, d, *J* = 8.4 Hz, Ar-H2′), 7.42 (1H, d, *J* = 8.1 Hz, Ar-H6). ^{13}C NMR (CD_3OD) *δ*: 21.32 (α-CH_3), 30.10 (C5′), 36.10 (C4′), 39.09 (CH_2), 45.72 (C3′), 52.20 (CH), 58.31 (C1′), 116.15 (Ar-C3′), 128.40 (Ar-C1), 128.54 (Ar-C5), 131.51 (Ar-C6), 131.66 (Ar-C2′), 134.03 (Ar-C1′), 138.19 (Ar-C3), 141.76 (Ar-C2), 143.24 (Ar-C4), 158.47 (Ar-C4′), 183.81 (CO_2Na), 219.16 (C=O). HR-FAB-MS (*m/z*): 361.1414 (M^++H, calcd for $C_{21}H_{22}NaO_4$: 361.1416). Anal. Calcd for $C_{21}H_{21}NaO_4 \cdot H_2O$: C, 68.28; H, 6.00. Found: C, 68.30, H, 6.09.

5.2.4. Synthesis of the alcohol derivative of 31 (32, 33)

A methyl ester intermediate derived from **31** was reduced by $NaBH_4$ (see below) and alkaline hydrolyzed.

5.2.4.1. Reduction of methyl ester intermediate derived from 31 with $NaBH_4$. To a stirred solution of methyl ester intermediate derived from **31** (1 equiv, ca. 1.8 mmol) in EtOH, $NaBH_4$ (1.3 equiv, ca. 2.4 mmol) was added, stirred for 1 h at room temperature, quenched by the addition of a few ice chips, and the resulting solution was extracted with CH_2Cl_2. The extracts were dried over anhydrous Na_2SO_4 and filtrated. The filtrate was evaporated to