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Synthesis and P2Y Receptor Activity of Nucleoside 5'-Phosphonate Derivatives

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Abstract

Ribose-based nucleoside 5'-diphosphates and triphosphates and related nucleotides were compared in their potency at the P2Y receptors with the corresponding nucleoside 5'-phosphonate derivatives. Phosphonate derivatives of UTP and ATP activated the P2Y₂ receptor but were inactive or weakly active at P2Y₄ receptors. Uridine 5'-(diphospho)phosphonate was approximately as potent at the P2Y₂ receptor as at the UDP-activated P2Y₆ receptor. These results suggest that removal of the 5'-oxygen atom from nucleotide agonist derivatives reduces but does not prevent interaction with the P2Y₂ receptor. Uridine 5'-(phospho)phosphonate as well as the 5'-methylphosphonate equivalent of UMP were inactive at the P2Y₄ receptor and exhibited maximal effects at the P2Y₂ receptor that were ≤50% of that of UTP suggesting novel action of these analogues.

The P2Y receptors are a family of eight G protein-coupled receptors (GPCRs) of class A that respond to diverse nucleotides.¹ The human P2Y₁, P2Y₁₂, and P2Y₁₃ receptors are activated preferentially by ADP, while the human P2Y₁₁ receptor is activated preferentially by ATP. The human P2Y₄, P2Y₆, and P2Y₁₄ subtypes respond exclusively to various uracil nucleotides, and the human P2Y₂ receptor responds to both UTP and ATP. Depending on the subtype, the effector coupling of these receptors is typically through G_q or G_i proteins, to stimulate phospholipase or to inhibit of adenylate cyclase, respectively.

P2Y receptors are targets for therapeutic approaches, some of which are currently being explored at an early stage.² Several clinical applications of P2Y receptor ligands are more advanced, such as P2Y₁₂ receptor antagonists as antithrombotic agents³ and P2Y₂ receptor agonists as drugs for cystic fibrosis and dry eye disease.⁴

To aid in the design of P2Y receptor ligands, we have reported rhodopsin-based molecular models of all known subtypes of P2Y receptors.^{5,6} Various amino acid residues have been

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Supporting material: Procedure for biological assays, the synthetic route for compounds **5** and **6**, and detailed procedures for compounds **3** - **7** are provided.

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proposed to coordinate the bound nucleotide ligands in P2Y₁ receptors and other P2Y subtypes. The medicinal chemistry of P2Y receptors that are responsive to uracil nucleotides has recently been explored.^{7–11} The conformational constraint or replacement of the hydroxyl moieties of the ribose moiety have been introduced as a means for increasing the selectivity at P2Y₁, P2Y₂, or P2Y₆ receptors.^{12,13}

In the present study, we have explored the removal of the 5'-oxygen or its replacement by carbon moieties, resulting in 5'-phosphonate derivatives. The activity of various phosphonate derivatives was compared with the native ribosides at various P2Y receptor subtypes. Potency was best preserved at the P2Y₂ receptor following this modification. However, the apparent efficacy of activation of the P2Y₂ receptor by several phosphonate derivatives was variable and significantly less than 100%, suggesting a potential change in the mode of interaction with the receptor. The introduction of the phosphonate linkage is also intended to increase stability of the alpha-phosphate toward hydrolysis by nucleotidases, which is frequently a limitation in the pharmacological use of nucleotide derivatives in *in vitro* and *in vivo* experiments.

Various adenine and uracil nucleotide phosphonate derivatives were synthesized and evaluated in functional assays of different P2Y receptors (Table 1). The derivatives in which the 5'-oxygen was omitted, i.e., **1**, **2**, and **4–6**, were prepared by known synthetic routes.¹⁴ The synthetic route to the elongated 5'-phosphonate derivatives **3** and **7** is shown in Scheme 1 following earlier reported strategies.^{15,19} 5', 6'-Vinyl phosphonate **9** was synthesized by oxidation of 2', 3'-*O*-isopropylideneuridine (**8**) to the 5'-aldehyde intermediate, which was immediately reacted with freshly prepared [(diethoxyphosphinyl)methylidene] triphenylphosphorane.¹⁶ The *E*-configuration of the resulting alkene could be inferred from the large coupling constant (³*J* = 17.3 Hz). Catalytic hydrogenation of the resulting vinyl phosphonate ester **9** in the presence of palladium on carbon gave the corresponding saturated phosphonate ester **10**. Simultaneous deprotection of the phosphonate diester and the acetonide was achieved upon treatment with TMSBr, yielding the corresponding 5'-phosphonate **7**.

To prepare the (diphospho)phosphonate derivative **3**, vinyl phosphonate **11**, obtained by hydrolysis of both the phosphonate esters and the acetonide upon treatment with TMSBr,¹⁵ was activated with CDI in DMF, followed by the addition of bis(tri-*n*-butylammonium) pyrophosphate.

For biological assays, the human P2Y₁, P2Y₂, P2Y₄, and P2Y₆ receptors, which are coupled to activation of phospholipase C (PLC) through G_q, were stably expressed in 1321N1 human astrocytoma cells, which lack a native response to nucleotides.^{17,18} Activity at the human P2Y₁₄ receptor was followed by quantification of phosphoinositide hydrolysis in COS-7 cells coexpressing a phospholipase C-activating chimeric G protein, Gαq/i, which is activated by G_i-coupled receptors.²⁰ Concentration effect curves were generated for each of the phosphonate derivatives at the relevant receptor(s), and the EC₅₀ values were compared to the potency of the corresponding nucleotide in the native riboside series (Table 1). Representative data for the uridine 5'-(diphospho)phosphonate **2** and the vinylciddiphosphophosphonate **3** at the P2Y₂ receptor are illustrated in Fig. 1.

Full agonist activity was observed with **1**, **2**, and **3** at the P2Y₂ receptor, although the potencies were reduced by 16–176-fold relative to ATP or UTP. Although UTP is also a potent agonist at the human P2Y₄ receptor, neither uridine 5'-(diphospho)phosphonate **2**, which is the simple phosphonate equivalent of UTP, nor the vinyl phosphonate derivative **3** exhibited activity at this receptor. Compounds **2** and **3** were inactive at the P2Y₆ receptor. Uridine 5'-phosphophosphonate **4**, which is the simple phosphonate equivalent of UDP, was a full agonist at the P2Y₆ receptor exhibiting a potency approximately 40-fold less than UDP. In contrast,

uridine 5'-(glucose-[1']phospho)-phosphonate **6** exhibited no activity at the UDP-glucose activated P2Y₁₄ receptor.

A surprising result from our tests of activity was that uridine 5'-phosphophosphonate **4** was essentially as potent for activation of the P2Y₂ receptor as for activation of the P2Y₆ receptor and had no activity at the P2Y₄ receptor. However, the maximal effect observed with **4** for activation of the P2Y₂ receptor was only ~50% of that observed with UTP (Fig. 2A). Although UMP has no effect, uridine 5'-methylene-phosphonate **7** also was a relatively potent (EC₅₀ = 1.6 ± 0.4 μM) agonist at the P2Y₂ receptor. Similar to **4**, the maximal effect observed at the P2Y₂ receptor with **7** was <50% of that observed with UTP (Fig. 2B). Analogue **7** had no effect on the P2Y₄ and P2Y₆ receptors.

Preliminary experiments examining the capacity of high concentrations of compounds **4** and **7** to antagonize activation of the P2Y₂ receptor by UTP failed to provide convincing evidence that these molecules interact with the orthosteric binding site of the receptor. Therefore, these novel analogues potentially activate the P2Y₂ receptor through an allosteric mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Activity of agonists **2** and **3** at P2Y₂ receptors as indicated by activation of PLC in stably transfected astrocytoma cells. The effect of UTP corresponds to 100%.

Figure 2. Partial agonist activity of **4** (A) and **7** (B) at P2Y₂ receptors as indicated by activation of PLC in stably transfected astrocytoma cells. The effect of UTP corresponds to 100%.

Scheme 1.

Reagents and conditions: (a) (i) IBX, CH₃CN; (ii) (EtO)₂P(O)C=PPh₃, DMSO (44%); (b) H₂, Pd/C, MeOH (84%); (c) TMSBr, CH₂Cl₂ (62%); (d) Bu₃N, CDI, DMF; (Bu₃NH)₂H₂P₂O₇; TEAB 1M (22%); (e) TMSBr, CH₂Cl₂ (65%).

Table 1

In vitro

pharmacological data for various nucleotide 5'-phosphonate derivatives analogues and the corresponding native ribosides (X = OCH₂) in the stimulation of PLC at recombinant human P2Y receptors expressed in 1321N1 astrocytoma cells.

B =			R	n	B	X	Receptor	EC ₅₀ , μM ^a	Reference comp. (X = OCH ₂) EC ₅₀ , μM ⁷⁻¹¹				
			Antagonist ¹²											
(diphospho)-phosphonate	HO	U	CH ₂	P2Y ₂	2	A	CH ₂	P2Y ₂	1.4 ± 0.6	0.085 ± 0.012	0.073 ± 0.02			
P2Y ₄				P2Y ₄				<50% at 10 μM			>10 ¹⁰			
(phospho)-phosphonate	HO	U	CH ₂	P2Y ₂	2	A	CH ₂	P2Y ₂	8.6 ± 3.1	0.049 ± 0.012	0.049 ± 0.012			
P2Y ₄				P2Y ₄				NE			0.073 ± 0.02			
(phospho)- vinylphosphonate	HO	U	<i>trans</i> -CH=CH	P2Y ₂	2	A	<i>trans</i> -CH=CH	P2Y ₂	1.0 ± 0.4	0.049 ± 0.012	0.049 ± 0.012			
P2Y ₄				P2Y ₄				<50% at 10 μM			0.09 ± 0.01 ²¹			
(phospho)- phosphonate	HO	U	CH ₂	P2Y ₂	1	A	CH ₂	P2Y ₂	3.4 ± 1.1 (partial ag)	NE	NE			
P2Y ₄				P2Y ₄				NE			NE			
P2Y ₆				P2Y ₆	1.9 ± 0.7		0.046 ± 0.020							

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n	B	X	Receptor	EC₅₀, μM^a	Reference comp. (X = OCH₃) EC₅₀, μM⁷⁻¹¹
1	U	CH ₂	P2Y ₂	<50% at 10 μM	NE ⁸
			P2Y ₄	NE	NE ⁸
			P2Y ₆	NE	1.26 ± 0.17 ^b

R

n	B	X	Receptor	EC ₅₀ , μM^a	Reference comp. (X = OCH ₃) EC ₅₀ , μM^{7-11}
1	U	CH ₃	P2Y ₁₄	NE	0.301 $\pm$ 0.07

no effect at 10 μ M.