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Evaluation of adenine as scaffold for the development of novel P2X3 receptor antagonists

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ABSTRACT

Ligands that selectively block P2X3 receptors localized on nociceptive sensory fibres may be useful for the treatment of chronic pain conditions including neuropathic pain, migraine, and inflammatory pain. With the aim at exploring the suitability of adenine moiety as a scaffold for the development of antagonists of this receptor, a series of 9-benzyl-2-aminoadenine derivatives were designed and synthesized. These new compounds were functionally evaluated at rat or human P2X3 receptors expressed in human embryonic kidney (HEK) cells and on native P2X3 receptors from mouse trigeminal ganglion sensory neurons using patch clamp recording under voltage clamp configuration. The new molecules behaved as P2X3 antagonists, as they rapidly and reversibly inhibited (IC_{50} in the low micromolar range) the membrane currents induced via P2X3 receptor activation by the full agonist α,β-methyleneATP. Introduction of a small lipophilic methyl substituent at the 6-amino group enhanced the activity, in comparison to the corresponding unsubstituted derivative, resulting in the 9-(5-iodo-2-isopropyl-4-methoxybenzyl)-N^6-methyl-9*H*-purine-2,6-diamine (**24**), which appears to be a good antagonist on recombinant and native P2X3 receptors with $IC_{50} = 1.74 \pm 0.21$ μM.

1. Introduction

P2X3 receptors belong to the purinergic P2X receptor class of ligand-gated channels activated by extracellular ATP to induce a rapid increase in membrane permeability to mono- and di-valent cations [1–3]. P2X3 receptors were cloned in 1995 [4,5] and shown to be almost exclusively localized on nociceptive sensory neurons and on afferent fibre terminals in lamina two of the spinal cord dorsal horn [6]. They contribute to pain sensation, visceral mechanosensory transduction, and gut peristalsis [7–10]. Extensive activation of such receptors is believed to be involved in a number of chronic pain conditions including neuropathic pain, which is typically resistant to standard pain treatment, migraine, and inflammatory pain [11]. Functional P2X3 receptors are predominantly expressed as homomers (with fastly desensitizing property upon prolonged exposure to ATP), and to a much lesser extent as heteromers with P2X2 (P2X2/3) [4,5,12]. However, the latter can be distinguished by their insensitivity to low concentrations of the reference non-hydrolysable and selective P2X3 agonist α,β-methyleneATP (α,β-meATP) [12–14]. Targeting these receptors with selective, potent antagonists can represent an innovative approach to treat chronic pain conditions of both neuropathic and inflammatory origin when P2X3 receptor function is reported to be enhanced [15,16]. In the last few years considerable effort has been dedicated to the development of potent and selective antagonists at P2X3 receptors; the first identified P2X3 antagonists were negatively charged and/or high molecular weight organic molecules like suramin, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), 2′,3′-*O*-(2,4,6-trinitrophenyl)-ATP (TNP-ATP) [17], and A-317491 (Fig. 1) [18]; on the other hand, some

Abbreviations: α,β-meATP, α,β-methyleneATP; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; TNP-ATP, 2′,3′-*O*-(2,4,6-trinitrophenyl)-ATP; PPP, triphosphate; HTS, high-throughput screening; TG, trigeminal ganglia; DCM, dichloromethane; NOE, nuclear Overhauser effect; TFAA, trifluoroacetic anhydride; TBAN, tetrabutylammonium nitrate; TEA, triethylamine; PTSA, *p*-toluenesulfonic acid; n_H, Hill coefficient; DRG, dorsal root ganglion; τ_{on}, rise time; $\tau_{des1;2}$, time constant of desensitization decay; CPU, central processing unit; MOE, Molecular Operating Environment; TLC, thin-layer chromatography; PTLC, preparative thin-layer chromatography.

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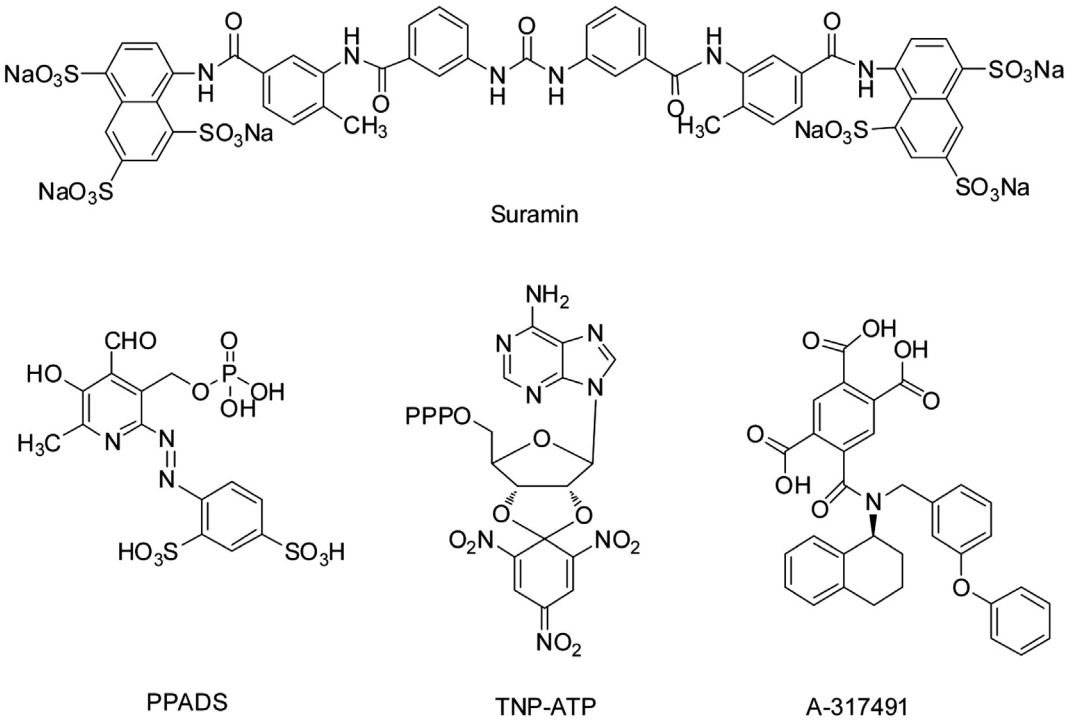

Fig. 1. Structure of some known P2X3 antagonists (PPP = triphosphate).

of them lacked selectivity and potency. The poor pharmacokinetic properties of these molecules (poor oral bioavailability, high protein binding, and uneven tissue distribution) would likely make them unattractive for their development as drugs. More recently, novel antagonists for homomeric P2X3 and heteromeric P2X2/3 receptors, structurally related to the diaminopyrimidine antibacterial drug trimethoprim, have been identified by a high-throughput screening (HTS) campaign [19]. Among them, a compound named RO-3 (Fig. 2A) represents an important step towards discovery of novel drug-like P2X antagonists [20,21] endowed with high affinity and selectivity. Furthermore, new acyclic-nucleotides based on the adenine skeleton and bearing in 9-position a phosphorylated four carbon chain (Fig. 2B) mimicking the ribose function have been described as partial agonists of P2X3 receptors [22].

In the present study, the information coming from the latter two series of above cited compounds was combined with the aim at testing the suitability of the adenine moiety as scaffold for the development of P2X3 antagonists. In detail, a new class of P2X3

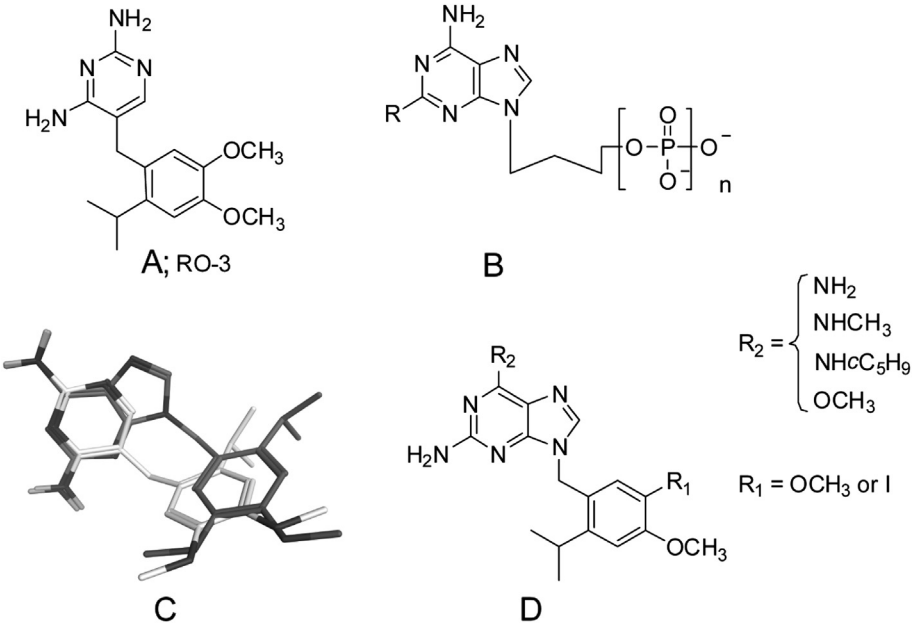

Fig. 2. A and B: known P2X3 ligands; C: 3D alignment of one of the designed purine derivatives (dark grey) with the pyrimidine analogue RO-3 (light grey). Non-polar hydrogen atoms are hidden; D: designed molecules.