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Antagonistic Properties of New Non-Phosphorylated Derivatives of Nitrogen-Containing Heterocycles Towards P2 Receptors

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ABSTRACT

P2 receptors are widely distributed in animal and human organisms; however, selective antagonists of these receptors are still lacking. One of the well-known and mostly used P2 receptor antagonist at present is pyridoxal-phosphate 6-azophenyl 2', 4'-disulphonic acid (PPDAS) which has no selectivity to either P2X or P2Y receptors. In this study, a series of new PPADS analogues for their potential antagonistic effects on P2 receptors was tested. The compounds (lab codes 2a-2g) were tested at a concentration of 10 mM *in vitro* for antagonistic activity towards P2X and P2Y receptors using isolated rat smooth muscle preparations of urinary bladder, vas deferens and duodenum. Contractions of urinary bladder and vas deferens preparations were evoked by either agonist of P2X-receptors, α,β -methylene-ATP (0.1-3 mM), or by electrical field stimulation (EFS, 0.5 msec, 100 V, 1-32 Hz) in the presence of M-cholinoblocker atropine (10 μ M) and alpha-adrenoblocker phentolamine (10 μ M). Relaxant responses of carbachol-precontracted duodenum preparations were induced by either ATP (1 μ M-1 mM) or by EFS (0.5 msec, 100 V) with frequencies of 0.5, 1, 2 and 4 Hz. Effects of tested compounds were compared with that of PPADS (10 μ M). It was found that the majority of the tested compounds showed some degree of antagonism against EFS-evoked contractions of rat isolated urinary bladder and vas deferens mediated via P2X receptors, however only compound 2c produced antagonism comparable with that of parent antagonist, PPADS. Further, compound 2c, unlike PPADS, did not antagonize P2Y-receptor-mediated relaxation in rat isolated duodenum preparations. It is concluded from this study that compound 2c is an effective antagonists of P2X but not P2Y receptors and its selectivity towards subtypes of P2 receptors needs to be proved in further experiments.

Key words: Azophenylsulfonic acids, P2-receptors, antagonists, pyridoxine derivatives

INTRODUCTION

P2 receptors, where ATP is a main endogenous agonist, are present in all tissues of any biological organism. Up to date 15 subtypes of P2 receptors have been identified which belong to two major classes, namely P2X and P2Y. Wide distribution and diversity of P2 receptors make them promising targets for

pharmacological intervention. Antagonists of P2Y₁₂ receptors (clopidogrel, ticlopidine etc.) are effective antiplatelet drugs and are widely used as alternatives to aspirin for prevention of myocardial infarction and stroke. Several other agonists and antagonists of P2 receptors are currently studied in clinical trials for treatment of different pathological conditions (Ziganshin and Ziganshina, 2009).

Development of P2 receptor antagonists is a promising area of modern medicinal chemistry and pharmacology. Despite considerable numbers of synthesized and studied P2 receptor antagonists with different chemical structures, each of them has certain disadvantages, in particular the lack of selectivity or efficiency of their antagonism or significant effect on the activity of ecto-ATPase (Ziganshin *et al.*, 1994a). One of the well-known P2 receptors antagonists is pyridoxal-phosphate 6-azophenyl 2', 4'-disulphonic acid (PPDAS)-an azosulphonyl derivative of pyridoxal phosphate (Ziganshin *et al.*, 1993; McLaren *et al.*, 1994). Various structural modifications of PPDAS have been made through functional group substitution on the sulphonyl ring (Connolly, 1995; Ziganshin *et al.*, 1998). However, the influence of substituents at the pyridoxal moiety on the P2 receptor antagonism was less studied. It was shown that a phosphate linkage is not required for P2 receptor antagonism through the inclusion of phosphonates (Kim *et al.*, 1998) and substitution of phosphate moiety by carboxylic acid groups (Jung *et al.*, 2013; Cho *et al.*, 2013). This study presents the biological activity of novel PPDAS analogs based on azosulphonyl derivatives of pyridoxine with acetal protection of hydroxymethyl groups. Such structure provides opportunities to vary the lipophilicity of resulting compounds which has significant impact on biological activity of pyridoxine derivatives.

MATERIALS AND METHODS

Tested compounds: Compounds with laboratory codes of 2a-2g were synthesized in the Centre for Research and Education in Pharmaceutics, Kazan (Volga Region) Federal University, Kazan, Russia (Pugachev *et al.*, 2013a, b). All compounds are close analogues of PPDAS with modifications in pyridoxal moiety. Compounds were diluted in distilled water to a concentration of 10^{-2} M; this was used as a stock solution for all experiments (Fig. 1).

General procedures: Antagonistic activity of the synthesized compounds towards P2 receptors was evaluated *in vitro* on isolated smooth muscle preparations of male Wistar rats weighing 150-200 g (nursery "Puschino", Moscow region). All experiments were performed in accordance with the workplace policies on the use of experimental animals. Method for studying the mechanical activity of isolated tissues was applied (Ziganshin *et al.*, 1993). All new compounds as well as PPDAS were tested at a concentration of 10^{-5} M in triple or quadruple experiments on tissues of at least three different animals.

Antagonistic activity against P2X receptors: The smooth muscle preparations of rat urinary bladder and vas deferens were placed in a 10 mL thermostatic bath with Krebs solution of the following composition (mM): NaCl 144.3, KCl 4.7, NaHCO₃ 5.0, MgCl₂ 0.6, NaH₂PO₄ 1.35, glucose 7.8 and CaCl₂ 2.5. The pH of the solution was 7.3-7.4, temperature

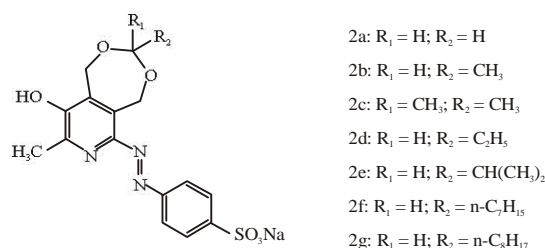


Fig. 1: Chemical structure of the tested compounds

$37.0 \pm 0.5^\circ\text{C}$. One end of the preparation was fixed while another end by a silk thread was attached to the transducer of mechanical activity. Mechanical activity of the muscle preparations was detected by a FSG-01 force-displacement transducer (Linton, Great Britain) and recorded by the MP100WSW Data Acquisition System (Biopack, Great Britain) and displayed on a computer screen. The preparations were allowed to equilibrate under the initial tension of 1 g for at least 60 min in the bath media while the Krebs solution was replaced every 15 min.

Contractions induced by electrical field stimulation:

Electrical Field Stimulation (EFS) was applied using a Digitimer MultiStim D330 stimulator (Digitimer Ltd, UK) via two platinum wire rings 2.5 mm in diameter, 15 mm apart, through which the muscles were threaded. Contractile activity of the tissue was elicited by applying rectangular impulses at frequencies of 0.5-32.0 Hz, 0.5 msec in length and 100 V in voltage. After initial contractions induced by EFS being recorded, EFS and recording were conducted again in the presence of either atropine sulfate (10 mM, urinary bladder) or phentolamine (10 mM, vas deferens) to exclude the effects of M-cholinergic or α -adrenergic receptors on electrical stimulation. The following EFS and recording were produced after 20 min incubation of muscle preparations with a given tested compound at a concentration of 10^{-5} M.

Contractions induced by α, β -methylene ATP (α, β -meATP):

A non-selective agonist of P2X receptors, α, β -meATP (0.1-3 mM), produced concentration-dependent contractions of isolated rat urinary bladder and vas deferens. Such concentration-contraction relationship for α, β -meATP was obtained before and after 20 min incubation of muscle preparations with tested compounds or PPDAS at a concentration of 10^{-5} M.

Antagonistic activity against P2Y-receptors:

Longitudinal smooth muscle layer of rat duodenum was gently removed by damp cotton ball and placed vertically in the 10 mL thermostatic bath with Krebs solution as written above. Smooth muscle preparations were allowed to equilibrate under the initial tension of 500 mg for at least 60 min in the bath media while the Krebs solution was replaced every 15 min. In preliminary experiments the concentration-response

relationship for carbachol-induced contractions of the longitudinal smooth muscles of duodenum was assessed. It was found that carbachol at a concentration of 0.3 μM causes persistent contractile responses; this concentration was used for further experiments to raise tissue tone. Relaxation responses were evaluated as a percentage of the maximal relaxation possible.

Relaxation induced by EFS: The EFS (0.5 msec, 100 V) with frequencies of 0.5, 1, 2 and 4 Hz induced frequency-dependent relaxation of carbachol-precontracted duodenum preparations. A frequency-relaxation relationship for EFS was constructed before and after 20 min incubation of the duodenum preparations with tested compounds or PPADS at a concentration of 10^{-5} M.

Relaxation induced by ATP: A non-selective agonist of P2 receptors, ATP (1 μM -1 mM), elicited a concentration-dependent relaxation of carbachol-precontracted duodenum preparation. A concentration-relaxation relationship for ATP was constructed before and after 20 min incubation of the duodenum preparations with tested compounds or PPADS at a concentration of 10^{-5} M.

Drugs: All agents for these experiments were purchased from Sigma-Aldrich.

Statistics: Data was expressed as Mean \pm Standard errors. The significances of differences were evaluated using the Student's t-test. The p value being not less than 0.05 were deemed significant. The n value reported in the text refer to the number of individual experiments.

RESULTS AND DISCUSSION

Antagonistic activity towards P2X receptors: PPADS at a concentration of 10 mM significantly down-shifted the frequency-response relationship curve for EFS-evoked contractions in the rat isolated urinary bladder and vas

deferens in the presence of atropine and phentolamine. The reduction of contractile responses in the presence of PPADS was up to 50% in vas deferens and up to 30% in urinary bladder comparing to corresponding control contractions.

It was found that all tested compounds showed some degree of antagonism against EFS-evoked contractions of rat isolated urinary bladder and vas deferens, although none of the compound was significantly better in their antagonism than that shown by PPADS. However, one of the tested compound (2c) produced antagonism towards P2X-mediated contractions of rat urinary bladder and vas deferens comparable with that of parent antagonist (PPADS).

To keep the paper short, further we present results only for compound 2c with comparison for that of PPADS.

Compound 2c was tested in experiments where contractions of rat isolated urinary bladder and vas deferens preparations were induced by a non-selective agonist of P2X receptors, α,β -meATP (0.1-3 μM). Pre-incubation of smooth muscle tissues with compound 2c (10 μM) caused downshift of the concentration-response curve for α,β -meATP, although this effect was less prominent than that of PPADS (Fig. 2).

Antagonistic activity towards P2Y receptors: To test potential antagonistic effects of compound 2c on P2Y receptor-mediated relaxation, we evaluated its activity on rat isolated longitudinal smooth muscle preparations of duodenum. It was found that compound 2c (10 μM) did not significantly affect P2Y receptor-mediated relaxation of duodenum induced by either EFS (0.5-8 Hz) or ATP (1-30 μM). In similar experiments, PPADS caused small but significant decrease of relaxant effects mediated by P2Y receptors (Fig. 3).

PPADS initially was introduced as a selective P2X receptor antagonist (Ziganshin *et al.*, 1993, 1994b), however soon its antagonistic activity against P2Y receptors was observed by Windscheif *et al.* (1995). Since then many analogues of PPADS were tested for their possible selective antagonistic activity towards different P2 receptors (Kim *et al.*, 1998; Ziganshin *et al.*, 1998), however not much progress was achieved.

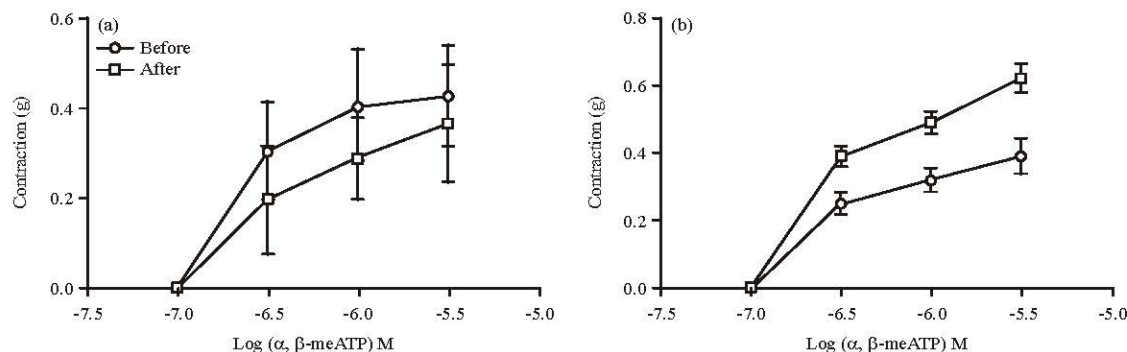


Fig. 2(a-b): Contractile responses of rat isolated vas deferens evoked by agonist of P2X receptors, α,β -meATP (10^{-7} - 3×10^{-6} M), before and after 20 min incubation of tissues with (a) Compound 2c and (b) PPADS at a concentration of 10^{-5} M. Results presented as Mean \pm SD, n = 3-4

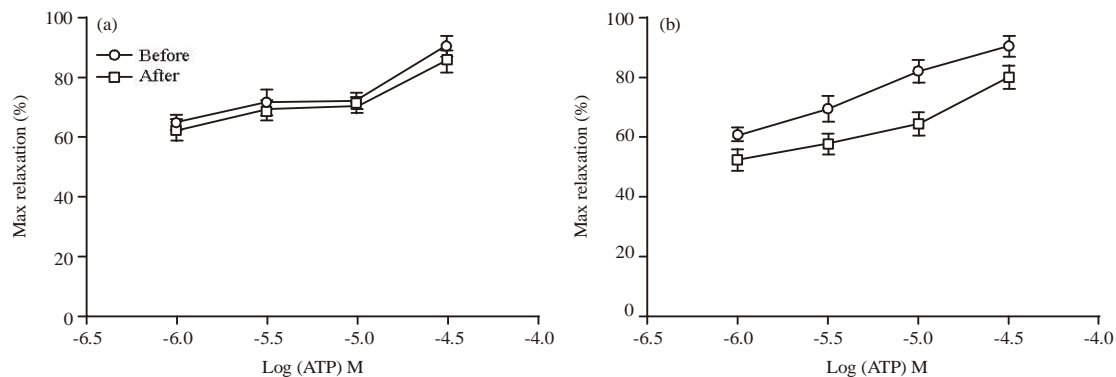


Fig. 3(a-b): Relaxant responses of carbachol-precontracted rat isolated longitudinal smooth muscle preparations of duodenum evoked by ATP (10^{-6} - 3×10^{-5} M) before and after 20 min incubation of tissues with (a) Compound 2c and (b) PPADS at a concentration of 10^{-5} M. Results presented as Mean \pm SD, n = 3-4

In this study, 7 new analogues of PPADS were tested with substitutions at the pyridoxal moiety for their antagonistic activity against P2X and P2Y receptor-mediated responses in rat isolated smooth muscle tissues. Although most of the tested compounds did not appear to be effective antagonists, one of compounds (2c) showed a notable antagonism against P2X receptor-mediated contractions of urinary bladder and vas deferens, both in experiments with EFS and with α, β -meATP. Although the antagonistic effect of compound 2c towards P2X receptors was not greater than that of PPADS, unlike PPADS, compound 2c did not significantly affect the P2Y receptor-mediated relaxation. Thus compound 2c might be more selective to some P2 receptor subtypes than PPADS. This fact confirms that phosphate moiety is not necessary for antagonistic activity (Kim *et al.*, 1998) and offers new opportunities for synthesis of novel purinoceptor antagonists. It is interesting to mention that unlike other biological activity of studied substances (Pugachev *et al.*, 2013a, b), in the present *in vitro* experiments, antagonism against P2 receptors seems to be not dependent on the lipophilicity of the compounds.

CONCLUSION

In conclusion, we have shown in this study that azophenilsulfonic acid derivatives demonstrated activity towards certain types of purinoceptors and in particular compound 2c is an effective antagonists of P2X but not P2Y receptors and it's selectivity towards subtypes of P2 receptors needs to be proved in further experiments.

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REFERENCES

- Cho, J.H., K.Y. Jung, Y. Jung, M.H. Kim, H. Ko, C.S. Park and Y.C. Kim, 2013. Design and synthesis of potent and selective P2X₃ receptor antagonists derived from PPADS as potential pain modulators. *Eur. J. Med. Chem.*, 70: 811-830.
- Connolly, G.P., 1995. Differentiation by pyridoxal 5-phosphate, PPADS and IsoPPADS between responses mediated by UTP and those evoked by alpha, beta-methylene-ATP on rat sympathetic ganglia. *Br. J. Pharmacol.*, 114: 727-731.
- Jung, K.Y., J.H. Cho, J.S. Lee, H.J. Kim and Y.C. Kim, 2013. Synthesis and structure-activity relationships of carboxylic acid derivatives of pyridoxal as P2X receptor antagonists. *Bioorg. Med. Chem.*, 21: 2643-2650.
- Kim, Y.C., E. Camaioni, A.U. Ziganshin, X. Ji and B.F. King *et al.*, 1998. Synthesis and structure-activity relationships of pyridoxal-6-aryloxy-5'-phosphate and phosphonate derivatives as P2 receptor antagonists. *Drug Dev. Res.*, 45: 52-66.
- McLaren, G.J., G. Lambrecht, E. Mutschler, H.G. Baumert, P. Sneddon and C. Kennedy, 1994. Investigation of the actions of PPADS, a novel P_{2x}-purinoceptor antagonist, in the guinea-pig isolated vas deferens. *Br. J. Pharmacol.*, 111: 913-917.
- Pugachev, M.V., N.V. Shtyrlin, L.P. Sysoeva, E.V. Nikitina and T.I. Abdullin *et al.*, 2013a. Synthesis and antibacterial activity of novel phosphonium salts on the basis of pyridoxine. *Bioorg. Med. Chem.*, 21: 4388-4395.
- Pugachev, M.V., N.V. Shtyrlin, S.V. Sapozhnikov, L.P. Sysoeva and A.G. Iksanova *et al.*, 2013b. Bis-phosphonium salts of pyridoxine: The relationship between structure and antibacterial activity. *Bioorg. Med. Chem.*, 21: 7330-7342.
- Windscheif, U., O. Pfaff, A.U. Ziganshin, C.H.V. Hoyle and H.G. Baumert *et al.*, 1995. Inhibitory action of PPADS on relaxant responses to adenine nucleotides or electrical field stimulation in guinea-pig taenia coli and rat duodenum. *Br. J. Pharmacol.*, 115: 1509-1517.

- Ziganshin, A.U., C.H.V. Hoyle, X. Bo, G. Lambrecht, E. Mutschler, H.G. Baumert and G. Burnstock, 1993. PPADS selectively antagonizes P_{2X}-purinoceptor-mediated responses in the rabbit urinary bladder. *Br. J. Pharmacol.*, 110: 1491-1495.
- Ziganshin, A.U., C.H.V. Hoyle and G. Burnstock, 1994a. Ecto-enzymes and metabolism of extracellular ATP. *Drug Dev. Res.*, 32: 134-146.
- Ziganshin, A.U., C.H.V. Hoyle, G. Lambrecht, E. Mutschler and H.G. Baumert *et al.*, 1994b. Selective antagonism by PPADS at P_{2X}-purinoceptors in rabbit isolated blood vessels. *Br. J. Pharmacol.*, 111: 923-929.
- Ziganshin, A.U., A.V. Rychkov, L.E. Ziganshina, Y.C. Kim, E. Kamaioni, G. Burnstock and K.A. Jacobson, 1998. Some 6-phenylazopyridoxal phosphate derivatives influence P₂-purinoreceptor-mediated effects. *Pharm. Chem. J.*, 32: 399-401.
- Ziganshin, A.U. and L.E. Ziganshina, 2009. [P2 receptors: A promising target for future drugs]. Geotar-Media, Moscow, Russia, (In Russian).