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Synthesis and evaluation of hedgehog signaling inhibitor with novel core system

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ABSTRACT

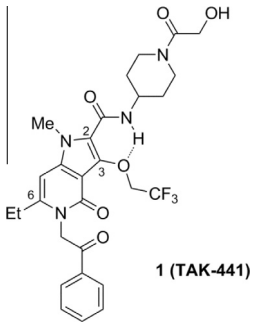
As we previously reported, *N*-methylpyrrolo[3,2-*c*]pyridine derivatives **1** (TAK-441) was discovered as a clinical candidate of hedgehog (Hh) signaling inhibitor by modification of the upper part. We next focused on modification of the lower part including core skeletons to discover new Hh signaling inhibitors with novel core rings. Efforts to find novel chemotypes by using X-ray single crystal structure analysis led to some potent Hh signaling inhibitors (**2c**, **2d**, **2e**, **2f**) with novel core ring systems, which had benzamide moiety at the 5-position as a key component for potent activity. The suppression of Gli1 expression with these new Hh signaling inhibitors were weaker than that of compound **1** (TAK-441) because of low pharmacokinetic property. We recognized again TAK-441 is a good compound as clinical candidate with good structural and pharmacokinetic advantages.

1. Introduction

The hedgehog (Hh) signaling pathway plays a significant role in the regulation of cell growth and differentiation during embryonic development. Abnormal activation of the Hh signaling pathway has been linked to several types of human cancers including pancreatic cancer,[1] prostate cancer,[2] colon cancer,[3] basal cell carcinoma[4] and medulloblastoma,[5] and the development of small-molecule inhibitors of this pathway represents a promising route toward novel anticancer therapeutics.[6]

The pyrrolo[3,2-*c*]pyridine-4-one derivative **1** (TAK-441) we had reported as a clinical candidate was discovered by modification of 2-, 3-, 6-substituents of a lead compound to improve activity and physicochemical property as hedgehog (Hh) signaling inhibitors (Fig. 1).[7,8] However, it was still unclear that exact role of the core skeleton including substituents at the 4- and 5-positions. Thus, we evaluated the modification of central core to confirm these properties and discovered new potent Hh signaling inhibitors with novel skeletons compared to that of **1**.

The pyrrole ring of **1** was essential for potent inhibitory activity because of the interaction by internal hydrogen bond between 2-amide NH and oxygen of 3-(2,2,2-trifluoro)methoxy group.[8] Thus, we fixed this upper part and focused on the lower part of core ring, especially six-membered rings. Besides novel core ring modification, we evaluated the effect of 4- or 5-substituents to in vitro inhibitory activity. In this report, we describe syntheses and SAR of novel hedgehog signaling inhibitors with multi-substituted bicyclic core rings (Fig. 2).



1 (TAK-441)

Figure 1. Chemical structure of **1** (TAK-441).

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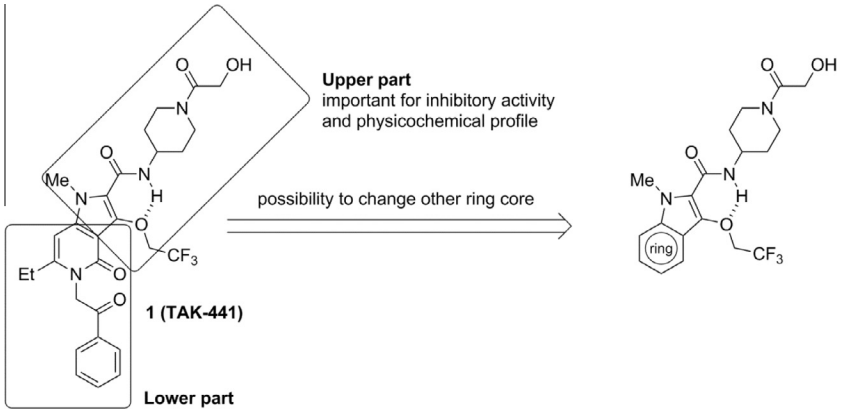


Figure 2. Change to novel core ring in **1** (TAK-441).

2. Chemistry

As we previously reported, the intramolecular hydrogen bond formed between (2,2,2-trifluoro)ethoxy group at 3-position and *N*-[1-(hydroxyacetyl)piperidinyl] amide at the 2-position on *N*-methyl pyrrole ring was important for potent in vitro activity.[7] Figure 3 illustrates our retrosynthetic analysis to access novel bicyclic cores with these two moieties. To construct the unique bicyclic core of **2**, it was necessary to synthesize intermediate **4**. We considered the Dieckmann condensation would be promising because the important substituents as mentioned before could be introduced in the desired position. Therefore, *o*-halo arylcarboxylic acid ester **6** was selected for a key intermediate which was converted to di-ester **5** by addition of sarcosine ester unit.[9] We thought this strategy could be utilized for the synthesis of various useful core rings. The syntheses for five types of carboxylic acid **3** are detailed in the next section.

The synthesis of pyrrolo[2,3-*d*]pyrimidine **3a** is shown in Scheme 1. Addition of propionitrile **7** with ammonia in acidic condition provided amidine hydrochloride **8**, followed by cyclization with diethyl malonate to afford **9** in 67% yield. The preparation of a key intermediate **6a** was conducted by stepwise conversion; introduction of formyl group, oxidation to carboxylic acid and esterification. After addition of sarcosine ethyl ester hydrochloride, subsequent Dieckmann condensation provided the bicyclic compound **4a** in 91% yield. The 3-hydroxyl group of **4a** was treated with (2,2,2-trifluoroethyl)trifluoromethanesulfonate to afford **10**. The stepwise saponification of chloropyridine and ethoxycarbonyl moieties gave carboxylic acid **3a**.

In our previous papers, we reported the synthesis of **1** using an intermediate with requisite phenacyl moiety on six-membered ring of the bicyclic core. The similar synthetic route using the intermediate **11** was initially attempted (Scheme 2). The phenacylation of **11** gave **12** in 57% yield.[10] The following hydrolysis under basic

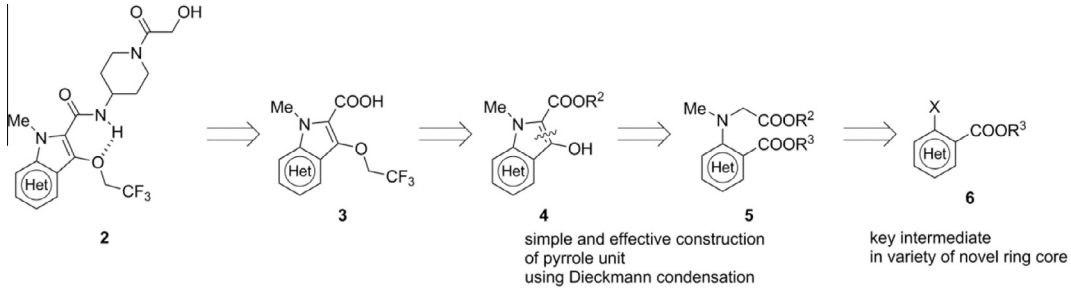
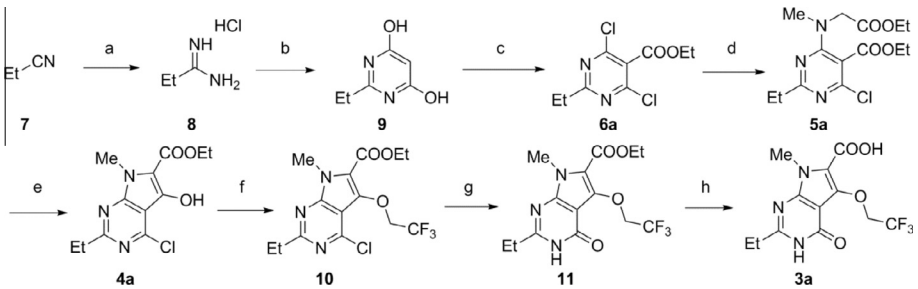


Figure 3. Retrosynthetic analysis of novel core.



Scheme 1. Synthesis of pyrrolo[2,3-*d*]pyrimidine derivative **3a**. Reagents and conditions : (a) (1) HCl, EtOH; (2) NH_3, MeOH, 70%; (b) $(COOEt)_2$, NaOMe, MeOH, 67%; (c) (1) DMF, $POCl_3$, 0 °C to reflux, 73%; (2) $NaClO_2$, NH_2SO_3H, *tert*-BuOH/H_2O; (3) $(COCl)_2$, DMF, THF; (4) Et_3N, EtOH, 53%; (d) $MeNHCH_2COOEt{\cdot}HCl$, Et_3N, THF, 99%; (e) NaOEt, EtOH, 91%; (f) CF_3CH_2OTf, Cs_2CO_3, DMF, 91%; (g) AcONa, AcOH, reflux, 96%; (h) NaOH, EtOH, 60 °C, 79%.