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Original article

Structure–activity relationships of novel P2-receptor antagonists structurally related to Reactive Blue 2

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In memoriam of the late Professor Dr. Felix Zymalkowski

Abstract

P2 membrane receptors for nucleotides represent significant targets for experimental pharmacology and drug research. In earlier publications, we have shown that Reactive Blue 2 (RB 2), one of the most widely used P2-receptor antagonists, displays only moderate affinity and does not discriminate between native P2X- and P2Y-receptor subtypes. In the present study we have pharmacologically evaluated a series of 15 synthesized and re-evaluated four commercially obtained and chromatographically purified RB 2 type anthraquinone derivatives on contractions of the rat vas deferens (RVD) elicited by α,β-methylene ATP (α,β-meATP), mediated by $P2X_1$-receptors, and relaxations of the carbachol-precontracted guinea-pig taenia coli (GPTC) elicited by adenosine 5′-O-(2-thiodiphosphate) (ADPβS), mediated by $P2Y_1$-like receptors. Based on the structure–activity relationships (SAR) it is concluded that hydrophobic interactions of aromatic π-electron systems, hydrogen bonds with nitrogen as donor and acceptor atoms, and, particularly, position, conformational distance and number of anionic sulfonate groups are of great importance for the blockade of the two native P2-receptor subtypes. We have also identified novel, for the most part reversible antagonists that bind with higher affinity and improved subtype selectivity in comparison to RB 2. In particular, 1-amino-4-{4-[4-chloro-6-(2-sulfonatophenylamino)-[1,3,5]triazine-2-ylamino]-2-sulfonatophenylamino}-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid trisodium salt (MG 50-3-1) is the most potent antagonist at the $P2Y_1$-like-receptors of the GPTC reported so far (IC_{50} = 4.6 nM). It is significantly less potent as reversible antagonist at the $P2X_1$-receptors of the RVD (IC_{50} = 2.8 μM). Thus, MG 50-3-1 represents a selective pharmacological tool and may be a lead compound for future investigations.

Keywords: Reactive Blue 2; P2-receptor antagonist; Rat vas deferens; Guinea-pig taenia coli; $P2X_1$-receptor; $P2Y_1$-like receptor

Abbreviations: AB 25, Acid Blue 25; ADPβS, adenosine 5′-O-(2-thiophosphate); α,β-meATP, α,β-methylene adenosine 5′-triphosphate; CB 3GA, Cibacron Blue 3GA; C.I., color index; COSY, correlation spectroscopy; DMF, dimethylformamide; ESI-MS, electrospray ionization mass spectra; FCC, flash column chromatography; GPTC, guinea-pig taenia coli; HETCOR, heteronuclear correlation; NOESY, nuclear overhauser enhancement spectroscopy; PPADS, pyridoxal-5′-phospate-6-phenylazo-2′,4′-disulfonate; RB 2, Reactive Blue 2; RB 4, Reactive Blue 4; RB 5, Reactive Blue 5; RP-FCC, reversed phase flash column chromatography; RP-TLC, reversed phase thin layer chromatography; RVD, rat vas deferens; SAR, structure–activity relationships; 2D-NMR, two dimensional nuclear magnetic resonance.

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1. Introduction

Plasma membrane nucleotide P2-receptors subdivided in ligand gated ion channels (P2X) and G-protein coupled receptors (P2Y) are interesting targets not only in experimental pharmacology but also in drug research [1–5]. To date, seven mammalian P2X-receptors termed $P2X_1$ to $P2X_7$ and eight P2Y-receptors termed $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ have been identified by molecular cloning [6–10]. However, experimental and therapeutic progress in the P2-receptor field is dependent on the availability of potent and selective antagonists. For instance, novel suramin analogues have been reported as potent $P2X_1$-selective antagonists [11–14], and 3′,5′-bisphosphate nucleotides have been developed as selective high affinity antagonists of the $P2Y_1$-receptor [15–18]. Nevertheless, the identification of antagonists within other chemical classes remains highly desirable.

The anthraquinone dye Reactive Blue 2 (RB 2) (1-amino-4-{4-[4-chloro-6-(3-/4-sulfonatophenylamino)- [1,3,5]triazin-2-ylamino]-3-sulfonato-phenylamino}-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid) (**1**) (Fig. 1) [20,21] is still one of the most widely used "classical" P2-receptor antagonists. A former study of a series of commercially obtained and chromatographically purified compounds [19], all structurally related to RB 2, identified P2-receptor ligands with higher affinity and improved subtype selectivity. Therefore, we have further investigated this chemical class of P2-receptor antagonists.

Unfortunately there has been some confusion concerning the identity and purity of commercially available RB 2 (**1**) [22–29]. Furthermore, it has been claimed to be P2Y-selective [30–32]. According to the Color Index (C.I.) of the Society of Dyers and Colorists, RB 2 (**1**) is defined as a mixture of two constitutional isomers with the sulfonate group at ring F either in *meta* **(1a)** or *para* **(1b)** position [33]. In fact, we have recently shown that the commercially available product is a 1:2 ring F *meta*/*para* sulfonate mixture and—more important—does not discriminate between native $P2X_1$-receptors of the rat vas deferens (RVD) and $P2Y_1$-like receptors of the guinea-pig taenia coli (GPTC) [34]. In contrast, the synthesized pure ring F *para* sulfonate isomer **(1b)** turned out to be a moderately selective antagonist at the $P2Y_1$-like versus the $P2X_1$-receptor.

In the present structure–activity relationships (SAR) study within this class of antagonists, the lead compound RB 2 (**1**) (Fig. 1) was structurally simplified to ascertain the minimal requirements of the anthraquinone core (rings ABC) and the (hetero)aromatic side chain (rings DEF) for P2-receptor subtype blockade. The concept of structural variations to identify molecules binding with higher affinity and improved subtype selectivity is shown in the general formula **A** (Fig. 1). Structures of all novel 13 synthesized anthraquinone derivatives **2**–**4** (ABC), **6–9**, **11**(ABCD), **12**, **15**, **16** (ABCDE), **18**, and **19** (ABCDEF) are listed together with two re-synthesized compounds **10** (ABCD), and **13** (ABCDE) [34] in ascending order of complexity (Schemes 1–5). These compounds were tested pharmacologically together with a comparative re-evaluation [19,34,35] of the four commercially obtained dyes Acid Blue 25 (AB 25) (**5**) (ABCD), Reactive Blue 4 (RB 4) (**14**) (ABCDE), Cibacron Blue 3GA (CB 3GA) (**17**), and Reactive Blue 5 (RB 5) (**20**) (ABCDEF) (Schemes 1,3 and 5).

As in our previous studies, both the synthesized and the commercially obtained compounds were purified by reversed phase flash column chromatography (RP-FCC) before use, since the dye content of the purchased dye stuffs was only about 35–65% and organic impurities and inorganic salts may have caused non-reliable pharmacological results in the literature. The chemical structures and purities were confirmed by ^{1}H- and ^{13}C-NMR, ESI-MS and RP-TLC.

Pharmacological effects of the pure compounds were studied on contractions of the RVD elicited by α,β-meATP, mediated by the $P2X_1$-receptor, which has been cloned [37–44], and relaxations of the carbachol-precontracted GPTC elicited by ADPβS, mediated by a P2Y-receptor which has not so far been cloned but displays close similarities with the cloned $P2Y_1$-receptor [19,45–50].

2. Chemistry

2.1. Chemical synthesis

2.1.1. Scheme 1

1-Amino-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid monosodium salt (**2**), the functionalized ABC anthraquinone core of the RB 2 isomer CB 3GA (1-amino-4-

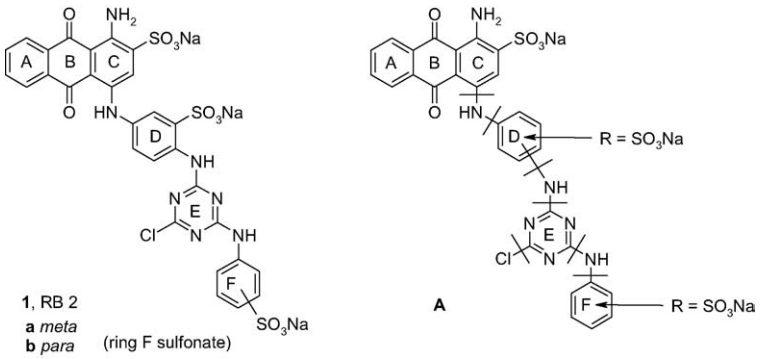

Fig. 1. Chemical structure of the lead compound RB 2 **(1)** as ring F *meta* **(1a)**/*para* **(1b)** constitutional mixture and general formula **A** with synthetic variations.