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Structure determination, synthesis, and biological evaluation of a metabolite of the selective α_{1D} adrenoceptor antagonist TAK-259

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ABSTRACT

5-Chloro-1-(5-chloro-2-(methylsulfonyl)benzyl)-2-imino-1,2-dihydropyridine-3-carboxamide hydrochloride (TAK-259) is a novel, selective, and orally active α_{1D} adrenoceptor antagonist with anti-urinary frequency effect. A metabolite (**2**) of TAK-259 was identified from monkey urine samples. To elucidate the structure of **2**, extraction and purification of the metabolite from TAK-259-treated monkey urine was conducted. Structural analysis of the purified compounds using NMR indicated the compound to be 2-amino-5-chloro-1-[5-chloro-2-(methylsulfonyl)benzyl]-4-oxo-1,4-dihydropyridine-3-carboxamide (**2a**). An authentic sample of compound **2a** was synthesized via regioselective alkylation of 4-methoxypyridin-2-amine. The biological activity of metabolite **2a** was also evaluated, and the compound found not to possess affinity toward any known α_1 adrenoceptor subtype.

1. Introduction

5-Chloro-1-(5-chloro-2-(methylsulfonyl)benzyl)-2-imino-1,2-dihydropyridine-3-carboxamide hydrochloride (TAK-259, **1**) is a novel, selective, and orally active α_{1D} adrenoceptor antagonist with anti-urinary incontinence effects.[1] A metabolite (**2**) of TAK-259 was detected in monkey plasma and urine after its oral administration, and was also discovered in metabolite identification studies conducted with human and monkey hepatocytes. Structure elucidation by LC–MS/MS analysis indicated oxidation of the dihydropyridine part as shown in the predicted structures **2a** and **2b** or their tautomers (Fig. 1).

There are a number of literature reports that describe the elucidation of the drug metabolites by chemical synthesis.[2–4] However, in this case, it would have been difficult to identify the metabolite by synthesizing **2a** and **2b** because there are few reports on the synthesis of hydroxy-2-imino-1,2-dihydropyridine rings or their tautomers.[5–8] At that time, a relatively high concentration of **2** was detected in the urine of the TAK-259 (**1**)-treated monkey. It was confirmed that **2** was stable in aqueous solutions (pH 1 and 9), and could be extracted with organic solvents such as ethyl acetate (EtOAc). The present study had three objectives. The first was to determine the structure of metabolite **2** by isolating the metabolite from monkey urine. The second aim was to synthesize **2** to confirm its structure based on the synthetic process. The third goal was to elucidate the biological properties of **2** such as potential affinity to the α_{1D} adrenoceptor and selectivity against the α_{1A} and α_{1B} adrenoceptors. Characterization of the selectivity of this metabolite was deemed important because any affinity for the α_{1A} and α_{1B} adrenoceptors would be associated with the risk of cardiovascular side

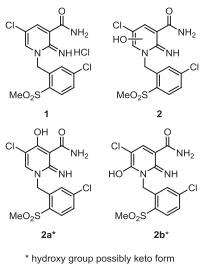

Fig. 1. Structures of TAK-259 (**1**) and its metabolite (**2**). **2a** and **2b**, possible structures of **2**.

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effects.[9] Therefore, this paper describes the isolation, identification, synthesis, and biological characterization of **2**.

2. Results and discussion

2.1. Metabolite isolation and identification

The metabolite was extracted from the urine samples of four monkeys that had been treated with **1** for one day with EtOAc. TOF/MS analysis of the combined extracts are shown in Fig. 2. Metabolite **2** was detected at 6.01 min with an *m*/*z* value of 390 (Fig. 2A and B), whereas the parent drug **1** was observed at 3.63 min with an *m*/*z* value of 374 (Fig. 2C). HPLC purification of the extracts was followed by crystallization to obtain **2** as a colorless solid. The structure of **2** was assigned as the keto form of **2a**, 2-amino-4-pyridone, by NMR analyses (Table 1). From the ^{1}H NMR spectrum, only one proton was observed on the pyridine ring, which indicates that oxidative metabolism occurred on the pyridine ring. The ^{13}C NMR spectrum suggested the presence of two carbonyl groups based on the two peaks found around 170 ppm. Since two carbonyl groups were observed, the metabolite structure was presumed to be a keto and not an enol form in DMSO. To determine the position of the carbonyl group, the nuclear Overhauser effect (NOE) was measured. Strong NOE correlations were observed between the H-7 benzyl protons and the H-6, H-13, and H-24 protons, indicating that the carbonyl group of the pyridone moiety is located at the 4-position (Fig. 3).

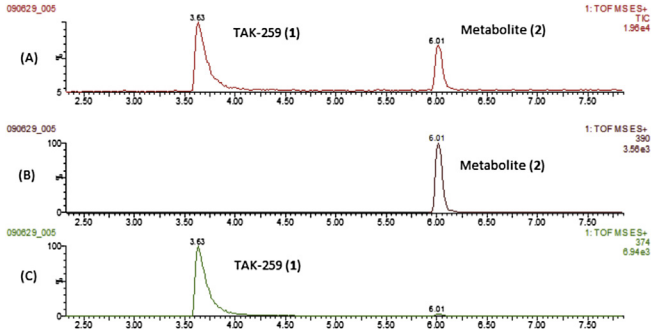


Fig. 2. Ion chromatograms of ethyl acetate (EtOAc) extracts from monkey urine. (A) Total ion, (B) extracted ion of *m*/*z* 390, and (C) extracted ion of *m*/*z* 374.

Table 1
Metabolite NMR spectra

1H	δ (ppm)	13C	δ (ppm)
24	3.39 (3H, s)	24	44.22
7	5.54 (2H, s)	7	51.06
13	6.76 (1H, d, *J*=2.2 Hz)	3	96.08
22	7.42 (1H, d, *J*=4.8 Hz)	5	118.31
11	7.74 (1H, dd, *J*=8.4, 2.2 Hz)	13	125.52
10	8.04 (1H, d, *J*=8.4 Hz)	11	128.63
6	8.06 (1H, s)	10	132.45
22	10.30 (1H, d, *J*=5.1 Hz).	6	136.43
		8	136.61
		9	136.86
		12	139.09
		2	156.18
		15	170.26
		4	171.38

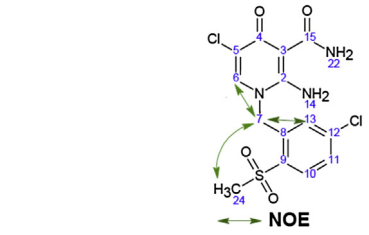


Fig. 3. Nuclear Overhauser effect (NOE) of metabolite 2a.

2.2. Synthesis

The retrosynthetic route we devised for **2a** is shown in Fig. 4. The compound **2a** would be synthesized by alkylation of 2-aminopyridine (**C**) with a benzyl halide (**B**) followed by the demethylation of **A**. Compound **C** could be prepared from commercially available 2-amino-4-methoxypyridine-3-carbonitrile (**D**) in two steps.

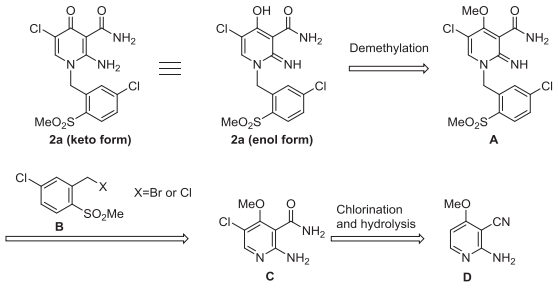
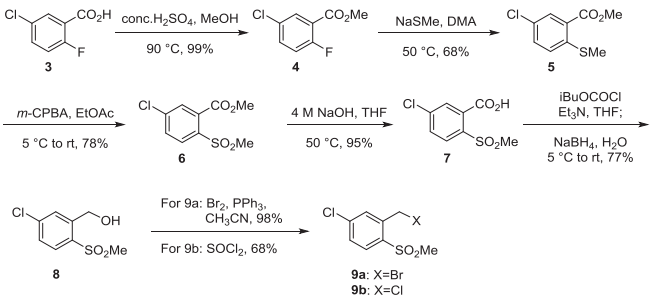


Fig. 4. Retrosynthesis of **2a**.

Initially, benzyl halides **B** (**9a** and **9b**) were prepared by the route shown in Scheme 1. Esterification of 5-chloro-2-fluorobenzoic acid (**3**) was performed in the presence of a catalytic amount of sulfuric acid in methanol to obtain **4**. A methylsulfanyl group was introduced by heating a mixture of **4** and



Scheme 1. Synthesis of benzyl halides **9a** and **9b**.