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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 $P2Y_2$ receptor but were inactive or weakly active at $P2Y_4$ receptor. Uridine 5′-(diphospho)phosphonate was approximately as potent at the $P2Y_2$ receptor as at the UDP-activated $P2Y_6$ receptor. These results suggest that removal of the 5′-oxygen atom from nucleotide agonist derivatives reduces but does not prevent interaction with the $P2Y_2$ receptor. Uridine 5′-(phospho)phosphonate as well as the 5′-methylenephosphonate equivalent of UMP were inactive at the $P2Y_4$ receptor and exhibited maximal effects at the $P2Y_2$ 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 $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors are activated preferentially by ADP, while the human $P2Y_{11}$ receptor is activated preferentially by ATP. The human $P2Y_4$, $P2Y_6$, and $P2Y_{14}$ subtypes respond exclusively to various uracil nucleotides, and the human $P2Y_2$ 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 $P2Y_{12}$ receptor antagonists as antithrombotic agents[3] and $P2Y_2$ 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 proposed to coordinate the bound nucleotide ligands in $P2Y_1$ 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 $P2Y_1$, $P2Y_2$, or $P2Y_6$ 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 $P2Y_2$ receptor following this modification. However, the apparent efficacy of activation of the $P2Y_2$ 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 α-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, that is, **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

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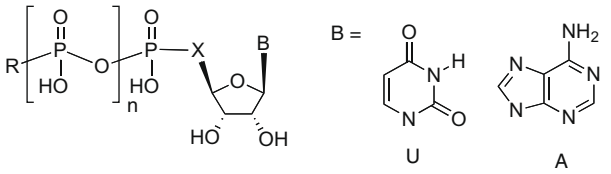
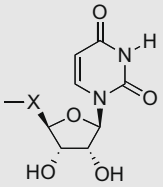
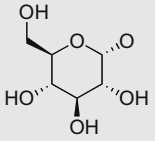
Table 1
In vitro pharmacological data for various nucleotide 5′-phosphonate analogues and the corresponding native ribosides (X = OCH_2) in the stimulation of PLC at recombinant human P2Y receptors expressed in 1321N1 astrocytoma cells

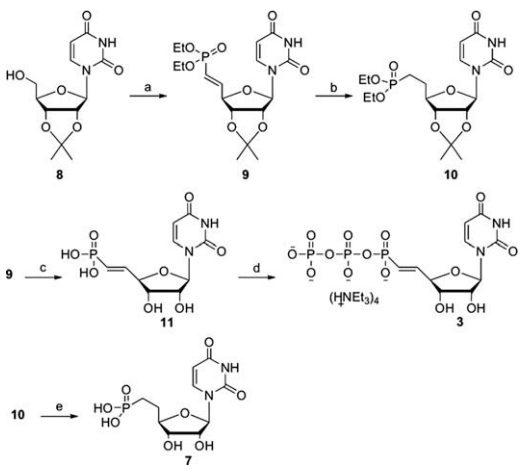


No	Name	R	*n*	B	X	Receptor	EC_{50}[a] (μM)	Reference compd (X = OCH_2) EC_{50}[7–11] (μM)
1	Adenosine 5′-(diphospho)-phosphonate	HO	2	A	CH_2	$P2Y_2$	1.4 ± 0.6	0.085 ± 0.012
						$P2Y_4$	<50% at 10 μM	Antagonist[12]
2	Uridine 5′-(diphospho)-phosphonate	HO	2	U	CH_2	$P2Y_2$	8.6 ± 3.1	0.049 ± 0.012
						$P2Y_4$	NE	0.073 ± 0.02
						$P2Y_6$	NE	>10[10]
3	Uridine 5′-(diphospho)-vinylphosphonate	HO	2	U	*trans*- CH=CH	$P2Y_2$	1.0 ± 0.4	0.049 ± 0.012
						$P2Y_4$	<50% at 10 μM	0.09 ± 0.01[21]
						$P2Y_6$	NE	NE
4	Uridine 5′-phospho-phosphonate	HO	1	U	CH_2	$P2Y_2$	3.4 ± 1.1 (partial ag)	NE
						$P2Y_4$	NE	NE
						$P2Y_6$	1.9 ± 0.7	0.046 ± 0.020
5	Di-(uridine-phosphonate)		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]
6	Uridine 5′-(glucose[1′]phospho)phosphonate		1	U	CH_2	$P2Y_{14}$	NE	0.301 ± 0.07
7	Uridine 5′-methylene-phosphonate	HO	0	U	$(CH_2)_2$	$P2Y_2$	1.6 ± 0.4 (partial ag)	NE
						$P2Y_4$	NE	NE
						$P2Y_6$	NE	NE

NE—no effect at 10 μM.

[a] Agonist potencies were calculated using a four-parameter logistic equation and the GraphPad software package (GraphPad, San Diego, CA). EC_{50} values (mean ± standard error) represent the concentration at which 50% of the maximal effect was achieved. A maximal effect (100% apparent efficacy) equivalent to that of the cognate agonist for the tested receptor was observed unless where indicated for two compounds (**1** and **3**) that exhibited <50% of maximal effect at the highest 10 μM concentration tested at the $P2Y_4$ receptor and for two compounds (**4** and **7**) that produced apparently saturable activation that was much less than that observed with UTP at the $P2Y_2$ receptor. The data for **4** and **7** are denoted as consistent with partial agonist (partial ag.) activity. *N* = 4–6 concn-effect curves for each analogue.

[b] Up_2U.



Scheme 1. Reagents and conditions: (a) (i) IBX, CH_3CN; (ii) $(EtO)_2P(O)C{=}PPh_3$, DMSO (44%); (b) H_2, Pd/C, MeOH (84%); (c) TMSBr, CH_2Cl_2 (62%); (d) Bu_3N, CDI, DMF; $(Bu_3NH)_2H_2P_2O_7$; TEAB 1 M (22%); (e) TMSBr, CH_2Cl_2 (65%).

inferred from the large coupling constant (^3J = 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 $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ 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 $P2Y_{14}$ 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

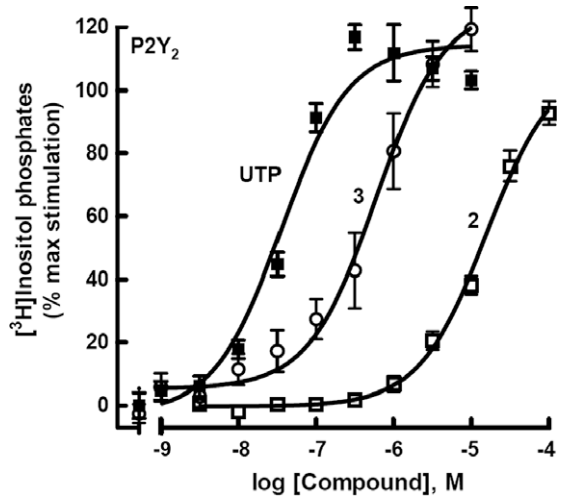


Figure 1. Activity of agonists **2** and **3** at $P2Y_2$ receptors as indicated by activation of PLC in stably infected astrocytoma cells. The effect of UTP corresponds to 100%.

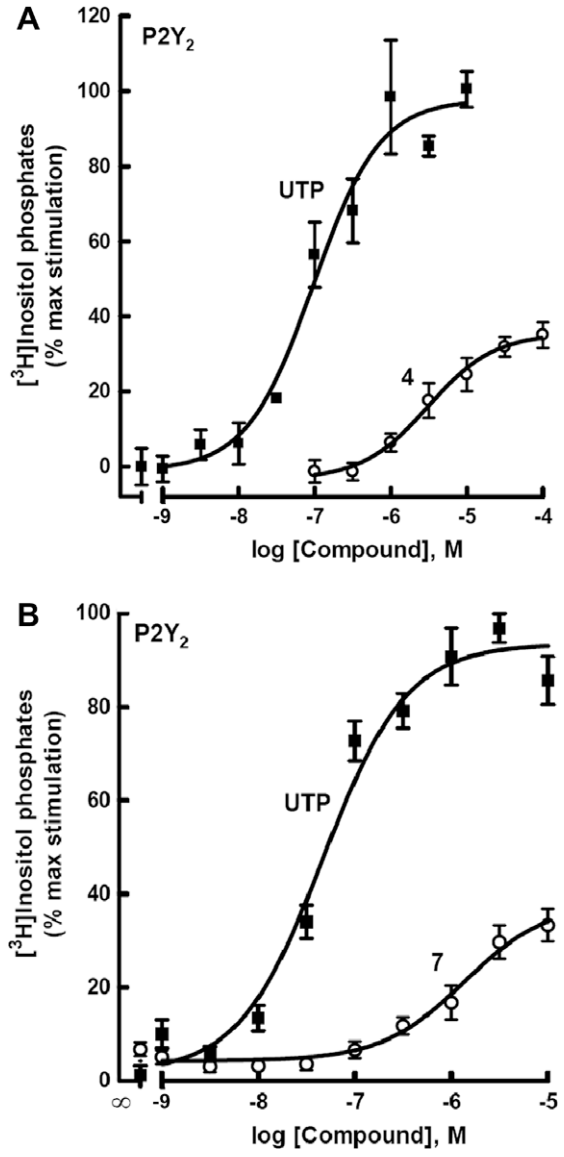


Figure 2. Partial agonist activity of **4** (A) and **7** (B) at $P2Y_2$ receptors as indicated by activation of PLC in stably infected astrocytoma cells. The effect of UTP corresponds to 100%.

relevant receptor(s), and the EC_{50} 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 vinylic diphosphophosphonate **3** at the $P2Y_2$ receptor are illustrated in Fig. 1.

Full agonist activity was observed with **1**, **2**, and **3** at the $P2Y_2$ 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 $P2Y_4$ 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 $P2Y_6$ receptor. Uridine 5′-phosphophosphonate **4**, which is the simple phosphonate equivalent of UDP, was a full agonist at the $P2Y_6$ 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 $P2Y_{14}$ receptor.

A surprising result from our tests of activity was that uridine 5′-phosphophosphonate **4** was essentially as potent for activation of the $P2Y_2$ receptor as for activation of the $P2Y_6$ receptor and had no activity at the $P2Y_4$ receptor. However, the maximal effect observed with **4** for activation of the $P2Y_2$ 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 (EC_{50} = 1.6 ± 0.4 μM) agonist at the $P2Y_2$ receptor. Similar to **4**, the maximal effect observed at the $P2Y_2$ receptor with **7** was <50% of that observed with UTP (Fig. 2B). Analogue **7** had no effect on the $P2Y_4$ and $P2Y_6$ receptors.

Preliminary experiments examining the capacity of high concentrations of compounds **4** and **7** to antagonize activation of the $P2Y_2$ 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 $P2Y_2$ receptor through an allosteric mechanism.

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Supplementary data

Supplementary data (procedure for biological assays, the synthetic route for compounds **5** and **6**, and detailed procedures for compounds **3**–**7** are provided) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.027.

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