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Identification of urine metabolites of TFAP, a cyclooxygenase-1 inhibitor

Hiroki Kakuta [a,*], Ryosuke Fukai [a], Zheng Xiaoxia [a], Fuminori Ohsawa [a], Takeshi Bamba [b], Kazumasa Hirata [c], Akihiro Tai [d]

[a] Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-Naka, Kita-Ku, Okayama, Okayama 700-8530, Japan
[b] Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
[c] Department of Environmental Biotechnology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
[d] Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, Shobara, Hiroshima 727-0023, Japan

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ABSTRACT

Only a few COX-1-selective inhibitors are currently available, and the research on COX-1 selective inhibitors is not fully developed. The authors have produced several COX-1 selective inhibitors including *N*-(5-amino-2-pyridinyl)-4-trifluoromethylbenzamide: TFAP (**3**). Although **3** shows potent analgesic effect without gastric damage, the urine after administration of **3** becomes red–purple. Since the colored-urine should be avoided for clinical use, in this research we examined the cause of the colored-urine. UV–vis spectra and LC–MS/MS analyses of urine samples and metabolite candidates of **3** were performed to afford information that the main reason of the colored urine is a diaminopyridine (**4**), produced by metabolization of **3**. This information is useful to design new COX-1 selective inhibitors without colored urine based on the chemical structure of **3**.

Cyclooxygenases (COXs) are molecular targets of non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin (**1**) and ibuprofen (**2**). Three subtypes of COXs are known which are expressed differently and are controlled by each gene.[1] COX-1 exists mainly in gastric membrane and kidney constantly, and controls synthesis of prostaglandins, which play role in mucosal protection.[2] COX-2 expression is induced predominantly by inflammation.[3] COX-3 has been identified as a target molecule of acetaminophen (paracetamol).[4] Although NSAIDs have been used as convenient anti-inflammatory agents, their side effect such as gastric disturbance remains a major problem.[5,6] These background promoted to develop COX-2 selective inhibitors as new NSAIDs without gastric damages over the world. However, the interest on COX-2 inhibitors gradually dies down because some COX-2 inhibitors induced heart attack or cardiovascular problems to die frequently.[7] The other hand, COX-1 selective inhibitors were thought as one cause of gastric damage by NSAIDs,[8–10] and research on COX-1 selective inhibitors is less-advanced.

Interestingly, in 2000, Wallace et al. reported that the independent inhibition of COX-1 or COX-2 does not induce gastric disturbance but both inhibition of them does.[11] In addition, based on the report that NSAIDs which possess potent COX-1 inhibitory activity have tendency to produce analgesic effect,[12] we developed novel COX-1 selective inhibitors and reported that those compounds show analgesic effect without gastric disturbance.[13–15] One of them is TFAP (**3**) (Fig. 1).[15] The IC_{50} value of this compound against COX-1 is about 0.80 μM, while that against COX-2 is over 200 μM. This compound is also orally available, and does not show significantly gastric damage with equivalently analgesic effect to indomethacin (**1**). However, at higher doses, purple-colored urine is observed. Figure 2a shows UV–vis spectra of diluted mice urines when **3** was administered at a dosage of 300 mg/kg or not

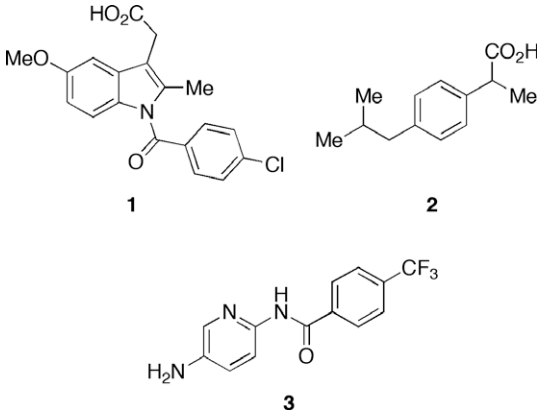

Figure 1. Chemical structures of indomethacin (**1**), ibuprofen (**2**), and TFAP (**3**) (COX-1 selective inhibitor).

* Corresponding author. Tel.: +81 (0) 86 251 7963; fax: +81 (0) 86 251 7926.
E-mail address: kakuta@pharm.okayama-u.ac.jp (H. Kakuta).

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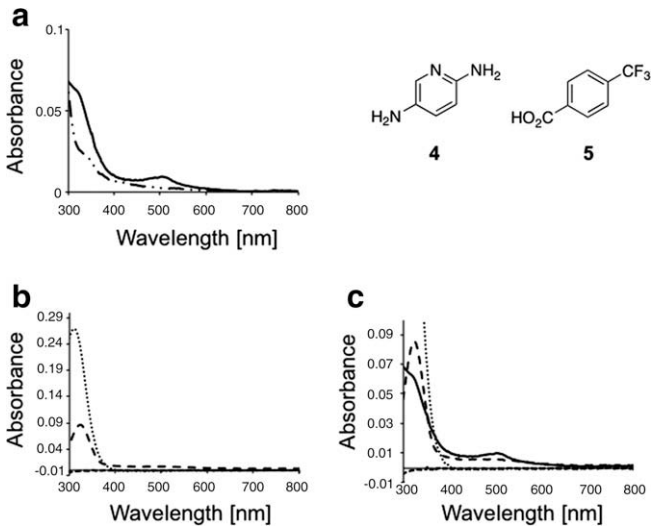


Figure 2. UV–vis spectra of (a) diluted rat urine with (line) or without (two dots and dash) administration of **3** (300 mg/kg) and (b) **3** (dots) and its metabolite candidates (**4**; long broken line and **5**; broken line), and chemical structures of **4** and **5**. Urine samples were detected after 1000-times dilution with buffer, and Compounds **3**–**5** were detected at 30 mM in buffer. Figure 2c is an enlarged figure of Figure 2b with a diluted urine sample of administration of **3**.

(vehicle).[16] Thus, in order to develop new COX-1 selective inhibitors without colored urine, we addressed the question what the cause of the colored urine is.

At first, though the cause of colored urine was thought from kidney disturbance, uric blood was not recognized (Data not shown). This result led us to focus the metabolites of **3** as a cause of the colored urine. Thus, the UV–vis spectra of expected metabolites of **3**, 2,5-diaminopyridine (DAP: **4**), 4-trifluorobenzoic acid (TFBA: **5**) were compared to that of **3** administered urine. Figure 2b shows the UV–vis spectra of each compound. The UV–vis absorption of **5** over 300 nm was not recognized, while **3** and **4** showed maximum absorption at 310 nm and 330 nm, respectively. In addition, **4** presented broad absorption from 400 nm to 600 nm (Fig. 2c). Comparing the UV–vis spectrum of diluted urine after administration of **3** to those of **3** or **4**, that of **3** administered urine is similar to that of **4**, suggesting that the colored urine by **3** is caused by **4**.

Next, these metabolites were examined using LC–MS/MS system and the metabolite candidate compounds in order to identify the urine metabolite of **3** administration. In the MS/MS analyses of **3**, **4**, and **5** standards, the fragments (*m*/*z* 92.70, 92.70, and 144.80, respectively) were observed as the major ion products. Therefore, the mass transitions were set at *m*/*z* 282.06/92.70 (**3**), 109.73/92.70 (**4**), 188.83/144.80 (**5**) for the specific detection of target compounds. The rat urine sample after 3 h of treatment with **3** was subjected to LC–MS/MS with the above conditions. The peaks derived from each compound were observed and the existence of **4** and **5** was confirmed in the urine sample (Fig. 3). However, unidentified peak at R_t 1.1 min was also observed (Fig. 3e). The unidentified peak showed $[M-H]^-$ ion at *m*/*z* 365, and its $[M-H]^-$ ion peak in a mass chromatogram at *m*/*z* 365 gave excellent agreement with a peak at R_t 1.1 min in a mass chromatogram at *m*/*z* 189 (Fig. 4a and b). As shown in Figure 4c, the major fragment ion at *m*/*z* 189 was assumed to be produced from the $[M-H]^-$ ion by elimination of a glucuronide moiety (176 amu). These data suggested that the unidentified peak was **5**-glucuronide. There is a report on a metabolite of NSAIDs in urine, which shows that their carboxyl moiety are glucuronized.[17] Therefore this information also must support **5**-glucuronide. Taken together, the metabolite scheme of **3** is suggested as shown in Scheme 1.

The metabolites of **3** are suggested to be **4**, **5**, and **5**-glucuronide (**6**), and the cause of purple-colored urine after administration of **3** is also suggested as **4**. This information is useful to design new COX-1 selective inhibitors without colored urine based on the chemical structure of **3**. New COX-1 inhibitors created on this information will give a chance of new research on COX-1 inhibitors. Now we are developing new COX-1 selective inhibitors without a structure **4**.

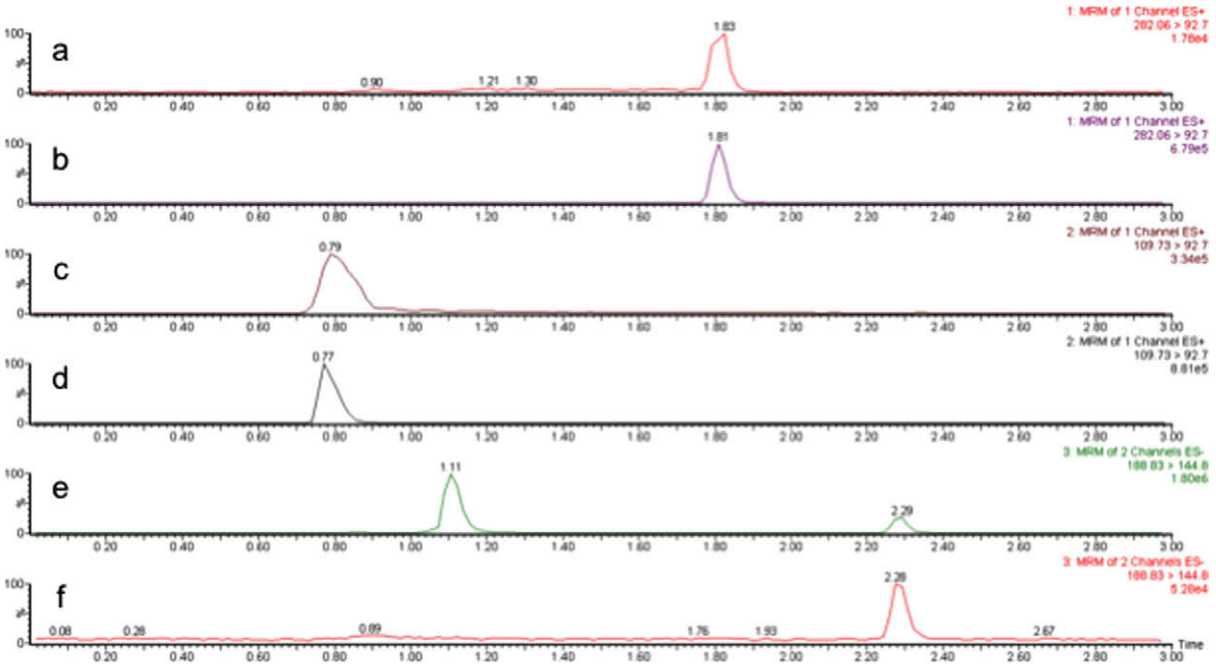


Figure 3. SRM chromatograms of rat urine sample after 3 h of treatment with **3**, and **3**, **4**, **5** standards. (a) *m*/*z* 282.06/92.70 mass transition, (b) **3** standard, (c) *m*/*z* 109.73/92.70 mass transition, (d) **4** standard, (e) *m*/*z* 188.83/144.80 mass transition, (f) **5** standard.

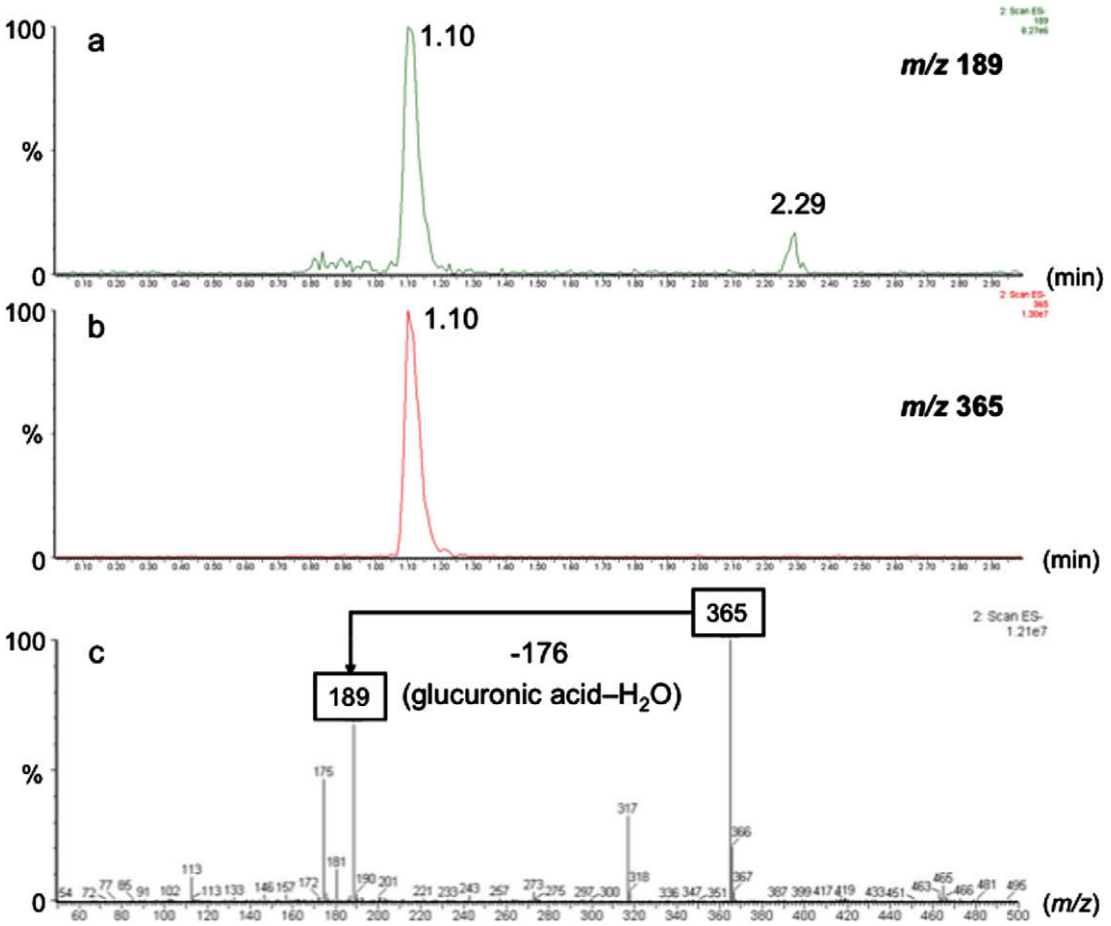
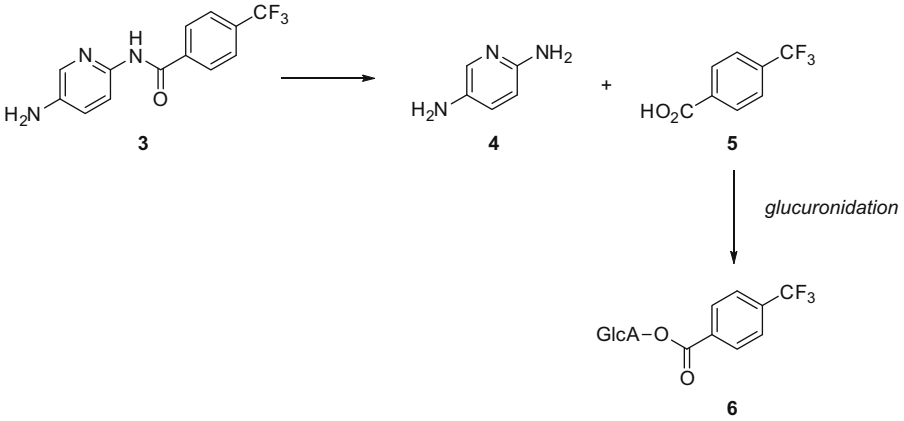


Figure 4. Mass chromatograms and mass spectrum of rat urine sample after 3 h of treatment with **3**. (a) mass chromatogram at *m*/*z* 189, (b) mass chromatogram at *m*/*z* 365, (c) mass spectrum of the peak at 1.10 min corresponding to **5**-glucuronide (**6**).



Scheme 1. Proposed metabolite scheme of **3**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.161.

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