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Pyrrolinone derivatives as a new class of P2X3 receptor antagonists. Part 1: Initial structure-activity relationship studies of a hit from a high throughput screening

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ABSTRACT

The P2X3 receptor is primarily expressed in the peripheral sensory nerves, and therefore, antagonists of this receptor may be useful for the treatment of chronic pain. Pyrrolinone derivatives have been identified as a novel class of P2X3 receptor antagonists. A lead structure with moderate activity was discovered through a high-throughput screening assay. A structure-activity study led to the discovery of several P2X3 receptor antagonists. Compound **34** showed potent and specific antagonistic activity and analgesic efficacy.

Adenosine triphosphate (ATP), produced in the mitochondria in cells, is used as a source of energy for various physiological activities, as a phosphorylation substrate for proteins, and as an extracellular neurotransmitter. ATP is released into the extracellular fluid by a variety of stimuli including inflammatory or cell damage. Extracellular ATP acts as a neurotransmitter for P2 receptors at the nerve terminal. P2 receptors are classified into two groups, P2X includes the ligand-gated ion channels[1] and P2Y includes G protein-coupled receptors.[2] P2X3, a subtype of P2X receptors, was first cloned in 1995.[3,4] P2X3 receptors are primarily expressed in the peripheral sensory nerve. P2X3 receptor knockout mice were reported for suppression of pain behaviors.[5] In 2002, A-317491, which is a specific P2X3 receptor antagonist, showed an analgesic effect by subcutaneous administration to rats in both an inflammation pain model and neuropathic pain model.[6] Thus, in an effort to develop a new analgesic drug, P2X3 receptor antagonists with various chemotypes were reported (Fig. 1).[7–9] Among them, AF-219 improved symptoms of pain and urinary urgency in patients with moderate to severe symptoms of interstitial cystitis/bladder pain syndrome (IC/BPS) in a Phase 2 clinical trial. Furthermore, AF-219 is in a Phase 2 clinical trial that examines its ability to improve refractory chronic cough.[10]

Building on the information gained from these previous studies, we aimed to develop a new P2X3 receptor antagonist as an analgesic drug free from the side effects observed for existing analgesics that act on the central nervous system. To discover novel chemotype P2X3 receptor antagonists, we performed high throughput screening of our chemical library using a FLIPR 384 Ca2+-influx assay system. Hit compound **1**, which has a pyrrolinone skeleton, was identified. The IC_{50} value of compound **1** showed the decrease by the increase in concentration of ATP. These results showed that compound **1** was orthosteric antagonist. In attempts to improve the antagonistic activity of compound **1**, a series of pyrrolinone derivatives were synthesized with various substituents to identify the structure exhibiting the strongest activity. Herein, we describe the synthesis and structure-activity relationships of pyrrolinone derivatives as P2X3 receptor antagonists.

Pyrrolinone skeletons were easily synthesized in a one-pot by condensation of an α-ketoester, aniline and aldehyde (Scheme 1).[11]

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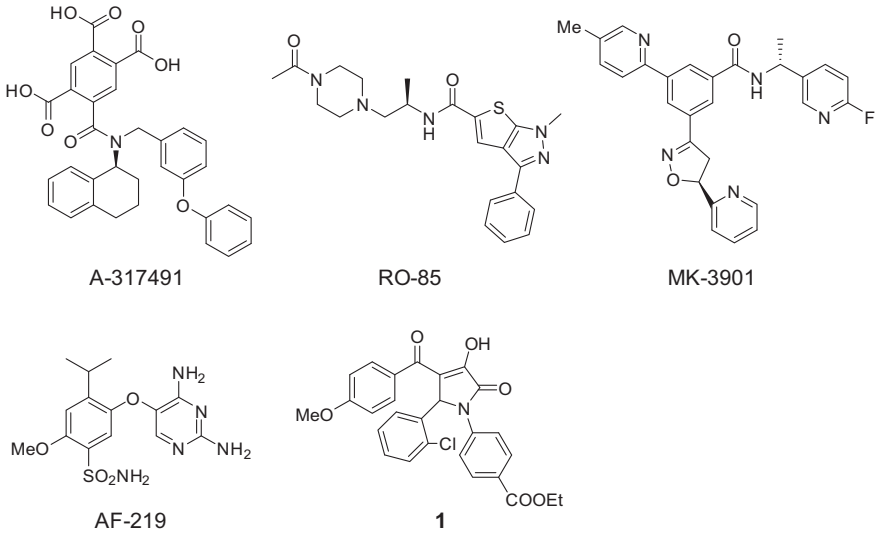
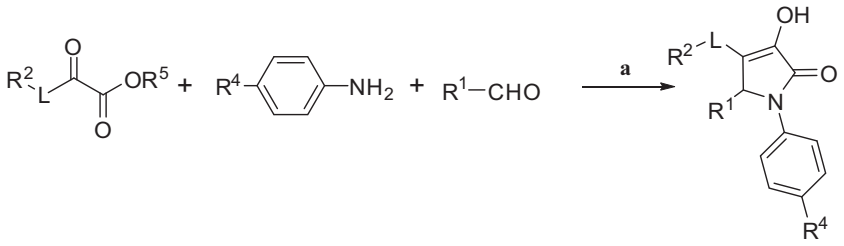


Fig. 1. Structures of P2X3 receptor antagonists and hit compound **1**.

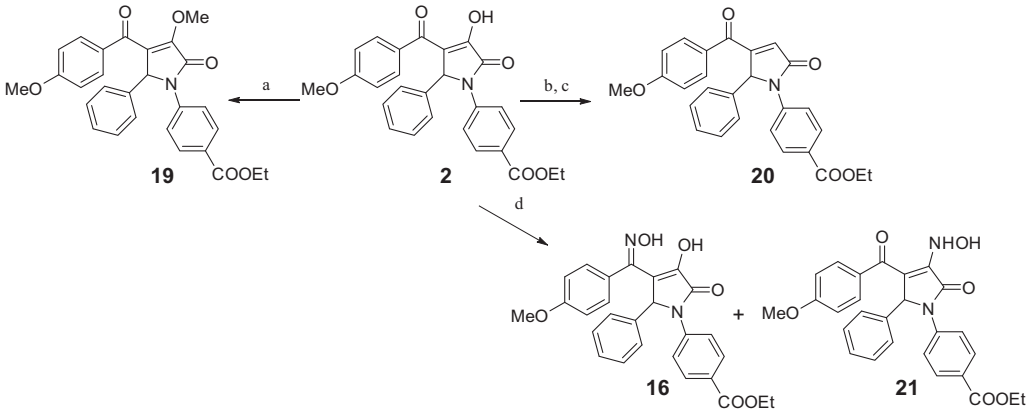


Scheme 1. Reagents and conditions for the synthesis of the pyrrolinone skeletons: (a) AcOH, dioxane, reflux.

The other pyrrolinone derivatives were synthesized from compound **2** as a starting material according to Scheme 2. Compound **19** was prepared by methylation of compound **2** using iodomethane in the presence of potassium carbonate. Dehydroxylated compound **20** was synthesized by acetylation of the enolic alcohol **2** and hydrogenation using Pd/C. Compound **2** was reacted with hydroxylamine to give compound **16** and **21** in moderate yields. To change the linker L at position-4 of the pyrrolinone ring, the Beckmann rearrangement was applied to the pyrrolinone derivatives (Scheme 3). The enolic alcohol **2** was converted to the benzyl ether by alkylation with benzyl bromide to give **35**. Oxime **36** was obtained by using the same conditions as described for compound **16**, and then amide compounds **37** and **38** were synthesized by Beckmann rearrangement using cyanuric chloride-$ZnCl_2$.[12] Both amide compounds were debenzylated by hydrogenation to give **17** and **18**.

Table 1 shows the effects of substitution of the pyrrolinone ring at each position and the ethoxycarbonyl moiety of the pyrrolinone derivatives on antagonistic activity.[13]

Among compounds with a substituent (R^1) at position-5 of the pyrrolinone ring (**1**–**9**), the phenyl (**2**), 4-fluorophenyl (**5**), and cyclohexyl (**7**) derivatives were the most active, followed by the



Scheme 2. Reagents and conditions for the conversion of compound **2**: (a) MeI, K_2CO_3, DMF, rt 3 h, 83%; (b) Ac_2O, pyridine, rt 3 h, 93%; (c) H_2, Pd/C, THF, rt, 3 days, 11%; (d) 50% NH_2OHaq, THF, reflux 7 h, **16** (20%), **21** (25%).