Tools and drugs for uracil nucleotide-activated P2Y receptors

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Contents

Abbreviations: ADP, Adenosine 5′-diphosphate; AP_4A, P^1,P^4-Di(adenosine-5′)-tetraphosphate; AR, Adenosine receptor; AR-C118925, 5-{[5-(2,8-Dimethyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)-3,4-dihydro-2-oxo-4-thioxo-1(2*H*)-pyrimidinyl]methyl}-*N*-(1*H*-tetrazol-5-yl)-2-furancarboxamide; ATP, Adenosine 5′-triphosphate; ATPγS, Adenosine 5′-(γ-thio)-triphosphate; BODIPY®, Boron-dipyrromethene; cAMP, 3′,5′-Cyclic adenosine monophosphate; CDP, Cytidine 5′-diphosphate; CFTR, Cystic fibrosis transmembrane conductance regulator; CNS, Central nervous system; CTP, Cytidine 5′-triphosphate; DAG, Diacylglycerol; DTPA, Diethylenetriaminepentaacetic acid; GDP, Guanosine 5′-diphosphate; GI tract, Gastrointestinal tract; GPCR, G protein-coupled receptor; GTP, Guanosine 5′-triphosphate; HEK, Human embryonic kidney; IDP, Inosine 5′-diphosphate; IMP, Inosine 5′-monophosphate; INS48823, P^1-((2-Benzyl-1,3-dioxolo-4-yl)uridine-5′-)-P^3-(uridine-5′-)-triphosphate;monobenzylacetal-Up_3U; IP_3, Inositol 1,4,5-trisphosphate; ITP, Inosine 5′-triphosphate; Me, Methyl; MeO, Methoxy; MeS, Methylthio; MRS2567, 1,2-Di-(4-isothiocyanatophenyl)ethane; MRS2578, *N*,*N*″-1,4-Butanediyl-*bis*[*N*′-(3-isothiocyanatophenyl)thiourea]; MRS2957, N^4-MeO-Cp_3U; P^1-(uridine 5′-)-P^3-(N^4-methoxycytidine-5′-)-triphosphate; n.d., No data was found; NECA, 5′-(*N*-Ethylcarboxamido)adenosine; P2YR, P2Y receptor; PAMAM, Polyamidoamine; PIP_2, Phosphatidylinositol 4,5-bisphosphate; PPADS, Pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; PPTN, 4-(4-(Piperidin-4-yl)-phenyl)-7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid; PSB-16133, Sodium 1-amino-4-[4-(2,4-dimethylphenylthio)]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate; PSB-716, 1-Amino-4-(2-methoxyphenyl)-2-sulfoanthraquinone; RB-2, Reactive Blue 2; SAR, Structure-activity relationship; TDP, Thymidine 5′-diphosphate; TIM-38, 3-Nitro-2-(trifluoromethyl)-2*H*-chromene; TMPS, Thymidine 5′-*O*-monophosphorothioate; TTP, Thymidine 5′-triphosphate; UDP, Uridine 5′-diphosphate; UDPβS, Uridine 5′-(β-thio)-diphosphate; UMP, Uridine 5′-monophosphate; UMPS, Uridine 5′-*O*-monophosphorothioate; UP_4U, P^1,P^4-Di(uridine-5′)-tetraphosphate; UTP, Uridine 5′-triphosphate; UTPγS, Uridine 5′-(γ-thio)-triphosphate.

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1. Introduction

1.1. Purinergic and pyrimidinergic receptors

The first reported observation that purines are capable of evoking cellular responses was published in 1929, when Drury and Szent-Györgyi investigated the effects of adenosine and adenosine 5′-monophosphate on the mammalian heart (Drury & Szent-Györgyi, 1929). However, it was not until 1976 that the existence a distinct family of purinergic receptors was proposed (Burnstock, 1976). Shortly thereafter, the distinction into P1 (adenosine receptors, xanthine-sensitive) and P2 subfamilies (nucleotide receptors, not blocked by xanthine derivatives) was made. A third class, P0 (activated by the nucleobase adenine), was later discovered in rodents through reverse pharmacology (Bender et al., 2002; Brunschweiger & Müller, 2006). Both P0 and P1 receptors are G protein-coupled receptors (GPCRs). The P2 family is subdivided into the metabotropic P2Y receptors (P2YRs) and the ligand-gated ion channels known as P2X receptors (Abbracchio & Burnstock, 1994). The latter group consists of seven subunits, termed P2X1-7, which form homo- or heterotrimeric ligand-gated cation channels (Aschrafi, Sadtler, Niculescu, Rettinger, & Schmalzing, 2004; Kaczmarek-Hájek, Lorinczi, Hausmann, & Nicke, 2012). They share little sequence homology with other proteins and form, besides Cys-loop and glutamate-gated channels, a third major family of ionotropic receptors (North, 1996). P2X channels are cation-selective (mainly Ca^{2+} and Na^{+}, but also K^{+}) and are activated by the nucleotide adenosine 5′-triphosphate (ATP) (Coddou, Yan, Obsil, Huidobro-Toro, & Stojilkovic, 2011; Kaczmarek-Hájek et al., 2012).

1.2. P2Y receptors

P2YRs belong to the δ-branch of class A rhodopsin-like GPCRs. They have an extracellular, glycosylated *N*-terminus, the characteristic seven hydrophobic transmembrane domains, three intra- and three extracellular loops, and an intracellular *C*-terminus that possesses consensus binding motifs for protein kinases. The mammalian P2YR family consists of eight subtypes: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$. The numbers missing in the sequence (p2y3, p2y5, p2y7-10) account for non-mammalian receptors or for GPCRs that have a certain degree of sequence homology but do not appear to bind nucleotides. Examples include the chicken p2y3 receptor and the *Xenopus laevis* p2y8 receptor, which may be homologs of the mammalian $P2Y_6R$ and the $P2Y_4R$, respectively (Bogdanov, Dale, King, Whittock, & Burnstock, 1997; Li, Olesky, Palmer, Harden, & Nicholas, 1998; Webb et al., 1996). The p2y7 and the p2y9 receptor are a leukotriene B_4 and a lysophosphatidic acid receptor (Akbar et al., 1996; Herold, Li, Schachter, Harden, & Nicholas, 1997; Noguchi, Ishii, & Shimizu, 2003). It is possible that further human P2YR subtypes have not been identified yet, as several orphan receptors share some sequence similarity with current P2YR members. It has to be noted that sequence homology between the current members of the P2YR family is moderate to low (21-48 % sequence identity) (Abbracchio et al., 2006).

The human P2YRs can be further categorized into two groups based on sequence homology and the type of G protein they are primarily coupled to (Fig. 1) (Costanzi, Mamedova, Gao, & Jacobson, 2004). The $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors signal mainly through $G_{q/11}$ with subsequent activation of phospholipase C_β. This enzyme in turn hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2), producing the second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 and DAG mediate the release of calcium ions from intracellular stores and activate protein kinase C, respectively. The $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors signal preferentially through $G_{i/o}$, thereby inhibiting adenylate cyclase and reducing 3′,5′-cyclic adenosine monophosphate (cAMP) levels (Abbracchio et al., 2006).

However, P2YR signaling is more complex and coupling to other second messenger systems has been described: $P2Y_2$ was reported to couple to G_0 and G_{16}, $P2Y_{11}$ to G_s, and $P2Y_2$, $P2Y_6$, and $P2Y_{12}$ were found to activate Rho through $G_{12/13}$ (Bagchi et al., 2005; Baltensperger & Porzig, 1997; Liao, Seye, Weisman, & Erb, 2007; Nishida et al., 2008; Sauzeau et al., 2000). Furthermore, P2YRs may form homo- or hetero-oligomers, as is the case for other GPCRs (a database of GPCR oligomers can be found at www.gpcr-okb.org (Khelashvili et al., 2010; Skrabanek et al., 2007)). This may alter their pharmacological, signaling, desensitization, and trafficking properties. The formation of $P2Y_6R$ homodimers was described (Kawashita, Tsuji, Kanno, Tsuchida, & Itoh, 2016). The $P2Y_1R$ and the $P2Y_2R$ were observed to coimmunoprecipitate with the A_1 adenosine receptor (A_1AR) in cotransfected human embryonic kidney (HEK) 293T cells (Suzuki, Namba, Tsuga, & Nakata, 2006; Yoshioka, Saitoh, & Nakata, 2001). The results suggested that the A_1AR can form heteromers with either one, the $P2Y_1R$ and the $P2Y_2R$. The formation of $A_1AR/P2Y_1R$ heteromers was also observed *in vivo* in colocalizaion and coimmunoprecipitation studies in rat brain tissue (Yoshioka, Hosoda, Kuroda, & Nakata, 2002). The heteromeric receptors were shown to possess novel pharmacological properties: $A_1AR/P2Y_1R$ heteromers behaved like A_1ARs with respect to signaling but showed the agonist profile of $P2Y_1Rs$. The $P2Y_1R$ agonist adenosine 5′-β-thio-diphosphate (ADPβS) displaced the adenosine receptor agonist [^{3}H]5′-(*N*-ethylcarboxamido)adenosine ([^{3}H]NECA) from its binding site on the A_1AR and induced $G_{i/0}$-mediated signaling, which is typical for A_1ARs (Yoshioka et al., 2001). However, the selective allosteric $P2Y_1R$ antagonist N^6-methyl-2′-deoxyadenosine 3′,5′-bisphosphate (MRS2179) failed to compete with [^{3}H]NECA for its binding site on the $A_1/P2Y_1$ heteromeric receptor (Yoshioka et al., 2001). In $A_1AR/P2Y_2R$ heteromers, binding of [^{3}H]NECA and of the selective A_1AR agonist [^{3}H]2-chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA) was inhibited by the $P2Y_2R$ agonists ATP and uridine 5′-triphosphate (UTP) (Suzuki et al., 2006). UTP prevented the $G_{i/0}$-mediated inhibition of adenylate cyclase in $A_1AR/P2Y_2R$ heteromers that is normally observed as a result of A_1AR activation by an A_1AR agonist. $P2Y_2R$-mediated intracellular Ca^{2+} release *via* $G_{q/11}$ was synergistically enhanced by the simultaneous activation of the $A_1AR/P2Y_2R$ heteromers by the $P2Y_2R$ agonist UTP and the AR agonist NECA (Suzuki et al., 2006). Heteromerization within the P2YR family appears to exist as well. Coimmunoprecipitation and Förster resonance energy transfer (FRET) experiments provided evidence for $P2Y_1R/P2Y_2R$, $P2Y_1R/P2Y_4R$, and $P2Y_1R/P2Y_{11}R$ heteromers (Ecke et al., 2008; Ribeiro-Filho et al., 2016). The physiological implications of P2YR heteromerization needs to be further elucidated, but it appears likely that it leads to allosteric modulation altering agonist affinity and specificity, and impacting intracellular signaling pathways.

1.3. P2Y receptor ligands

Another approach to categorizing the human P2YR family is based on their preference for natural ligands. The $P2Y_1$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ receptors are activated by adenine di- or triphosphates. In contrast, uracil nucleotides are the endogenous agonists for the $P2Y_4$, $P2Y_6$, and $P2Y_{14}$ receptors. The $P2Y_2R$ responds to both ATP and UTP. The P2 receptor family was initially named P2 purinoceptors. With the discovery of the uracil nucleotide-activated subtypes, the inadequacy of this nomenclature became evident and the receptor family was renamed accordingly (purine and pyrimidine receptors). The pronounced differences in agonist preferences, which is uncommon in other GPCR families, likely results from the relatively low sequence homology shared between members of the P2YR family. Yet, it has proven difficult with most P2YR subtypes to develop potent agonists and antagonists that are highly selective over other subtypes. A major issue with many current P2YR ligands – mainly concerning agonists but also some classes of antagonists – is their low oral bioavailability due to several negative charges (e.g. phosphate or sulfonate groups that are deprotonated at physiological pH). Nucleotides are subject to degradation by ectonucleotidases, which results in the production of nucleoside di- or monophosphates and eventually leads to the

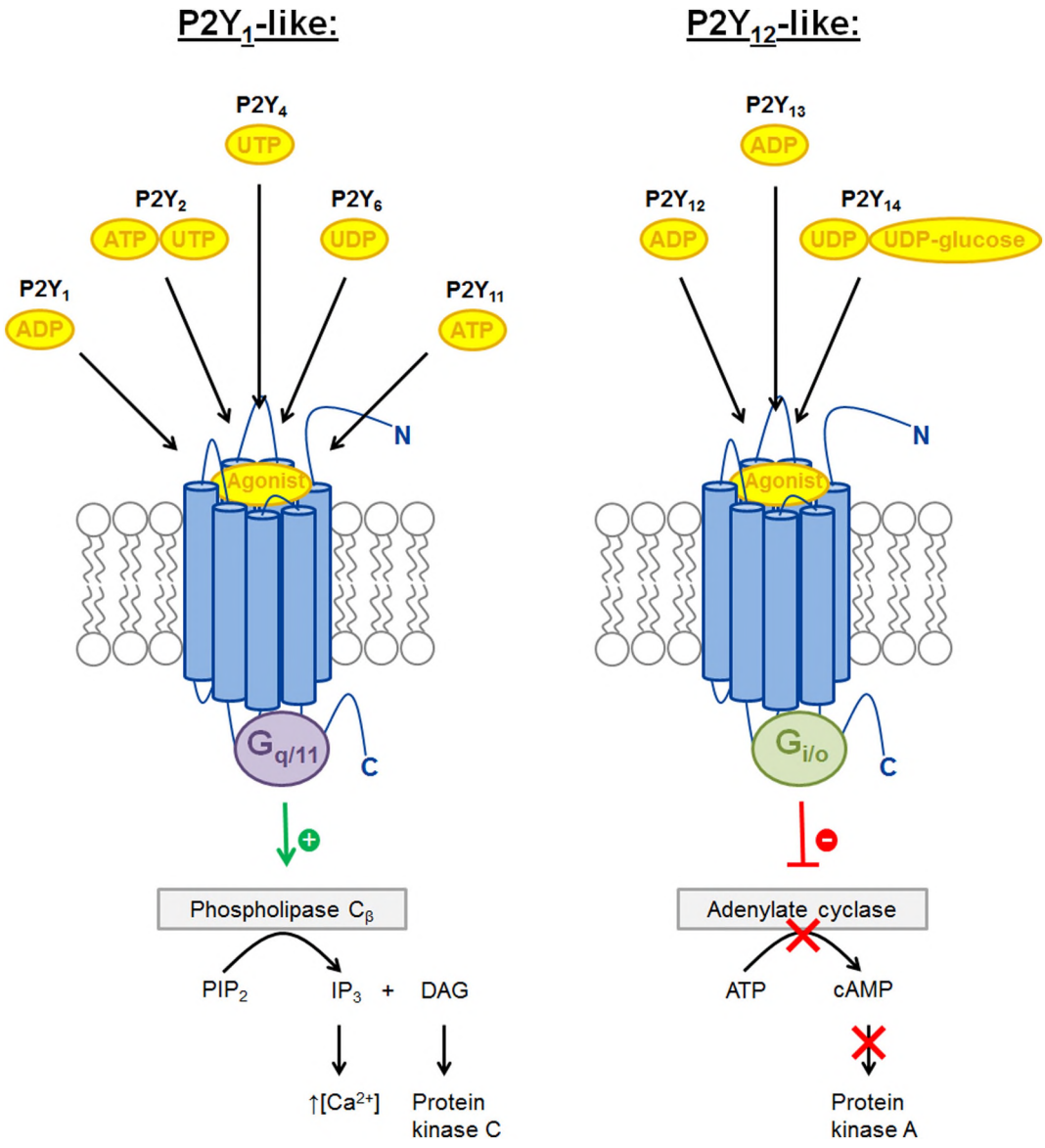


Fig. 1. Overview of the P2YR subtypes categorized into two groups based on sequence homology and main signaling cascades.

formation of the nucleosides adenosine or uridine (Harden, Lazarowski, & Boucher, 1997; also see reviews by Zimmermann, 2016 and Zimmermann, Zebisch, & Sträter, 2012). Some of these metabolites in turn can activate other receptors and thereby cause ambiguity in pharmacological experiments. Moreover, ectokinases may phosphorylate nucleosides and nucleotides, further causing difficulties in the correct interpretation of the measured biological responses (Harden et al., 1997; Lazarowski, Boucher, & Harden, 2000; Nicholas, Watt, Lazarowski, Li, & Harden, 1996). Additional complicating factors in cell-based experiments could also be the result of nucleotide release *via* connexins, pannexins, maxi-channels, ATP-binding cassette transporters, exocytosis of secretory granules, or vesicular transport. This may occur in response to stress experienced by the cells, for example through physical movement (e.g. shaking), hypoxia, or changes in pH or nutrient medium (Lazarowski, Boucher, & Harden, 2003; Lazarowski, Watt, Stutts, Boucher, & Harden, 1995; for more information, refer to the recent review by Burnstock & Knight, 2017). In addition, nucleotides are released from cells that undergo necrosis or apoptosis, acting as signalling molecules to promote phagocytic clearance of the dying cells (Elliott et al., 2009; Idzko et al., 2007). To complicate matters even further, some commercial preparations of nucleoside diphosphates were found to contain significant amounts of the corresponding nucleoside triphosphates, and *vice versa*. This had previously led to incorrect conclusions regarding pharmacological properties of P2Y receptors (Nicholas et al., 1996).

1.4. Scope and structure of this review

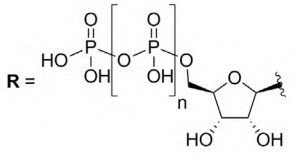
Given the significant clinical potential of the P2YR family, substantial research efforts directed at developing P2YR ligands for use as pharmacological tools and drugs have led to the discovery and development of a significant number of agonists but so far only a moderate number of antagonists. An exception is the $P2Y_{12}R$, for which antagonists are already marketed as antithrombotic drugs. The present review summarizes the current state of ligand development for the class of uracil nucleotide-activated P2Y receptors – the $P2Y_2R$, $P2Y_4R$, $P2Y_6R$, and $P2Y_{14}R$ – and discusses the compounds' advantages and limitations. In addition, a series of tables provide a comprehensive overview of the available receptor ligands, their structures and their properties, to allow for direct comparison. For simplicity, compounds are represented as free acids or free bases. It should be noted, however, that they are synthesized, purchased and tested in their more stable salt forms. The

Table 1
Overview of the human uracil nucleotide-activated P2YR subtypes.

Receptor	Entrez gene ID	Uniprot ID[a]	Amino acid length	Main physiological agonists	Main tissue distribution[b]	Therapeutic potential[e]
$P2Y_2$	5029	P41231	377	ATP, UTP, (Ap_4A, Up_4U)	Endocrine tissue, immune system, skeletal & cardiac muscle, lung, GI tract, male & female reproductive tract, bladder, kidney, skin[c] Prominent expression in epithelial & glandular cells[c]	**Agonists**: Neurodegenerative disorders, dry eyes, ocular hypertension, retinal degeneration, cystic fibrosis, myocardial infarction **Antagonists**: Atherosclerosis, nephrogenic diabetes insipidus, psoriasis, osteoporosis, cancer, inflammation, pain
$P2Y_4$	5030	P51582	365	UTP, (Up_4U)	GI tract; lower expression in CNS, lung, heart, prostate, skin, adipose tissue, skeletal muscle, spleen & immune cells[d]	**Agonists**: Neurodegenerative disorders, cystic fibrosis **Antagonists**: Neurodegenerative disorders, myocardial infarction, constipation, diarrhea, cancer
$P2Y_6$	5031	Q15077	328	UDP, (Up_3U)	Spleen, placenta, kidney; lower expression in CNS, lung, heart, GI tract, adipose tissue, bone[d]	**Agonists**: Neurodegenerative disorders, ocular hypertension, glaucoma, cystic fibrosis, blood pressure, cancer, diabetes, infectious diseases **Antagonists**: Neurodegenerative disorders, cerebral vasospasms, cardiac hypertrophy, cardiac fibrosis, atherosclerosis, inflammation, pain, cancer, obesity
$P2Y_{14}$	9934	Q15391	338	UDP, UDP-glucose	CNS, endocrine tissue, immune system, muscle (skeletal, cardiac, smooth), lung, pancreas, GI tract, kidney, bladder, male & female reproductive tract, skin[c]	**Agonists**: Autoimmune diseases, diabetes **Antagonists**: Inflammation, diabetes, osteoporosis

[a] UniProt Consortium, 2015.
[b] Moore et al., 2001; Uhlén et al., 2015.
[c] Detected at both protein and mRNA level.
[d] Detected at mRNA level.
[e] Refer to the text for more details and for references.

most important compounds listed in these tables will be discussed in more detail. Agonists are collected in Tables 2 to 9, categorized according to structural modifications. These include physiological mononucleotides (Fig. 2 and Table 2) and synthetic derivatives thereof, with substitutions at the nucleobase (Fig. 3 and Table 3), the ribose moiety (Fig. 4 and Table 4), or the phosphate chain (Fig. 5 and Table 5). Nucleotide derivatives with combined substitutions at different sites are depicted in Fig. 6 and Table 6. Physiological dinucleotides and synthetic analogues are listed in Fig. 7 and Table 7. Nucleotide sugars are summarized in Fig. 8 and Table 8, while non-nucleotide agonists reported very recently are shown in Fig. 9 and Table 9. For each compound, published EC_{50} values and, where available, K_i or K_B values at the $P2Y_2R$, $P2Y_4R$, $P2Y_6R$, and $P2Y_{14}R$ are presented. These potencies are given as a range if more than two different values for a compound were found in the literature. Since EC_{50} values of GPCRs are influenced by receptor expression and can vary significantly between different test systems (Fujioka & Omori, 2012), a direct comparison is facilitated by means of a correlation factor given in brackets. This factor expresses the multiplicity by which the potency of the compound directly compares to that of the endogenous agonists ATP (superscript a), UTP (superscript b), UDP (superscript c), or UDP-glucose (superscript d) assessed in the same assay system. The correlation factor for a compound that is n-fold more potent (i.e. smaller EC_{50}, K_i, or K_B) is shown as $(\uparrow nx)^{a,b,c, \text{ or } d}$, while compounds of n-fold lower potency (i.e. larger EC_{50}, K_i, or K_B) are described



2-01 ATP n = 2
2-02 ADP n = 1
2-03 GTP n = 2
2-04 GDP n = 1
2-05 ITP n = 2
2-06 IDP n = 1
2-07 IMP n = 0
2-08 UTP n = 2
2-09 UDP n = 1
2-10 UMP n = 0
2-11 CTP n = 2
2-12 CDP n = 1
2-13 TTP n = 2
2-14 TDP n = 1

Fig. 2. Structures of physiological mononucleotides (for data, refer to Table 2).

as (↓nx)[a,b,c, or d]. In cases where large series of structural analogues were published, only the most important compounds are shown.

The development of antagonists for the uracil nucleotide-activated P2YRs has yielded significantly fewer compounds so far as compared to the number of published agonists, but they display greater structural variability (Fig. 10 and Table 10). The antagonists were organized according to receptor selectivity. Antagonist potencies are mostly given as IC_{50} values; in some cases they are shown as pA_2, K_i, K_B values, or as the percentage of inhibition at a given concentration.

Table 2
Physiological mononucleotides for the uracil-activated P2YRs. Shown are the EC_{50} or, in some cases, K_i and K_B values in μM at the human receptor, unless stated otherwise. The correlation factor by which the potency is n-fold higher (↑nx) or lower (↓nx) compared to that of the endogenous agonist in the same test system is given in brackets. Particularly interesting values are shown in bold.

Physiological mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
2-01	ATP	0.015 - 0.230 (1x-↓4x)[b] **Mouse:** 0.7 - 17.7 (↑2x-↓14x)[b] **Rat:** 2.7 (1x)[b] 0.2 (1x)[b] **Dog:** ≈0.2 (1x)[b] ≈2 (1x)[b] **Pig:** 2.7 (↓5x)[b] 80 % efficacy	antagonist (see Table 10) **Mouse:** 0.435 (1x[a] / ↓2x[b]) 0.7 (1x[a] / ↓2x[b]) **Rat:** 0.51 - 1.8 (1x[a] / 1x-↓3x[b])	>3 mM (↓>10,000x)[c] **Mouse:** inactive at 100 μM (↓>2300x)[c] **Rat:** 500 (n.d.)[c] inactive (n.d.)[c]	0.252 (↓100x[c] / ↓115x[d]) inactive at 1 μM (n.d.[c] / ↓>10x[d])		Bogdanov et al., 1998; Chambers et al., 2000; Chang et al., 1995; Chen, Krull, Xu, Levy, & Lightman, 1996; Communi, Parmentier et al., 1996; Erb, Lustig, Sullivan, Turner, & Weisman, 1993; Filippov, Webb, Barnard, & Brown, 1999; Hamel et al., 2011; Ivanov, Fricks, Kendall Harden, & Jacobson, 2007; Jacobson et al., 2006; Janssens, Paindavoine, Parmentier, & Boeynaems, 1999; Kennedy et al., 2000; Knoblauch et al., 1999; Lazarowski et al., 1995; Lazarowski et al., 2001; Lazarowski & Harden, 1994; Lustig et al., 1993; Nicholas et al., 1996; Sakuma et al., 2017; Shaver et al., 2005; Shen et al., 2004; Suarez-Huerta, Pouillon, Boeynaems, & Robaye, 2001; Webb, Henderson, Roberts, & Barnard, 1998; Wildman, Unwin, & King, 2003; Zambon et al., 2000

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Table 2 (*continued*)

Physiological mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
2-02	ADP	inactive at 100 μM (↓500 - >6600x[a] / ↓1000 - >6600x[b]) **Mouse:** ≈100 (n.d.)[a b]; >100 (↓>140x[a] /↓>90x[b]) partial agonist **Rat:** 9 (↓45x)[a b] **Dog:** ≈10 (↓5x[a] / ↓5x[b]) **Pig:** 29.3 (↓11x[a] / ↓55x[b])	inactive at 100 μM (↓>500x)[b] partial agonist with 15 % efficacy **Mouse:** inactive at 100 μM (↓>230x[a] / ↓>390x[b]) **Rat:** partial agonist with 34 % efficacy inactive at 100 μM (↓>95x[a] / ↓>160x[b])	30 - 100 (↓100-500x)[c] inactive at 100 μM (↓>200x)[c] **Mouse:** >1 (↓>24x)[c] **Rat:** 50 (n.d.)[c] inactive (↓>130x)[c]	0.0139 (↓6x)[c,d] inactive at 1 μM (n.d.[c] / ↓>10x[d])		Bogdanov et al., 1998; Chambers et al., 2000; Chang et al., 1995; Chen et al., 1996; Communi, Parmentier et al., 1996; Filippov et al., 1999; Hamel et al., 2011; Lazarowski et al., 2001; Lazarowski & Harden, 1994; Lin, Lustig, Sportiello, Weisman, & Sun, 1993; Lustig et al., 1993; Nguyen et al., 1995; Nicholas et al., 1996; Robaye et al., 1997; Shaver et al., 2005; Shen et al., 2004; Webb et al., 1998; Zambon et al., 2000
2-03	GTP	2.64 (↓31x[a] / ↓54x[b]) 12.3 (↓53x[a] / ↓88x[b]) **Mouse:** >100 (n.d.)[a b] inative at 1 mM (↓>670x[a] / ↓>1100x[b]) **Rat:** 9.7 (↓4x[a] / ↓3x[b]) 20 (↓100x)[a b]	6.59 (↓12x)[b] inactive at 100 μM (↓>500x)[b] **Mouse:** 7 (↓16x[a] / ↓27x[b]) **Rat:** 1.4 (1x)[a,b] 2.28 (↓5x[a] / ↓11x[b])	n.d. **Rat:** inactive at 300 μM (↓>130x)[c]	0.814 (↓340x[c] / ↓370x[d])		Chen et al., 1996; Erb et al., 1993; Hamel et al., 2011; Jacobson et al., 2006; Kennedy et al., 2000; Lazarowski et al., 1995; Lazarowski et al., 2001; Lazarowski & Harden, 1994; Lin et al., 1993; Shen et al., 2004; Wildman et al., 2003

Table 2 (*continued*)

Physiological mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
		Pig: 1.3 (↑2x[a] / ↓3x[b]) 63 % efficacy					
2-04	GDP	66.0 (↓290x[a] / ↓470x[b]) **Rat:** inactive at 100 μM (↓>500x)[a,b] **Pig:** inactive at 100 μM (↓>37x[a] / ↓>190x[b])	n.d.	44.6 (↓150x)[c] **Rat:** inactive at 300 μM (↓>130x)[c] **Mouse:** >1 (↓>24x)[c]	0.0800 (↓33x[c] / ↓36x[d]) ≈30 (↓400x[c] / ↓93x[d])		Carter et al., 2009; Chen et al., 1996; Hamel et al., 2011; Lazarowski et al., 1995; Lazarowski et al., 2001; Lazarowski & Harden, 1994; Robaye et al., 1997; Shen et al., 2004
2-05	ITP	≈10 (n.d.)[a,b] **Mouse:** ca. 100 (n.d.)[a,b] **Rat:** 20.9 (↓8x[a] / ↓6x[b]) **Pig:** 1.7 (↑2x[a] / ↓3x[b])	7.38 - 32.8 (↓13x)[b] >1000 (↓>5000x)[b] **Mouse:** 2 (↓5x[a] / ↓8x[b]) **Rat:** 1.4 - 1.82 (1x-↓4x[a] / 1x-↓9x[b])	n.d.	n.d.		Bogdanov et al., 1998; Brunschweiger & Müller, 2006; Communi, Motte et al., 1996; Kennedy et al., 2000; Lazarowski et al., 2001; Lin et al., 1993; Nguyen et al., 1995; Shen et al., 2004; Wildman et al., 2003
2-06	IDP	n.d. **Pig:** 35.7 (↓13x[a] / ↓67x[b])	n.d.	34.4 (↓120x)[c]	0.010 (↓4x[c] / ↓5x[d]) inactive at 1 μM (1x[c] / ↓>10x[d])		Chambers et al., 2000; Hamel et al., 2011; Robaye et al., 1997; Shen et al., 2004
2-07	IMP	n.d.	n.d.	n.d.	5.43 (↓2300x[c] / ↓2500x[d])		Hamel et al., 2011

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Table 2 (*continued*)

Physiological mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
2-08	UTP (INS316)	0.014 - 0.64 (↑4x-1x)[a]	0.069 - 2.5	6 (↓20x)[c] >10 (↓>100x)[c]	0.0175 - 0.381 (↓7-54x[c] / ↓3-16x[d]) inactive at 1 μM (n.d.[a] / ↓>10x[d]) K_i = 0.009 (↑2x)[c,d] K_i = 0.251 (↓2x[c] / ↓4x[d])	Investigated in clinical studies to stimulate mucociliary clearance by targeting the $P2Y_2R$ in patients with mild chronic bronchitis*	Bogdanov et al., 1998; Chambers et al., 2000; Chang et al., 1995; Charlton et al., 1996b; Chen et al., 1996; Communi, Motte et al., 1996; Communi, Parmentier et al., 1996; Eliahu et al., 2009; El-Tayeb et al., 2006; Erb et al., 1993; Filippov et al., 1999, 1997, 1998; WO002009066298A1, 2008; WO002012073237A1, 2011; WO002012032513A1, 2011; Fricks et al., 2008; Guile et al., 2001; Hamel et al., 2011; Ivanov, Ko et al., 2007; Jacobson et al., 2006; Janssens et al., 1999; US020090148850A1, 2008; Kennedy et al., 2000; Kim et al., 2002; Knoblauch et al., 1999; Ko et al., 2007; Ko et al., 2008; Lazarowski et al., 1995; Lazarowski et al., 2001; Lazarowski & Harden, 1994; Lustig et al., 1993; Marucka et al., 2011; Nguyen et al., 1995; Nicholas et al., 1996; Patel et al., 2001; Pendergast et al., 2001; Sakuma et al., 2017; Shaver et al., 2005; Shen et al., 2004; Suarez-Huerta et al., 2001; Webb et al., 1998; Wildman et al., 2003; Zambon et al., 2000 *Johnson, Donohue, & Shaffer, 2002
		Mouse: 0.9 - 1.25 (↑14x- ↓2x)[a]	**Mouse:** 0.260 (↑2x)[a] 0.4 (↑2x)[a]	**Mouse:** ≈0.8 (↓20x)[c]	**Mouse:** 0.049 - 0.0303 (↓2-3x[c] / ↓4-6x[d])		
		Rat: 0.50 - 3.6 (1x)[a] 0.2 (1x)[a]	**Rat:** 0.20 - 2.6 (1x-↑3x)[a]	**Rat:** 0.020 - >100 (↓25->500x)[c]	**Rat:** competitive antagonist		
		Dog: ≈2 (1x)[a]			**Chimpanzee:** 0.0246 - 0.0445 (↓6-8x[c] / ↓4-6x[d]) K_i = 0.011 (1x[c] / ↑2x[d])		
		Pig: 0.53 (↑5x)[a]					

Table 2 (*continued*)

Physiological mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
2-09	UDP	4.20 (↓280x)[a,b] 16.5 (↓72x[a] / ↓120x[b]) inactive at 100 (↓500x[a] / ↓1000x[b]) **Mouse:** 3.21 (↑6x[a] / ↓3x[b])	9.48 - 19.5 (↓8-70x)[b] inactive at 100 μM (↓>130x)[b] **Mouse:** inactive at 100 μM	0.007 - 0.530 **Mouse:** 0.0259 - 0.042	0.0024 - 0.160 (1x-↑15x)[d] K_i = 0.014 - 0.63 (↑3-4x)[d] **Mouse:** 0.0084 - 0.018		Besada et al., 2006; Bogdanov et al., 1998; WO002007002945A2, 2006; WO002007002945A2, 2006; Brüser et al., 2017; Carter et al., 2009; Chambers et al., 2000; Chen et al., 1996; Communi, Motte et al., 1996; Communi, Parmentier et al., 1996; Das et al., 2010, 2010; Eliahu et al., 2009; El-Tayeb et al., 2006; El-Tayeb et al., 2011; Erb et al., 1993; Filippov et al., 1999; WO002009066298A1, 2008; WO002012073237A1, 2011; WO002012032513A1, 2011; Fricks et al., 2008; Gao et al., 2010; Hamel et al., 2011; Hoffmann, Soltysiak, West, & Jacobson, 2004; Hou et al., 2002; US020090148850A1, 2008; Kiselev et al., 2015; Knoblauch et al., 1999; Ko et al., 2007; Ko et al., 2008; Lazarowski et al., 1995; Lazarowski et al., 2001; Lazarowski & Harden, 1994; Nguyen et al., 1995; Nicholas et al., 1996; Patel et al., 2001; Robaye et al., 1997; Shaver et al., 2005; Shen et al., 2004; Webb et al., 1998; Zambon et al., 2000
		partial agonist with 61 % efficacy inactive at 1 mM (↓>670x[a] / ↓>1100x[b]) **Rat:** 16 (↓80x)[a,b] **Dog:** ≈10 (↓≈50x[a] / n.d.[b]) **Pig:** 1.5 (↑2x[a] / ↓3x[b]) 35 % efficacy	(↓>230x[a] / ↓>390x[b]) **Rat:** 6.3 (↓4x[a] / ↓2x[b]) partial agonist inactive at 100 μM (↓>95x[a] / ↓>160x[b])	**Rat:** 0.0059 - 2.3	(↓2x)[d] **Rat:** 5.23 (1x)[d] partial agonist **Chimpanzee:** 0.0031 - 0.0068 (1x-↑2x)[d] K_i = 0.013 (↑2x)[d]		

(*continued on next page*)

Table 2 (*continued*)

Physiological mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
2-10	UMP	inactive at 100 μM (n.d.[a] / ↓>2300x[b]) **Rat:** inactive at 100 μM (↓>500x)[a,b] **Dog:** inactive at 100 μM (↓>500x[a] / n.d.[b])	inactive at 100 μM (↓>1150x)[b]	inactive at 100 μM (↓>2400x)[c] **Rat:** inactive at 300 μM (↓>130x)[c]	0.0540 - 0.703 (↓14-100x[c] / ↓7-25x[d]) inactive (n.d.)[c,d] K_i = 0.695 (↓50x[c] / ↓35x[d]) **Mouse:** 0.0634 - 0.082 (↓5-8x[c] / ↓7-13x[d]) **Chimpanzee:** 0.169 - 0.289 (↓43-54x[c] / ↓27-45x[d]) K_i = 1.15 (↓88x[c] / ↓50x[d]) K_i = 5.10 (↓31x[c] / ↓84x[d])		Chen et al., 1996; Communi, Motte et al., 1996; El-Tayeb et al., 2006; Hamel et al., 2011; US020090148850A1, 2008; Ko et al., 2007; Lazarowski & Harden, 1994; Zambon et al., 2000
2-11	CTP	5.63 (↓>66x[a] / ↓>120x[b]) **Mouse:** almost inactive at 100 μM (n.d.)[a,b] inactive at 1 mM (↓>670x[a] / ↓>1100x[b]) **Rat:** 6.8 (↓3x[a] / ↓2x[b]) >100 (↓>500x)[a,b] **Pig:** 8.4 (↓3x[a] / ↓16x[b])	antagonist inactive at 100 μM (↓>500x)[b] **Mouse:** 25 (↓58x[a] / ↓96x[b]) **Rat:** 1.2 (1x)[a,b] 7.24 (↓14x[a] / ↓36x[b])	n.d. **Rat:** inactive at 300 μM (↓>130x)[c]	3.7 (↓1500x[c] / ↓1700x[d]) inactive at 1 μM (n.d.[c] / ↓>10x[d])		Chambers et al., 2000; Chen et al., 1996; Erb et al., 1993; Hamel et al., 2011; Jacobson et al., 2006; Kennedy et al., 2000; Lazarowski et al., 2001; Lazarowski & Harden, 1994; Lin et al., 1993; Nguyen et al., 1995; Shen et al., 2004; Wildman et al., 2003

Table 2 (*continued*)

Physiological mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
2-12	CDP	n.d. **Pig:** 87.1 (↓32x[a] / ↓160x[b]) **Rat:** inactive at 100 μM (↓>500x)[a,b]	n.d.	88.0 (↓290x)[c] partial agonist **Mouse:** inactive at 100 μM (↓>2400x)[c] **Rat:** inactive at 300 μM (↓>130x)[c]	0.319 (↓130x[c] / ↓150x[d]) ≈50 (↓680x[c] / ↓160x[d]) inactive at 1 μM (1x[c] / ↓>10x[d])		Carter et al., 2009; Chambers et al., 2000; Chen et al., 1996; Hamel et al., 2011; Lazarowski et al., 2001; Lazarowski & Harden, 1994; Robaye et al., 1997; Shen et al., 2004
2-13	TTP	n.d. **Pig:** 8.6 (↓3x[a] / ↓16x[b]) 74 % efficacy	n.d.	n.d.	n.d.		Shen et al., 2004
2-14	TDP	n.d. **Pig:** 31.4 (↓12x[a] / ↓59x[b])	n.d.	7.7 (↓26x)[c]	2.17 (↓900x[c] / ↓960x[d])		Hamel et al., 2011; Robaye et al., 1997; Shen et al., 2004

[a]relative to ATP; [b]relative to UTP; [c]relative to UDP; [d]relative to UDP-glucose.

2. The $P2Y_2$ receptor

The mouse $P2Y_2R$, formerly referred to as the P_{2U} receptor, was first cloned in 1993 from a cDNA library obtained from NG108-15 cells, a mouse N18TG2 neuroblastoma x rat C6 glioma hybrid cell line (Lustig, Shiau, Brake, & Julius, 1993). The human receptor was cloned shortly thereafter (Parr et al., 1994). $P2Y_2R$ mRNA is predominantly expressed in the gastrointestinal (GI) tract and the immune system, in muscle (mostly skeletal and cardiac muscle), and in endocrine tissue (thyroid, parathyroid, and adrenal glands). Lower $P2Y_2R$ mRNA expression was also detected in the CNS, lungs, liver and gallbladder, kidney and bladder, male and female reproductive organs, placenta, skin, bone, and adipose tissue (Moore et al., 2001; Uhlén et al., 2015). At protein level, the $P2Y_2R$ was mostly detected in epithelial and glandular cells of the lungs (nasopharynx and bronchi), gastrointestinal tract (salivary glands, oral mucosa, esophagus), tonsils, breasts, male and female reproductive tracts (prostate, epididymis, fallopian tubes, to a lower extent also in vagina, cervix, uterus, endometrium), and in placenta trophoblasts. Low $P2Y_2R$ protein expression was also observed in immune tissue (bone marrow, spleen, lung macrophages, lymphoid tissue of the appendix) and skin keratinocytes (Uhlén et al., 2015). An overview of the tissue distribution and therapeutic significance of the $P2Y_2R$ and the other uracil nucleotide-activated P2YRs is provided in Table 1 (for discussion, see below).

A relatively large number of agonists – mostly derivatives of the endogenous ligand UTP – and a few antagonists have been described so far. Those of greater potential are discussed in the subsequent sections, while a comprehensive overview is provided in Tables 2 to 10.

2.1. $P2Y_2$ receptor agonists

2.1.1. Therapeutic potential of $P2Y_2$ receptor agonists

$P2Y_2R$ activation bears significant therapeutic potential for different applications, including cystic fibrosis. The $P2Y_2R$ is involved in epithelial ion transport. Na^+ absorption by the airway epithelium is reduced as a result of $P2Y_2R$ activation, and knockout mice were found to exhibit defective Cl^- secretion. Thus, $P2Y_2R$ agonism was expected to compensate for the malfunctioning of the Cl^- channel cystic fibrosis transmembrane conductance regulator (CFTR) in cystic fibrosis patients (Cressman et al., 1999; Kunzelmann & Mall, 2003). In the eye, $P2Y_2Rs$ mediate tear production and reduce intraocular pressure. $P2Y_2R$ agonists are therefore useful for the treatment of dry eyes, and may also be beneficial for treating ocular hypertension and retinal degeneration (Jacobson & Civan, 2016; Lau, Samarawickrama, & Skalicky, 2014; Pintor et al.,

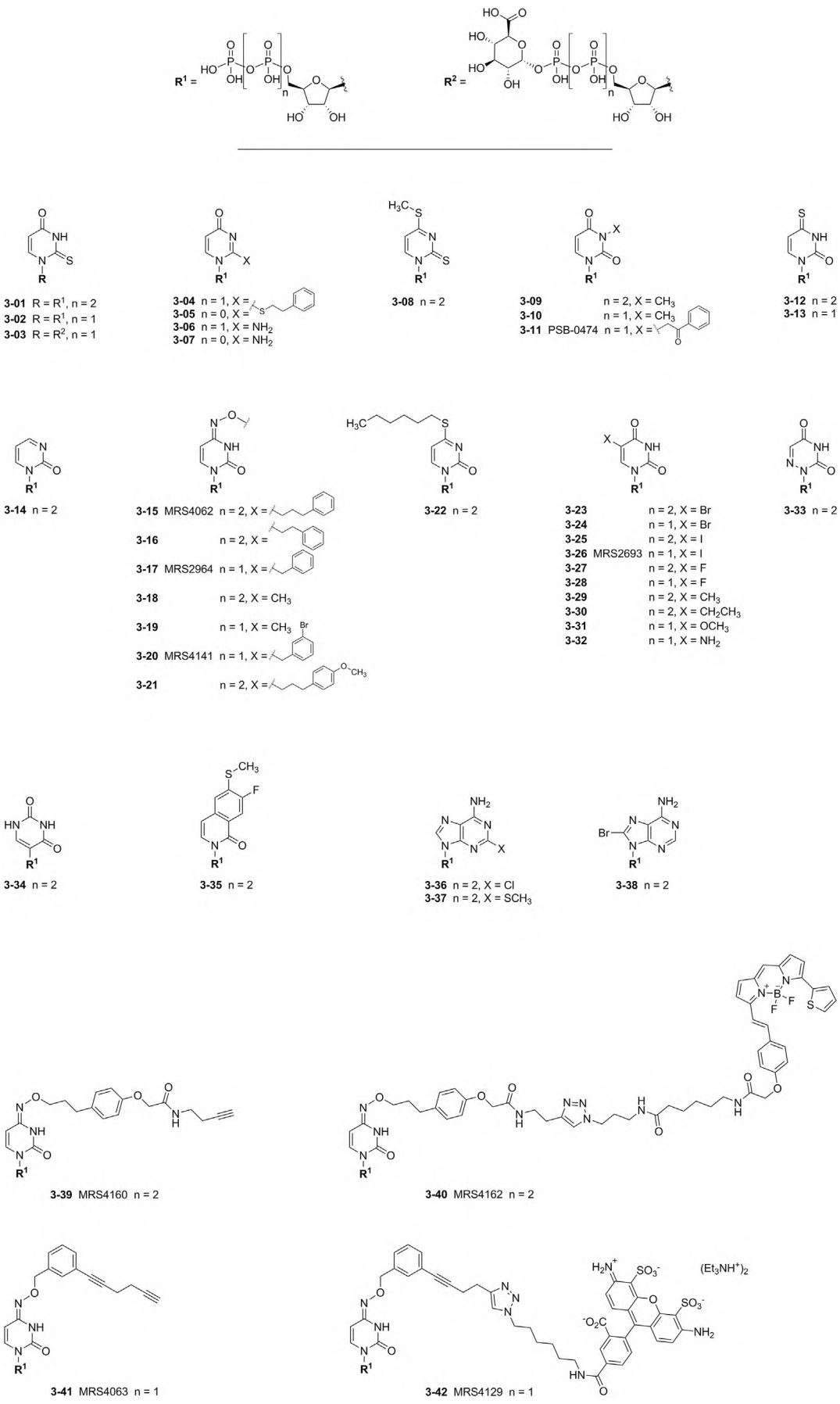

Fig. 3. Structures of agonists at uracil nucleotide-activated P2YRs with substitutions at the nucleobase (for data, refer to Table 3).

Table 3
Base-modified nucleotides as agonists for the uracil-activated P2YRs. Shown are the EC_{50} or, in some cases, K_i and K_B values in μM at the human receptor, unless stated otherwise. The correlation factor by which the potency is n-fold higher (↑nx) or lower (↓nx) compared to that of the endogenous agonist in the same test system is given in brackets. Particularly interesting values are shown in bold.

Base-substituted mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
3-01	2-S-UTP	≈0.007 - 0.050 (↑2x[a] / 1x[b])	1.77 (↓20x)[b] 0.35 (↓5x)[b]	≈1.5 (↓36x)[c]	n.d.	10-fold selective for $P2Y_2R$ Commercially available	El-Tayeb et al., 2006; Jacobson et al., 2006; Sakuma et al., 2017
3-02	2-S-UDP	≈50 (n.d.[a] / ↓1200x[b])	≈40 (↓460x)[b]	0.239 (↓6x)[c] 0.447 (1x)[c]	**0.00192** (↑83x[c] / ↑210x[d]) **0.002** (↑37x[c] / ↑160x[d])	**125-fold $P2Y_{14}R$ selective**	Carter et al., 2009; Das et al., 2010; El-Tayeb et al., 2006
3-03	2-S-UDP-glucuronic acid	n.d.	n.d.	n.d.	0.42 (n.d.[c] / 1x[d])		Ko et al., 2007
3-04	2-Phenylethyl thio-UDP	0.544 (n.d.[a] / ↓30x[b])	≥100 (↓≥1100x)[b]	≈2.5 (↓52x)[c]	n.d.		El-Tayeb et al., 2011
3-05	2-Phenylethyl thio-UMP	1.32 (n.d.[a] / ↓74x[b])	>100 (↓>1100x)[b]	>100 (↓>2100x)[c]	n.d.		El-Tayeb et al., 2011
3-06	2-Amino-UDP (iso-CDP)	0.604 (n.d.[a] / ↓34x[b])	≥100 (↓≥1100x)[b]	≥100 (↓≥2100x)[c]	n.d.	**170-fold $P2Y_2R$ selective**	El-Tayeb et al., 2011
3-07	2-Amino-UMP (iso-CMP)	>100 (n.d.[a] / ↓>5600x[b])	4.98 (↓56x)[b]	>100 (↓>2100x)[c]	n.d.	**>20-fold $P2Y_4R$ selective** 2-(Me-amino)-UMP inactive at $P2Y_4R$	El-Tayeb et al., 2011
3-08	2-S-4-MeS-UTP	0.91 (n.d.[a] / ↓15x[b])	5.35 (↓59x)[b]	>10 (↓>33x)[c]	n.d.		Ko et al., 2008
3-09	3-Me-UTP	0.564 (n.d.[a] / ↓13x[b]) 1.20 (↓14x[a] / ↓25x[b])	2.92 (↓34x)[b] 3.40 (↓47x)[b]	≈8 (↓5300x)[c]	n.d.		El-Tayeb et al., 2006; Jacobson et al., 2006
3-10	3-Me-UDP	n.d.	n.d.	3.3 (↓250x)[c]	n.d.		Besada et al., 2006
3-11	3-Phenacyl-UDP (PSB-0474)	≈40 (n.d.[a] / ↓930x[b])	>100 (↓>1200x)[b]	**0.070** (↓2x)[c]	n.d. **Rat:** inactive at 10 μM (↓>2x[c] / ↓>2x[d])	**570-fold $P2Y_6R$ selective** Commercially available	El-Tayeb et al., 2006; Gao et al., 2010

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Table 3 (*continued*)

Base-substituted mononucleotides							
No.	**Name**	**EC_{50} in µM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
						Potency of 3-phenacyl-UTP in the µM range at $P2Y_2R$, $P2Y_4R$, and $P2Y_6R$	
3-12	4-S-UTP	0.026 - 0.71 (↑3x[a] / ↑2x-↓6x[b])	0.023 (↑3x)[b]	n.d.	n.d.		Brunschweiger & Müller, 2006; Jacobson et al., 2006; Shaver et al., 1997
3-13	4-S-UDP	n.d.	n.d.	0.08 (↓6x)[c] 2.36 (↓5x)[c]	0.320 (↓2x[c] / 1x[d])		Besada et al., 2006; Das et al., 2010
3-14	Zebularine triphosphate	8.9 (↓100x[a] / ↓180x[b])	inactive at 10 µM (↓>140x)[b]	n.d.	n.d.		Jacobson et al., 2006
3-15	N^4-phenylpropoxy-CTP (MRS4062)	0.640 (n.d.[a] / ↓12x[b])	**0.023** (↑4x)[b]	0.740 (n.d.)[c]	n.d.	28-fold selective for $P2Y_4R$ Commercially available	Jayasekara et al., 2013; Maruoka et al., 2011
3-16	N^4-phenylethoxy-CTP	1.20 (n.d.[a] / ↓22x[b])	**0.073** (1x)[b]	1.21 (n.d.)[c]	n.d.	16-fold selective for $P2Y_4R$	Jayasekara et al., 2013; Maruoka et al., 2011
3-17	N^4-benzyloxy-CDP (MRS2964)	2.13 (n.d.[a] / ↓36x[b])	1.15 (↓13x)[b]	**0.026** (↑12x)[c]	n.d.	44-fold selective for $P2Y_6R$	Jayasekara et al., 2013; Maruoka et al., 2010
3-18	N^4-MeO-CTP	0.05 (n.d.[a] / 1x[b])	0.05 (↑2x)[b]	0.08 (↑4x)[c]	n.d.		Jayasekara et al., 2013; Maruoka et al., 2010
3-19	N^4-MeO-CDP	3.60 (n.d.[a] / ↓60x[b])	6.45 (↓72x)[b]	**0.070** (↑4x)[c]	3.32 (↓21x[c] / ↓8x[d])	4-fold more potent than UDP at $P2Y_6R$ 47-fold selective for $P2Y_6R$	Das et al., 2010; Jayasekara et al., 2013; Maruoka et al., 2010
3-20	MRS4141	6.20 (n.d.)[a b]	>10 (n.d.)[b]	0.039 (n.d.)[c]	n.d.		Jayasekara et al., 2013
3-21	N^4-(3-(4-methoxyphenyl)-propyl)oxy-UTP	0.047 (n.d.)[a b]	0.023 (↑4x)[b]	0.277 (n.d.)[c]	n.d.		Jayasekara et al., 2014
3-22	4-(Hexyl-S)-UTP	0.84 (n.d.[a] / ↓7x[b])	n.d.	n.d.	n.d.		Shaver et al., 1997

Table 3 (*continued*)

Base-substituted mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
3-23	5-Br-UTP	0.347 - 2.06 (↓9x[a] / ↓8x-15[b])	2.1 - 49 (↓14-75x)[b]	0.291 (↓7x)[c] 0.800 (↓3x)[c] **Rat:** 9 (↓4x)[c] inactive at 100 μM (↓>530x)[c]	n.d.	Patented	Communi, Parmentier et al., 1996; El-Tayeb et al., 2006; Jacobson et al., 2006; US000005620676A, 1995; Lazarowski et al., 1995; Lazarowski & Harden, 1994; Nguyen et al., 1995; Nicholas et al., 1996
3-24	5-Br-UDP	3.67 (n.d.[a] / ↓85x[b])	7.20 (↓83x)[b] inactive at 1 mM (↓>1300x)[b]	0.151 (↓4x)[c] 0.800 (↓3x)[c] **Rat:** 0.13 (↑2x)[c]	n.d.		Communi, Parmentier et al., 1996; El-Tayeb et al., 2006; Nicholas et al., 1996
3-25	5-I-UTP	0.83 (↓10x[a] / ↓17x[b])	4.0 (↓55x)[b]	n.d.	n.d.		Jacobson et al., 2006
3-26	5-I-UDP (MRS2693)	n.d.	n.d.	0.015 (1x)[c]	n.d.	Commercially available	Besada et al., 2006
3-27	5-F-UTP	6 (n.d.[a] / ↓60x[b])	0.6 (1x)[b]	>100 (↓>710x)[c]	n.d.	Possibly a **partial agonist** Patented	Ginsburg-Shmuel et al., 2010; US000005620676A, 1995
3-28	5-F-UDP	2 (n.d.[a] / ↓20x[b])	3.5 (↓7x)[b]	10 (↓71x)[c]	n.d.	Possibly a **partial agonist**	Ginsburg-Shmuel et al., 2010
3-29	5-Methyl-UTP	0.48 (↓6x[a] / ↓10x[b])	3.9 (↓53x)[b]	n.d.	n.d.		Jacobson et al., 2006
3-30	5-Ethyl-UTP	n.d. **Mouse:** 99 (↓6x[a] / ↓79x[b])	n.d.	n.d.	n.d.		Knoblauch et al., 1999
3-31	5-MeO-UDP	inactive (n.d.)[a,b]	≥20 (↓≥40x)[b]	**0.08** (↑2x)[c]	n.d.		Ginsburg-Shmuel et al., 2010
3-32	5-Amino-UDP	n.d.	n.d.	0.61 (↓2x)[c]	n.d.		Ko et al., 2008
3-33	6-Aza-UTP	8.6 (↓100x[a] / ↓180x[b])	>10 (↓>140x)[b]	n.d.	n.d.		Jacobson et al., 2006
3-34	Pseudouridine 5'-triphosphate	0.78 (↓9x[a] / ↓16x[b])	3.0 (↓41x)[b]	n.d.	n.d.		Jacobson et al., 2006

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Table 3 (*continued*)

Base-substituted mononucleotides							
No.	**Name**	**EC_{50} in µM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
3-35	Nucleotide triphosphate with 'unnatural' bicyclic aromatic base substitution	**0.003** (n.d.[a] / ↑2x[b])	>20 (↓>510x)[b]	>20 (n.d.)[c]	n.d.	**>6600-fold $P2Y_2R$ selective** Patented	Brookings et al., 2007; Davenport et al., 2007; WO002002062816A1, 2002; WO002003011885A1, 2002
3-36	2-Cl-ATP	2.30 (↓10x[a] / ↓16x[b]) "low activity"	n.d.	n.d.	n.d.	Patented	Guile et al., 2001; US000005620676A, 1995; Lazarowski et al., 1995
3-37	2-MeSATP	inactive (n.d.)[a b] **Rat:** 9.4 (↓4x[a] / ↓3x[b]) partial agonist with 34 % efficacy >100 (↓>500x)[a b] **Mouse:** ≈100 (n.d.)[a b] ≈1000 (↓1400x[a] / ↓900x[b]) **Dog:** >10 (↓>5x[a] / ↓>5x[b]) **Pig:** inactive at 100 µM (↓37x[a] / ↓190x[b])	inactive at 1 mM (↓>1300x)[b] **Rat:** 1.4 (1x)[a b] partial agonist with 24 % efficacy 2.1 (1x)[a b] partial agonist inactive at 100 µM (↓>95x[a] / ↓>160x[b])	100 (↓330x)[c] **Rat:** 50 (n.d.)[c] ≈1000 (↓5300x)[c] inactive at 300 µM (↓>130x)[c]	n.d.	Patented	Bogdanov et al., 1998; Chang et al., 1995; Chen et al., 1996; Communi, Parmentier et al., 1996; Guile et al., 2001; US000005620676A, 1995; Lazarowski & Harden, 1994; Lin et al., 1993; Lustig et al., 1993; Nicholas et al., 1996; Shen et al., 2004; Webb et al., 1998; Wildman et al., 2003; Zambon et al., 2000

Table 3 (*continued*)

Base-substituted mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
3-38	8-Br-ATP	23.0 (↓100x[a] / ↓160x[b])	n.d.	0.800 (↓3x)[c]	n.d.	Patented	Communi, Parmentier et al., 1996; US000005620676A, 1995; Lazarowski et al., 1995
3-39	MRS4160	0.109 (n.d.)[a,b]	0.040 (↑2x)[b]	0.183 (n.d.)[c]	n.d.	Suitable for chain extension by click tethering Derivative of **3-21**	Jayasekara et al., 2014
3-40	MRS4162	0.066 (n.d.)[a,b]	0.070 (1x)[b]	0.023 (n.d.)[c]	n.d.	**Fluorescent probe** Derivative of **3-21**	Jayasekara et al., 2014
3-41	MRS4063	3.9 (n.d.)[a,b]	0.952 (n.d.)[b]	0.100 (↑3x)[c]	n.d.	Suitable for chain extension using click tethering	Jayasekara et al., 2013
3-42	MRS4129	2.5 (n.d.)[a,b]	>10 (n.d.)[b]	0.009 (↑33x)[c]	n.d.	**Fluorescent probe**	Jayasekara et al., 2013

[a]relative to ATP; [b]relative to UTP; [c]relative to UDP; [d]relative to UDP-glucose.

2003; Pintor, Carracedo, Alonso, Bautista, & Peral, 2002). The concept of using $P2Y_2R$ agonists to promote secretion and mucus clearance for the treatment of dry eyes, ocular hypertension, and retinal degeneration has been patented, as well as their use in the treatment of sinusitis, ear infections, bronchitis, pneumonia, gastrointestinal tract disorders, vaginal dryness, and for joint lubrication (Cowlen, Yerxa, Jones, & Brown, 2002; Drutz, Rideout, & Jacobus, 1997; Jacobus & Leighton, 1997; Pendergast, Rideout, Siddiqi, & Yerxa, 1998; Pendergast, Shaver, Drutz, & Rideout, 1999; Peterson, 2001; Peterson & Yerxa, 2002; Shaffer, Boucher, Rideout, & Jacobus, 1997; Yerxa et al., 2000; Yerxa, Peterson, Rideout, & Pendergast, 2009). $P2Y_2R$ agonism may possibly also find application in cardiovascular incidents, as it was shown to reduce post-ischemic myocardial damage *in vivo* and protect cardiomyocytes from hypoxia *in vitro* (Cohen et al., 2011; Hochhauser et al., 2013). Moreover, a neuroprotective role was postulated for the $P2Y_2R$ *via* the induction of α-secretase-dependent amyloid precursor protein processing in astrocytoma cells, microglia-mediated clearance of β-amyloid, and modulation of the ubiquitin-proteasome system (Ajit et al., 2014; Camden et al., 2005; Diego-García et al., 2017; Kim et al., 2012). In addition, a reduced $P2Y_2R$ expression was observed post mortem in the parietal cortex of patients who had suffered from Alzheimer's disease (Lai et al., 2008).

2.1.2. Mononucleotides and analogues

Almost all of the current $P2Y_2R$ agonists are nucleotide derivatives, which are generally not ideal for drug development or *in vivo* studies due to their susceptibility to enzymatic degradation and their lack of oral bioavailability as a result of the several negative charges at physiological pH.

The endogenous agonists for the $P2Y_2R$ are ATP (**2-01**, compound **01** in Fig. 2 and Table 2) and UTP (**2-08**, compound **08** in Fig. 2 and Table 2) with similar potencies. Many commercial preparations of uridine 5′-diphosphate (UDP, **2-09**, Fig. 2 and Table 2) were found to contain significant amounts of UTP that can activate the $P2Y_2R$. Furthermore, UDP can be phosphorylated to UTP by nucleoside diphosphokinase. These effects initially caused misinterpretations regarding the potency of UDP. Following purification of the test compounds, and in experimental conditions that excluded enzymatic conversion, both UDP and adenosine 5′-diphosphate (ADP, **2-02**, Fig. 2 and Table 2) were found inactive at the human $P2Y_2R$ (Lazarowski et al., 1995; Nicholas et al., 1996). Adenosine 5′-(γ-thio)-triphosphate (ATPγS, **5-03**, compound **03** in Fig. 5 and Table 5) and uridine 5′-(γ-thio)-triphosphate (UTPγS, **5-05**, Fig. 5 and Table 5) are also full agonists. The γ-thiophosphate group increases stability towards ectonucleotidases (Lazarowski et al., 1995; Malmsjö et al., 2000). A thio-substitution at the α-phosphate group caused a reduction in potency (Jacobson et al., 2006).

UTP (**2-08**, compound **08** in Fig. 2 and Table 2) is not only an agonist for the $P2Y_2R$ but activates the related $P2Y_4R$ as well. Relatively simple nucleobase modifications (Fig. 3 and Table 3) to UTP may enhance the selectivity for the $P2Y_2R$: 2-thio-UTP (**3-01**) was described as a $P2Y_2R$ agonist with potency similar to UTP (EC_{50} of 35-50 nM) and 10- to 30-fold selectivity versus the related $P2Y_4$ and $P2Y_6$ receptors (El-Tayeb, Qi, & Müller, 2006; Jacobson et al., 2006). Moreover, 2-amino-UDP (**3-06**), although 34-fold less potent (EC_{50} of 604 nM), is at least 170-fold selective (El-Tayeb, Qi, Nicholas, & Müller, 2011). Further modifications, not only on the pyrimidine ring but additionally through substitutions at the ribose moiety, led to one of the most potent and selective $P2Y_2R$ agonists known to date: 2′-amino-2′-deoxy-2-thio-UTP (MRS2698, **6-01**, Fig. 6 and Table 6; EC_{50} of 8 nM). It is 6-fold more potent than UTP and >300-fold selective versus the $P2Y_4$ and $P2Y_6$ receptors (Ivanov et al., 2007). Several thousandfold improvement in selectivity with potencies similar to UTP could be achieved by

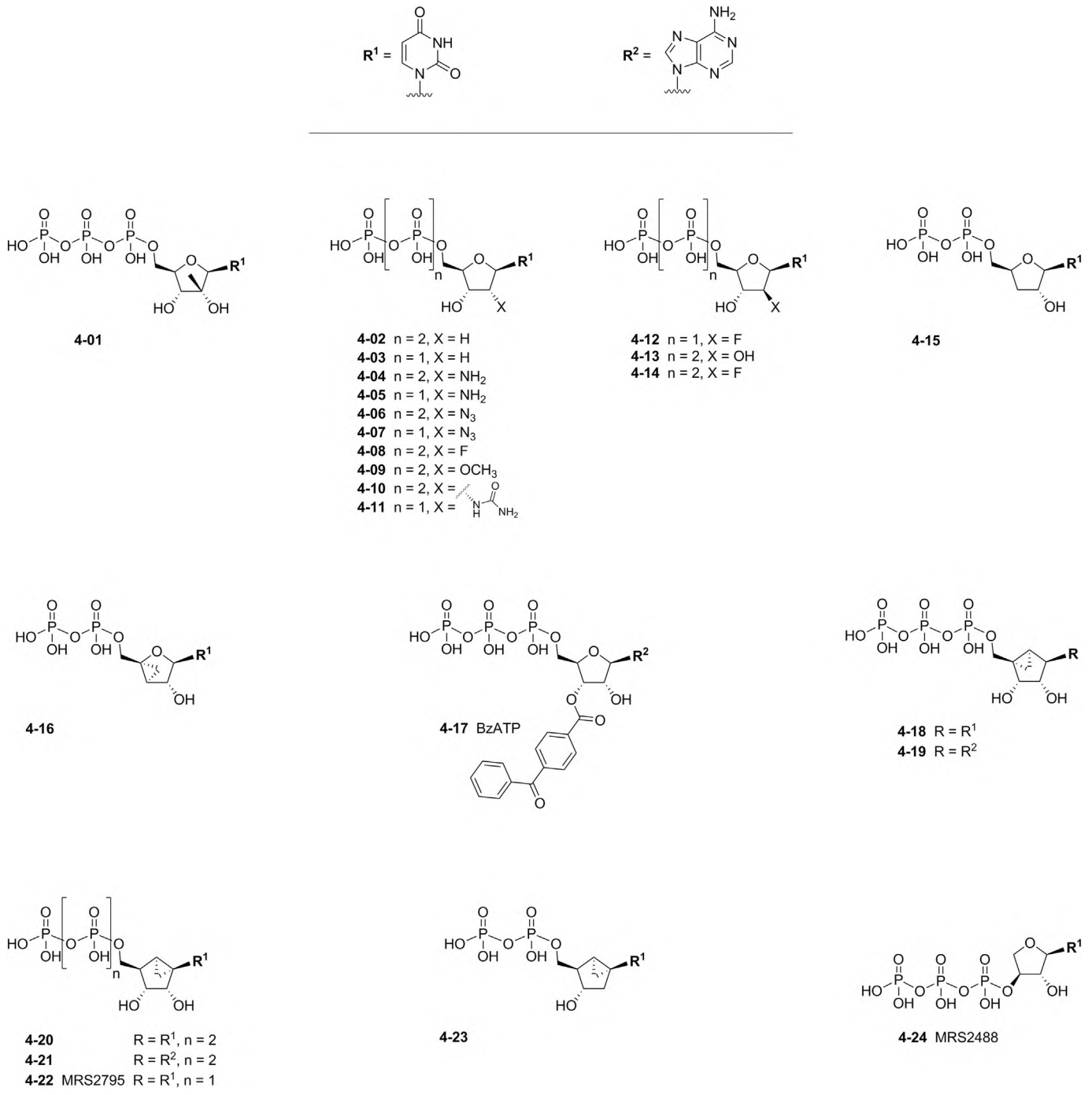


Fig. 4. Structures of agonists at uracil nucleotide-activated P2YRs with substitutions at the ribose ring (for data, refer to Table 4).

replacing the nucleobase with various 'unnatural' bicyclic aromatic residues, as in compound **3**-**35** (Fig. 3 and Table 3) (Brookings et al., 2007). These structures could serve as templates for the development of more selective compounds than the uracil-derived $P2Y_2R$ agonists.

Improved ectonucleotidase stability was achieved in a series of nucleobase-substituted nucleotide derivatives through β,γ-dichloro- and β,γ-difluoromethylene substitutions (El-Tayeb et al., 2011). The most promising compound of this series is 4-thio-β,γ-difluoromethylene-UTP (PSB-1114, **6**-**05**, Fig. 6 and Table 6) with >50-fold selectivity versus the $P2Y_4$ and $P2Y_6$ receptors. PSB-1114, which is available from commercial sources, is with an EC_{50} of 134 nM approx. 8-fold less potent than UTP (El-Tayeb et al., 2011).

Masking of the terminal phosphate group may also be a strategy to reduce the susceptibility of nucleotides to ectonucleotidase hydrolysis. Up_4-δ-3-chlorophenyl phosphoester (**5**-**17**, Fig. 5 and Table 5) exhibited an EC_{50} value of 840 nM at the $P2Y_2R$ and is 15-fold less potent than UTP (Maruoka et al., 2011). It showed no effect on the $P2Y_4R$ at 10 μM, but the selectivity versus the $P2Y_6R$ was merely 4-fold (Maruoka et al., 2011). Up_4-δ-phenyl ester (MRS2768, **5**-**02**, Fig. 5 and Table 5) also displayed some selectivity for the $P2Y_2R$, as no effect was observed at 10 μM for the $P2Y_4R$ and $P2Y_6R$ subtypes. However, the potency is with an EC_{50} of 1.89 μM at the $P2Y_2R$ lower compared to many other agonists (Ko et al., 2008). Moreover, the stability of such esters in biological media may be limited.

2.1.3. Dinucleotides

Nucleoside triphosphates can be mimicked by dinucleotide tetraphosphates, as both classes of compounds bear four negative

Table 4
Mononucleotides with modifications at the ribose ring as agonists for the uracil-activated P2YRs. Shown are the EC_{50} or, in some cases, K_i and K_B values in μM at the human receptor, unless stated otherwise. The correlation factor by which the potency is n-fold higher (↑nx) or lower (↓nx) compared to that of the endogenous agonist in the same test system is given in brackets. Particularly interesting values are shown in bold.

Ribose-modified mononucleotides							
No.	Name	EC_{50} in μM (potency higher (↑) or lower (↓) relative to endogenous agonist)				Comments	References
		P2Y$_2$	P2Y$_4$	P2Y$_6$	P2Y$_{14}$		
4-01	2′-Me-UTP	1.45 (n.d.[a] / ↓24x[b])	1.26 (↓14x)[b]	inactive at 10 μM (↓>33x)[c]	n.d.		Ko et al., 2008
4-02	2′-Deoxy-UTP	1.08 (↓13x[a] / ↓22x[b])	1.9 (↓26x)[b]	n.d. **Rat:** inactive at 300 μM (↓>130x)[c]	n.d.		Jacobson et al., 2006; Lazarowski & Harden, 1994
4-03	2′-Deoxy-UDP	n.d.	n.d.	1.72 (↓130x)[c] **Rat:** ≈1000 (↓440x)[c]	n.d.		Besada et al., 2006; Costanzi et al., 2005; Lazarowski & Harden, 1994
4-04	2′-Amino-2′-deoxy-UTP	0.062 (1x)[a,b]	1.2 (↓16x)[b]	inactive at 10 μM (↓>33x)[c]	n.d.		Jacobson et al., 2006; Ko et al., 2008
4-05	2′-Amino-2′-deoxy-UDP	n.d.	n.d.	3.9 (↓300x)[c]	n.d.		Besada et al., 2006
4-06	2′-Azido-2′-deoxy-UTP	5.0 (↓59x[a] / ↓100x[b])	1.1 (↓15x)[b]	n.d.	n.d.		Jacobson et al., 2006
4-07	2′-Azido-2′-deoxy-UDP	n.d.	n.d.	1.5 (↓120x)[c]	n.d.		Besada et al., 2006
4-08	2′-Deoxy-2′-F-UTP	0.78 (↓9x[a] / ↓16x[b])	0.54 (↓7x)[b]	n.d.	n.d.		Jacobson et al., 2006
4-09	2′-Deoxy-2′-MeO-UTP	14.3 (↓170x[a] / ↓290x[b])	8.2 (↓110x)[b]	n.d.	n.d.		Jacobson et al., 2006
4-10	2′-Deoxy-2′-ureido-UTP	1.74 (n.d.[a] / ↓29x[b])	4.64 (↓52x)[b]	>10 (↓>33x)[c]	n.d.		Ko et al., 2008
4-11	2′-Deoxy-2′-ureido-UDP	n.d.	n.d.	4.70 (↓16x)[c]	n.d.		Ko et al., 2008
4-12	2′-F-2′-deoxyara-UDP	n.d.	n.d.	5.5 (↓423x)[c]	n.d.		Besada et al., 2006
4-13	Arabino-UTP	0.087 (1x[a] / ↓2x[b])	0.71 (1x)[b]	n.d.	n.d.		Jacobson et al., 2006
4-14	2′-Deoxyarabino-2′-F-UTP	0.52 (↓6x[a] / ↓11x[b])	0.52 (↓7x)[b]	n.d.	n.d.		Jacobson et al., 2006
4-15	3′-Deoxy-UDP	n.d.	n.d.	2.5 (↓192x)[c]	n.d.		Besada et al., 2006
4-16	3′,4′-Cyclopropyl-UDP	n.d.	n.d.	3.5 (↓269x)[c]	n.d.		Besada et al., 2006
4-17	3′-O-(4-benzoyl)-benzoyl-ATP (BzATP)	n.d. **Rat:**	n.d. **Rat:**	n.d.	n.d.	ATP analogue used as photoaffinity label	Erb et al., 1993; Lin et al., 1993; Wildman et al., 2003

(*continued on next page*)

Table 4 (*continued*)

Ribose-modified mononucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
		4.7 (↓2x[a] / 1x[b]) **Mouse:** 104 % receptor activation at 100 μM	antagonist (see Table 10)				
4-18	(*N*)-methano-carba-UTP	0.0159 (↓5x[a] / ↓2x[b])	0.085 (↓2x)[b]	inactive at 100 μM (↓>6700x)[c]	n.d.		Kim et al., 2002
4-19	(*N*)-methano-carba-ATP	0.091 (1x[a] / ↓11x[b])	>10 (↓>200x)[b]	n.d.	n.d.	Methanocarba-adenosine analogues patented	WO002001051490A1, 2001; Kim et al., 2002
4-20	(*S*)-methano-carba-UTP	0.08 (n.d.[a] / 1x[b])	0.30 (↓3x)[b]	1.37 (↓5x)[c]	n.d.		Maruoka et al., 2010
4-21	(*S*)-methano-carba-ATP	3.7 (↓44x[a] / ↓460x[b])	inactive at 100 μM (↓>2000x)[b]	n.d.	n.d.		Kim et al., 2002
4-22	(*S*)-methano-carba-UDP (MRS2795)	n.d.	n.d.	0.042 (↑2x)[c]	inactive at 10 μM (↓>19x)[c]	(*N*)-methano-carba-UDP inactive at $P2Y_6R$	Besada et al., 2006; Das et al., 2010; Maruoka et al., 2010
4-23	2′-Deoxy-(*S*)-methanocarba-UDP (MRS2633)	n.d.	n.d.	0.230 (↓18x)[c]	n.d.		Besada et al., 2006; Costanzi et al., 2005
4-24	L-*α*-threo furanosyl-UTP (MRS2488)	9.9 (n.d.[a] / ↓1200x[b])	26 (↓530x)[b]	inactive at 10 μM (n.d.)[c]	n.d.		Ohno et al., 2004

[a]relative to ATP; [b]relative to UTP; [c]relative to UDP; [d]relative to UDP-glucose.

charges at physiological pH (Brunschweiger & Müller, 2006). The naturally occurring dinucleotides P^1,P^4-di(adenosine-5′)-tetraphosphate (AP_4A, **7-01**, Fig. 7 and Table 7) and P^1,P^4-di(uridine-5′)-tetraphosphate (Up_4U, **7-03**, Fig. 7 and Table 7) are potent $P2Y_2R$ agonists, albeit non-selective versus the $P2Y_4R$. Up_4U is also known as diquafosol or INS365, and is marketed since 2010 under the trade name of Diquas® in Japan, Korea, Thailand, and Vietnam as a treatment for dry eye syndrome (see reviews by Lau et al., 2014, Keating, 2015, and Nichols, Yerxa, & Kellerman, 2004). It is an analogue of the diadenosine polyphosphates naturally found in human tears that were shown to promote tear secretion (Mundasad et al., 2001; Pintor et al., 2002; Tauber et al., 2004; Yamane et al., 2015). AP_4A was shown to induce controlled hypotension in patients and exert modulating effects on blood pressure during anesthesia (Kikuta, Ohiwa, Okada, Watanabe, & Haruki, 1999). Dinucleotides are less susceptible to enzymatic hydrolysis *in vivo* than mononucleotides with a free terminal phosphate group. Replacement of one of the two uridine moieties with 2′-deoxycytidine to form Up_4dC (denufosol, INS37217, **7-07**, Fig. 7 and Table 7) further enhanced ectonucleotidase stability. Denufosol was shown to significantly enhance tracheal mucus transport for more than 8 hours in an animal model (Deterding et al., 2007; Yerxa et al., 2002). It was subsequently evaluated in two phase III clinical trials as a therapy for cystic fibrosis patients. In the first trial (TIGER-1), it was found to significantly improve lung function in cystic fibrosis patients with normal to mildly impaired lung function. However, this could not be confirmed in the second phase III clinical trial: in the TIGER-2 study, denufosol failed to meet the primary endpoint, a significant change in baseline FEV1 (forced expiratory volume in one second) at week 48 (Accurso et al., 2011; Kellerman et al., 2008; Ratjen et al., 2012; for a detailed account on the denufosol clinical trials, refer to the commentary by Moss, 2013). A series of further dinucleotides with potencies in the high nanomolar or low micromolar range at the $P2Y_2R$ is described in the patent literature (Pendergast et al., 1998; Rideout, Yerxa, Shaver, & Douglass III, 2003).

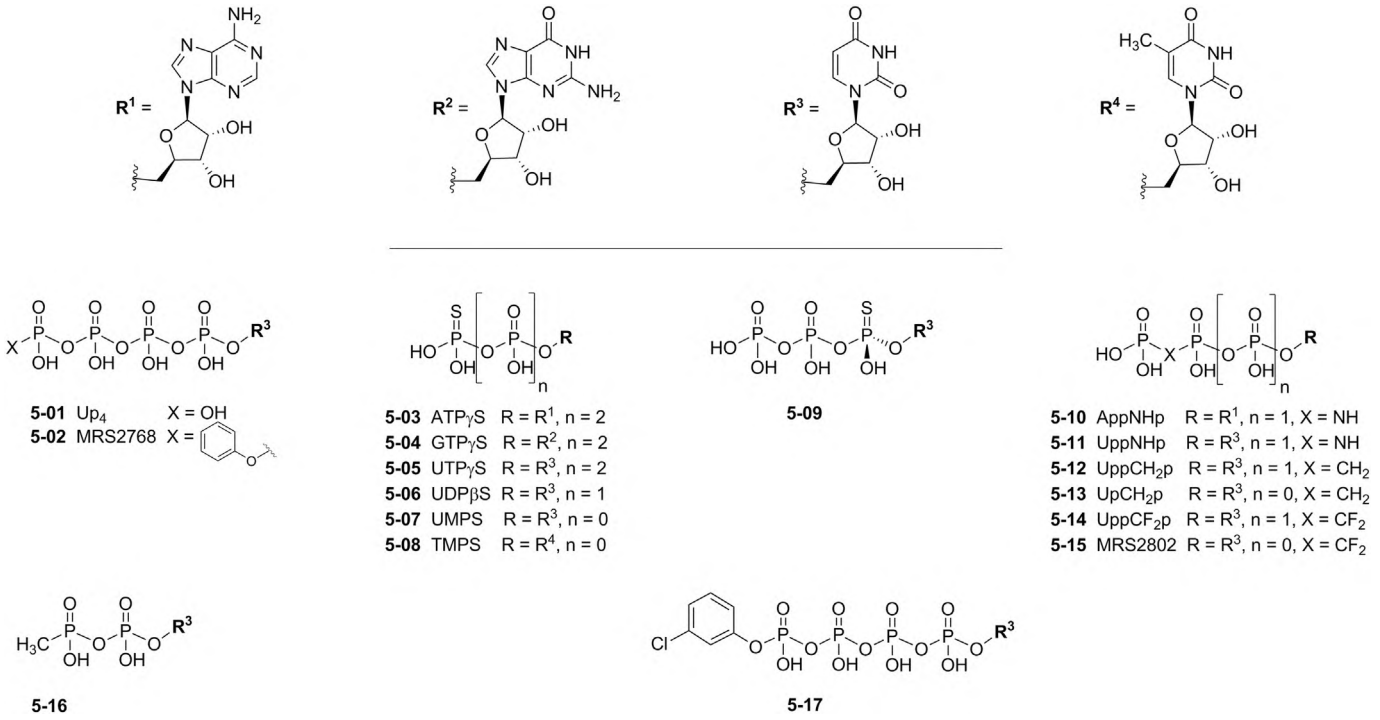


Fig. 5. Structures of agonists at uracil nucleotide-activated P2YRs with substitutions at the phosphate chain (for data, refer to Table 5).

2.1.4. An allosteric $P2Y_2$ receptor partial agonist

A carbon-phosphorus bond cannot be hydrolyzed by ectonucleotidases. Thus, in an attempt to enhance metabolic stability, the α-phosphate group of UTP was replaced by an isosteric phosphonate (Cosyn et al., 2009). It was subsequently discovered that the entire triphosphate group could be replaced by a smaller 5′-methylenephosphonate moiety. This modification also enhanced the compounds' selectivity for the $P2Y_2R$ over the $P2Y_4R$ (van Poecke et al., 2012). For a series of uridine 5′-methylenephosphonates with varying substitutions in the 5-position of the uracil base the potencies were in the high nanomolar to low micromolar range. The most potent compound, SVP333 (**6-11**, Fig. 6 and Table 6), displayed an EC_{50} value of 400 nM (van Poecke et al., 2012). The maximal receptor stimulation elicited by these compounds was not higher than half of that observed with UTP, suggesting that they act as partial agonists. High concentrations (100 μM) did not affect UTP-mediated receptor activation, as would otherwise be expected from orthosteric partial agonists. They were thus described as allosteric partial agonists (van Poecke et al., 2012). Replacing the 5-(4-fluoropheny)uracil ring of SVP333 (**6-11**, Fig. 6 and Table 6) with quinazoline-2,4-dione led to a reduction in potency (Song et al., 2014).

2.1.5. Discovery of a non-nucleotide allosteric $P2Y_2$ receptor modulator

Very recently, the discovery of a weakly potent non-nucleotide agonist for the $P2Y_2R$ was reported (Sakuma, Nakagawa, Oikawa, Noda, & Ikeda, 2017). The 4(1*H*)-quinoline derivative 2-((ethyl(4-fluorobenzyl)amino)methyl)-7,8-dimethylquinolin-4(1*H*)-one (**9-01**, Fig. 9 and Table 9), referred to as "Compound 89", exhibited an EC_{50} value of 10.5 μM at the human $P2Y_2R$, and of 2.7 μM at the mouse $P2Y_2R$ in calcium mobilization assays using recombinant 1321N1 astrocytoma cells. It was described as a partial agonist with 66 % (human $P2Y_2R$) and 51 % (mouse $P2Y_2R$) efficacy. However, these maximum efficacy values were calculated by extrapolation and could not be determined experimentally due to the low potency and limited solubility. **9-01** was inactive at 30 μM at the human $P2Y_1R$, $P2Y_4R$, $P2Y_6R$, $P2Y_{11}R$, and $P2Y_{12}R$, and was thus reported to be selective for the $P2Y_2R$ (Sakuma et al., 2017). In recombinant 1321N1 astrocytoma cells, it activated the $P2Y_2R$ in the absence of an orthosteric agonist but also exhibited positive allosteric modulation in combination with the agonists ATP, UTP, and AP_4A; it thus appears to function as an ago-allosteric agonist (for more information on allosteric modulation of GPCRs, see Müller, Schiedel, & Baqi, 2012 and Christopoulos et al., 2014). In neonatal rat cardiomyocytes, **9-01** behaved as a positive allosteric modulator only (Sakuma et al., 2017). Being the only non-nucleotide $P2Y_2R$ agonist described in the literature so far, **9-01** might serve as a lead structure for the development of analogues with higher potency that could be useful tool compounds with the potential to become therapeutics.

2.1.6. N^4-Substituted cytidine 5′-triphosphate (CTP) derivatives

Recently, a series of CTP derivatives with varying (3-arylpropyl)oxy substitutions on the cytosine amino group (N^4) was published (Jayasekara et al., 2014). These compounds revealed that sterically demanding groups in this region of the nucleotide were tolerated by the $P2Y_2R$, $P2Y_4R$, and $P2Y_6R$. The potencies and selectivity profiles could be modulated by structural changes of the N^4-substituent. Among these compounds showed N^4-(3-(4-methoxyphenyl)propyl) oxy-UTP (**3-21**, Fig. 3 and Table 3) relatively high potencies on the $P2Y_2R$ (EC_{50} of 47 nM), the $P2Y_4R$ (EC_{50} of 23 nM), and the $P2Y_6R$ (EC_{50} of 277 nM) (Jayasekara et al., 2014). Since steric bulk in this region does not significantly impede binding to those receptors, **3-21** was selected for the attachment of fluorophores and other reporter moieties as prosthetic groups. MRS4162 (**3-40**, Fig. 3 and Table 3) is a fluorescent derivative that features a boron-dipyrromethene (BODIPY®) 630/650 moiety (Jayasekara et al., 2014). MRS4162 exhibited potencies in the mid-nanomolar range on the $P2Y_2R$, $P2Y_4R$, and $P2Y_6R$. It was shown in a preliminary feasibility study using flow cytometry to label 1321N1 astrocytoma cells that express $P2Y_6Rs$ but not those without P2YR expression (Jayasekara et al., 2014). MRS4162 is thus a useful tool for the labeling and quantification of the $P2Y_6R$ (and most likely also the $P2Y_2R$ and $P2Y_4R$) in living cells, similar to the development of fluorescent ligands for adenosine receptors (Dale, Hill, & Kellam, 2012; Kozma et al., 2012). However, it will be difficult to differentiate

Table 5
Nucleotide derivatives with substitutions at the phosphate groups as agonists for the uracil-activated P2YRs. Shown are the EC_{50} or, in some cases, K_i and K_B values in μM, unless stated otherwise. The correlation factor by which the potency is n-fold higher (↑nx) or lower (↓nx) compared to that of the endogenous agonist in the same test system is given in brackets. Particularly interesting values are shown in bold.

Modifications at the phosphate groups							
No.	**Name**	**EC_{50} in μM**				**Comments**	**References**
		(potency higher (↑) or lower (↓) relative to endogenous agonist)					
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
5-01	Up_4	2.61 (n.d.[a] / ↓44x[b])	4.64 (↓52x)[b]	7.56 (↓25x)[c]	n.d.		Ko et al., 2008
5-02	Up_4-δ-phenyl ester (MRS2768)	1.89 (n.d.[a] / ↓32x[b])	>10 (↓>110x)[b]	>10 (↓>33x)[c]	n.d.	Commercially available	Ko et al., 2008
5-03	ATPγS	0.570 - 1.72 (↓7-8x[a] / ↓12-29x[b]) **Rat:** 10.5 (↓4x[a] / ↓3x[b]) **Mouse:** 94 % receptor activation at 100 μM 7.9 (↓11x[a] / ↓7x[b]) 10.4 (↓7x[a] / ↓12x[b]) **Dog:** ≈1 (↓≈5x[a] / n.d.[b]) **Pig:** 1.0 (↑3x[a] / ↓2x[b]) 54 % efficacy	inactive (n.d.)[b] **Rat:** 2.1 (1x)[a,b] partial agonist 5.4 (↓4x[a] / ↓3x[b]) partial agonist with 24 % efficacy	n.d. **Rat:** inactive at 300 μM (↓>130x)[c]	n.d.		Bogdanov et al., 1998; Communi, Motte et al., 1996; Erb et al., 1993; Janssens et al., 1999; Lazarowski et al., 1995; Lazarowski & Harden, 1994; Lin et al., 1993; Lustig et al., 1993; Shen et al., 2004; Wildman et al., 2003; Zambon et al., 2000
5-04	GTPγS	26.5 (↓120x[a] / ↓190x[b]) **Pig:** 1.2 (↑3x[a] / ↓2x[b]) 80 % efficacy	n.d.	n.d.	n.d.		Lazarowski et al., 1995; Shen et al., 2004
5-05	UTPγS	0.240 (1x[a] / ↓2x[b])	1.6 (↓2x)[b]	n.d. **Rat:** weak agonist	n.d.	α-Thiophosphate unfavorable for $P2Y_2R$ & $P2Y_4R$	Erb et al., 1993; Jacobson et al., 2006; Lazarowski et al., 1996; Nicholas et al., 1996
5-06	UDPβS	inactive at 10 μM (n.d.)[a,b]	inactive at 10 μM (n.d.)[b]	**0.028** (↑2x)[c]	**0.026** (↓3x[c] / ↓12x[d])	Initially thought to be a competitive	Carter et al., 2009; Fricks et al., 2008;

Table 5 (*continued*)

Modifications at the phosphate groups							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
						antagonist at $P2Y_{14}R$	Hou et al., 2002
5-07	UMPS	n.d.	n.d.	62 % receptor activation at 10 μM	81 % receptor activation at 10 μM	AMPS & CMPS are also agonists with EC_{50} >10 μM at $P2Y_{14}R$; TMPS is an antagonist	Gendaszewska-Darmach et al., 2016; Gendaszewska-Darmach & Szustak, 2016
5-08	TMPS	inactive at 1 mM (n.d.)[a,b]	inactive at 1 mM (n.d.)[b]	partial agonist with 50 % efficacy	antagonist (see Table 10)	AMPS, UMPS & CMPS are also $P2Y_{14}R$ agonists	Gendaszewska-Darmach et al., 2016; Gendaszewska-Darmach & Szustak, 2016
5-09	R_p-*α*-S-UTP	5.4 (↓64x[a] / ↓110x[b])	27 (↓370x)[b]	n.d.	n.d.	2'-Deoxy-analogues less potent or inactive at $P2Y_2R$ & $P2Y_4R$	Jacobson et al., 2006
5-10	*β*,*γ*-Imido-ATP (AppNHp)	5.66 (↓25x[a] / ↓40x[b]) **Mouse:** inactive	n.d.	n.d.	n.d.		Erb et al., 1993; Lazarowski et al., 1995
5-11	*β*,*γ*-Imido-UTP (UppNHp)	1.45 (n.d.)[a,b]	n.d.	n.d.	n.d.		Brunschweiger & Müller, 2006
5-12	*β*,*γ*-Methylene-UTP ($UppCH_2p$)	73.3 (n.d.)[a,b]	n.d.	n.d.	n.d.		Brunschweiger & Müller, 2006
5-13	*α*,*β*-Methylene-UDP ($UpCH_2p$)	n.d.	n.d.	0.339 (↑2x)[c] 0.66 (↓2x)[c]	0.011 (↑15x[c] / ↑36x[d])		Das et al., 2010; Ko et al., 2008
5-14	*β*,*γ*-Difluoro methylene-UTP ($UppCF_2p$)	4.9 (n.d.[a] / ↓270x[b])	n.d.	n.d.	n.d.		El-Tayeb et al., 2011
5-15	*α*,*β*-Difluoro methylene-UDP (MRS2802)	n.d.	n.d.	inactive at 10 (↓>33x)[c]	0.063 (↑3x[c] / ↑6x[d])		Carter et al., 2009; Das et al., 2010; Ko et al., 2008
5-16	Up_2-*β*-Me-phosphonate	n.d.	n.d.	8.0 (↓15x)[c]	4.58 (↓29x[c] / ↓12x[d])		Das et al., 2010
5-17	Up_4-*δ*-3-chlorophenyl phosphoester	0.840 (n.d.[a] / ↓15x[b])	inactive at 10 μM (↓>130x)[b]	3.69 (n.d.)[c]	n.d.	4-fold selective for $P2Y_2R$	Maruoka et al., 2011

[a]relative to ATP; [b]relative to UTP; [c]relative to UDP; [d]relative to UDP-glucose.

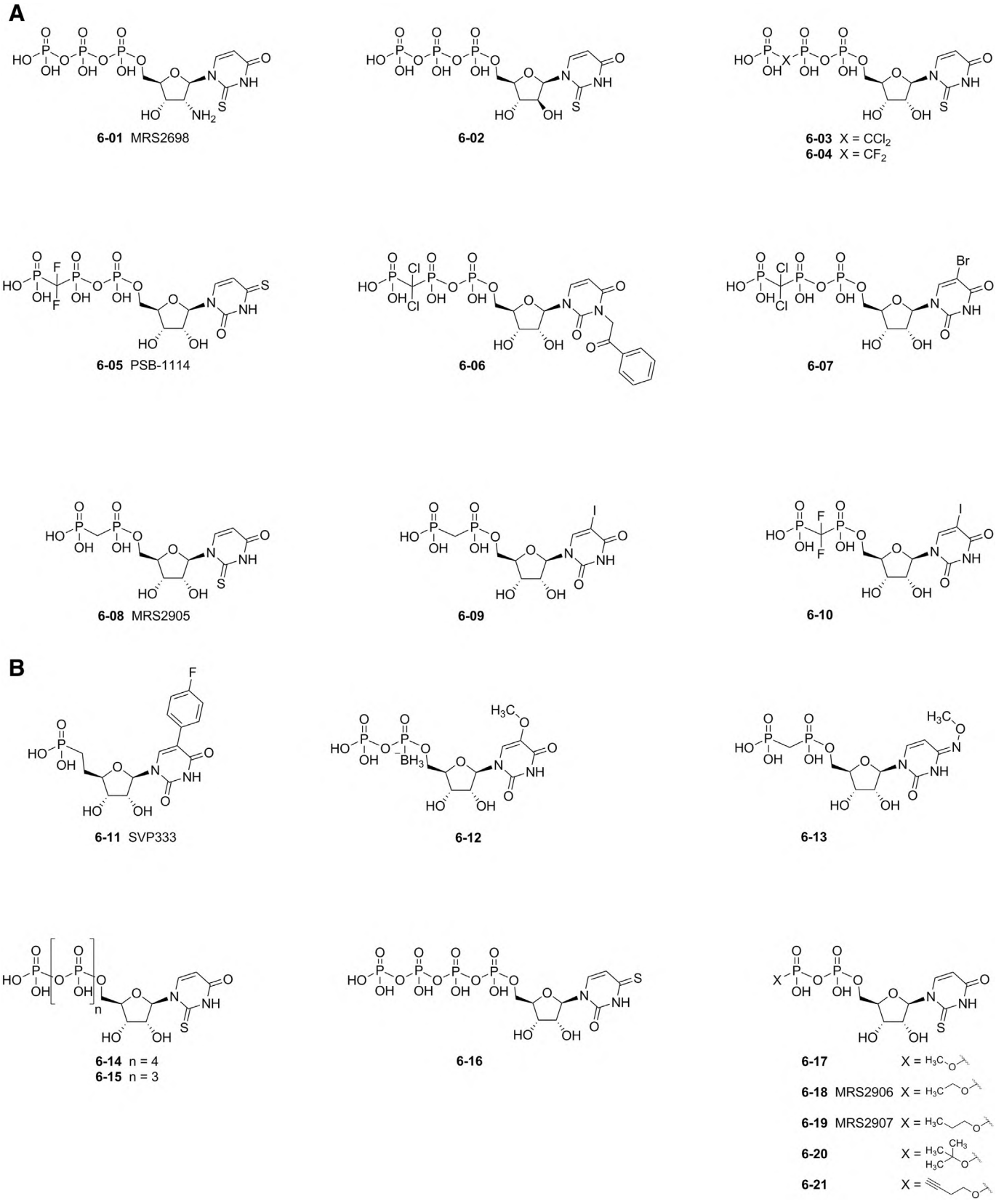


Fig. 6. Structures of agonists at uracil nucleotide-activated P2YRs with combined substitutions at the base, ribose ring, and/or phosphate chain (for data, refer to Table 6).

the response for cell lines where different P2YRs are simultaneously expressed. Developing analogues with improved selectivity may be a next step, as would be the development of assays for routine application of this fluorescent probe. Comprehensive information on fluorescent probes for P2YRs (as well as adenosine receptors) and their use were published by Ciruela, Fernández-Duenas, & Jacobson, 2015.

Table 6
Mononucleotide derivatives with combined modifications at the base, ribose ring, and/or phosphate chain as agonists for the uracil-activated P2YRs. Shown are the EC_{50} or, in some cases, K_i and K_B values in μM, unless stated otherwise. The correlation factor by which the potency is n-fold higher (↑nx) or lower (↓nx) compared to that of the endogenous agonist in the same test system is given in brackets. Particularly interesting values are shown in bold.

Mononucleotide derivatives with combined substitutions							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
6-01	2'-Amino-2'-deoxy-2-S-UTP (MRS2698)	**0.008** (↑11x[a] / ↑6x[b])	2.4 (↓33x)[b]	inactive at 10 μM (↓>440x)[c]	n.d.	**300-fold $P2Y_2R$ selective**	Ivanov, Ko et al., 2007; Ko et al., 2008
6-02	2-S-arabino-UTP	0.14 (n.d.[a] / ↓2x[b])	7.93 (↓88x)[b]	inactive at 10 μM (↓>33x)[c]	n.d.		Ko et al., 2008
6-03	2-S-β,γ-dichloro-methylene-UTP	2.51 (n.d.[a] / ↓42x[b])	inactive at 10 μM (↓>110x)[b]	inactive at 10 μM (↓>33x)[c]	n.d.		Ko et al., 2008
6-04	2-S-β,γ-difluoro-methylene-UTP	1.63 (n.d.[a] / ↓27x[b])	8.11 (↓90x)[b]	5.15 (↓17x)[c]	n.d.		Ko et al., 2008
6-05	4-S-β,γ-difluoro-methylene-UTP (PSB-1114)	0.134 (n.d.[a] / ↓8x[b])	9.3 (↓110x)[b]	7.0 (↓150x)[c]	n.d.	50-fold selective for $P2Y_2R$ Enhanced ectonucleotidase stability Commercially available	El-Tayeb et al., 2011
6-06	*N*3-phenacyl-β,γ-dichloromethylene-UTP	0.826 (n.d.[a] / ↓46x[b])	7.3 (↓83x)[b]	0.142 (↓3x)[c]	n.d.		El-Tayeb et al., 2011
6-07	β,γ-Dichloro-methylene-5-Br-UTP	0.354 (n.d.[a] / ↓20x[b])	3.99 (↓45x)[b]	0.120 (↓3x)[c]	n.d.		El-Tayeb et al., 2006
6-08	α,β-Methylene-2-S-UDP (MRS2905)	n.d.	n.d.	1.99 (↓4x)[c]	**0.00092** (↑170x[c] / ↑440x[d])	Commercially available α,β-Methylene-UDP 12-fold less potent at $P2Y_{14}R$	Das et al., 2010
6-09	α,β-Methylene-5-I-UDP	n.d.	n.d.	0.13 (↑2x)[c]	n.d.		Maruoka et al., 2010
6-10	α,β-Difluoro-methylene-5-I-UDP	n.d.	n.d.	0.127 (↑4x)[c]	0.142 (1x[c] / ↑3x[d])		Das et al., 2010
6-11	SVP333	0.4 (n.d.[a] / ↓20x[b])	>100 (n.d.)[b]	>100 (n.d.)[c]	n.d.	**Allosteric** partial agonist	van Poecke et al., 2012
6-12	5-MeO-uridine 5'-	inactive at 100 μM	inactive at 100 μM	**0.008** (↑19x)[c]	n.d.	**>12,500-fold**	WO002012073237A1,

(*continued on next page*)

Table 6 (*continued*)

Mononucleotide derivatives with combined substitutions							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
	O-(*α*-borano diphosphate), R_p isomer	(n.d.[a] / ↓>710x[b])	(n.d.[a] / ↓110x[b])			**$P2Y_6$R selective** Patented	2011; Ginsburg-Shmuel et al., 2012
6-13	*α,β*-Methylene-*N*^{4}-MeO-CDP	>10 (n.d.[a] / ↓>170x[b])	inactive at 10 μM (↓110x)[b]	0.678 (↓2x)[c]	n.d.		Maruoka et al., 2010
6-14	2-S-Up_5	0.57 (n.d.[a] / ↓10x[b])	5.27 (↓59x)[b]	7.33 (↓24x)[c]	n.d.		Ko et al., 2008
6-15	2-S-Up_4	0.60 (n.d.[a] / ↓10x[b])	5.52 (↓61x)[b]	6.83 (↓23x)[c]	n.d.		Ko et al., 2008
6-16	4-S-Up_4	0.070 (n.d.[a] / 1x[b])	0.28 (↓3x)[b]	6.46 (↓22x)[c]	n.d.		Ko et al., 2008
6-17	2-S-Up_2-OMe	n.d.	n.d.	inactive at 10 μM (↓19x)[c]	0.056 (↑3x[c] / ↑7x[d])		Das et al., 2010
6-18	2-S-Up_2-OEt (MRS2906)	n.d.	n.d.	inactive at 10 μM (↓19x)[c]	0.039 (↑4x[c] / ↑10x[d])	4-S-analogue of **6-17** inactive at $P2Y_{14}$R	Das et al., 2010
6-19	2-S-Up_2-OPr (MRS2907)	n.d.	n.d.	9.1 (↓17x)[c]	0.040 (↑4x[c] / ↑10x[d])		Das et al., 2010; Fricks et al., 2009
6-20	2-S-Up_2-$OC(CH_3)_3$	n.d.	n.d.	2.04 (↓4x)[c]	0.032 (↑5x[c] / ↑13x[d])		Das et al., 2010
6-21	2-S-Up_2-$O(CH_2)_2C{\equiv}CH$	n.d.	n.d.	inactive at 10 μM (↓19x)[c]	0.011 (↑15x[c] / ↑36x[d])		Das et al., 2010

[a]relative to ATP; [b]relative to UTP; [c]relative to UDP; [d]relative to UDP-glucose.

2.2. $P2Y_2$ receptor antagonists

2.2.1. Therapeutic potential of $P2Y_2$ receptor antagonists

$P2Y_2$R antagonists could function, among other possible indications, as anti-metastatic cancer therapeutics. This hypothesis is based in part on the observation that $P2Y_2$Rs expressed on the endothelium open the endothelial barrier in response to ATP. The nucleotide is released from platelets following induction by tumor cells, thereby facilitating tumor cell extravasation from the blood stream into surrounding tissue to form secondary tumor sites (Schumacher, Strilic, Sivaraj, Wettschureck, & Offermanns, 2013). In addition, the proliferation and migration of different tumor and non-tumor cells, as well as the induction of cell cycle progression in vascular smooth muscle cells was attributed to $P2Y_2$R activation (Greig, Linge, Cambrey, & Burnstock, 2003; Malam-Souley et al., 1996; Martínez-Ramírez, Garay, García-Carrancá, & Vázquez-Cuevas, 2016; Miyagi et al., 1996; Muscella, Elia, Greco, Storelli, & Marsigliante, 2003; Qiu et al., 2018; Robles-Martinez et al., 2017; Schafer, Sedehizade, Welte, & Reiser, 2003; Shen et al., 2004; Tu et al., 2000; Wilden, Agazie, Kaufman, & Halenda, 1998; Zhang et al., 2017). Another therapeutic application for $P2Y_2$R antagonists may be psoriasis, as $P2Y_2$R activation was shown to induce keratinocyte proliferation and neutrophil migration (Chen et al., 2006; Dixon et al., 1999). $P2Y_2$R antagonists may in fact be useful for a range of excessive inflammatory reactions, including atherosclerosis. This is because the $P2Y_2$R was found to activate cytosolic phospholipase A_2, which in turn promotes the release of arachidonic acid and the subsequent synthesis of prostaglandins and leukotrienes (Seye et al., 2002; Welch, Carlson, Shi, Myatt, & Kishore, 2003; Xing, Post, Ostrom, Samardzija, & Insel, 1999; Xu et al., 2002; Xu et al., 2003). The potential of $P2Y_2$R blockade in the treatment of atherosclerosis is further emphasized by the following observations: the receptor appears to mediate the uptake of low-density lipoprotein in vascular smooth muscle cells and contribute to vascular inflammation by increasing vascular cell adhesion molecule-1 expression. Moreover, plaques were more stable in mice with an endothelial cell-specific deficiency of $P2Y_2$Rs (Chen et al., 2017; Dissmore et al., 2016). $P2Y_2$R function was also linked to the development of neointimal hyperplasia and pulmonary fibrosis (Agca, Qian, Agca, & Seye, 2016). In addition, $P2Y_2$R activation in human esophageal epithelial cells led to the release of the pro-inflammatory chemokine interleukin-8, which is believed to contribute to mucosal inflammation and gastroesophageal reflux disease (Wu, Oshima, Fukui, Watari, & Miwa, 2016). Furthermore, the $P2Y_2$R is expressed in collecting ducts of the kidney, where it opposes the actions of antidiuretic hormone (arginine vasopressin) and, consequently, reduces water reuptake into the blood (Kishore et al., 2015). Thus, patients suffering from nephrogenic diabetes insipidus acquired, for example, through chronic use of lithium in a bipolar disorder therapy, may benefit from $P2Y_2$R antagonists; a concept that has been patented (Kishore, Carlson, & Zhang, 2012). $P2Y_2$R antagonists could further prove useful in the treatment of osteoporosis and pain. They could prevent the $P2Y_2$R-mediated inhibition of bone formation and its involvement in thermal nociception (Hoebertz, Mahendran, Burnstock, & Arnett, 2002; Malin et al., 2008; Orriss et al., 2017; Shi et al., 2017). Refer to Table 1 for an overview of the possible indications for $P2Y_2$R modulation.

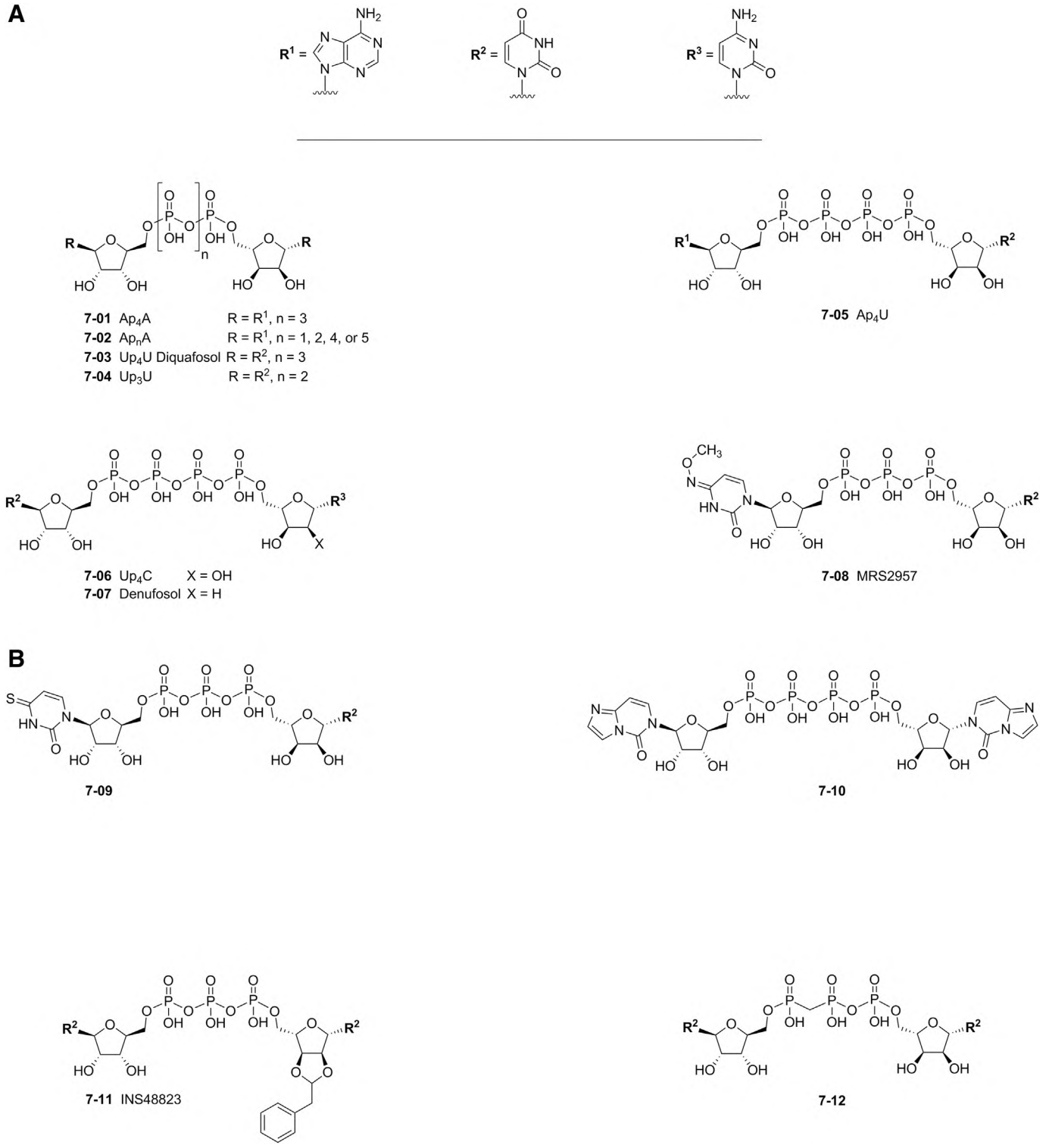


Fig. 7. Structures of dinucleotide agonists at uracil nucleotide-activated P2YRs (for data, refer to Table 7).

2.2.2. AR-C118925

In comparison to the relatively large number of agonists reported for the $P2Y_2R$, only few antagonists have been described to date. The most potent antagonist reported in the literature is the thiouracil derivative 5-{[5-(2,8-dimethyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)-3,4-dihydro-2-oxo-4-thioxo-1(2*H*)-pyrimidinyl]methyl}-*N*-(1*H*-tetrazol-5-yl)-2-furancarboxamide (AR-C118925, **10-01**, compound 01 in Fig. 10 and Table 10). For AR-C118925, IC_{50} values in the mid-nanomolar to low micromolar range were reported (Kemp, Sugar, & Jackson, 2004; Rafehi, Burbiel, Attah, Abdelrahman, & Müller, 2017). The selectivity of this compound for the $P2Y_2R$ over other P2YR subtypes is approx. 50- to 500-fold. It is particularly high versus the $P2Y_4R$ (500-fold), which is the P2YR subtype that is structurally and pharmacologically most closely related to the $P2Y_2R$ (Rafehi, Burbiel, et al., 2017). Being a structural analogue of the endogenous agonist UTP (refer to Kindon et al., 2017 for an account on the systematic development of AR-C118925

Table 7
Physiological and synthetic dinucleotide polyphosphate compounds and analogues as agonists for the uracil-activated P2YRs. Shown are the EC_{50} or, in some cases, K_i and K_B values in μM, unless stated otherwise. The correlation factor by which the potency is n-fold higher (↑nx) or lower (↓nx) compared to that of the endogenous agonist in the same test system is given in brackets. Particularly interesting values are shown in bold.

Dinucleotides							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
7-01	Ap_4A	0.054 - 0.720 (↓3-4x[a] / 1x-↓13x[b]) **Rat:** 6.5 (↓2x)[a,b]	inactive at 100 μM (↓1800-14300x)[b] low activity at 100 μM (n.d.)[b] **Rat:** 1.24 - 3.0 (↓2x[a] / 1x-↓6x[b]) inactive (n.d.)[a,b]	low activity at 100 μM (n.d.)[c]	inactive at 1 μM (n.d.[c] / ↓10x[d])	>140-fold $P2Y_2R$ selective Boranophosphate analogue patented	Bogdanov et al., 1998; Chambers et al., 2000; Communi et al., 1995; US020060287271A1, 2006; Janssens et al., 1999; Kennedy et al., 2000; Lazarowski et al., 1995; Patel et al., 2001; Sakuma et al., 2017; Shaver et al., 2005; Webb et al., 1998; Wildman et al., 2003
7-02	Ap_nA, where n = 2, 3, 5, or 6	12.6 - 39.8 (n.d.[a] / ↓160-500x[b]) 30 % efficacy of Ap_3A **Rat:** 20.3 - 1029 (↓8-380x[a] / ↓6x-290[b])	>1000 (n.d.)[b] low activity at 100 μM (n.d.)[b] **Rat:** 2.6 (↓2x-670[a] / ↓2-560x[b]) partial agonists with efficacy <25 % or inactive at 1 mM	Only Ap_3A and Ap_5A active (n.d.)[c]	inactive at 1 μM (n.d.[c] / ↓10x[d])	Boranophosphate analogues patented	Chambers et al., 2000; Communi et al., 1995; US020060287271A1, 2006; Janssens et al., 1999; Patel et al., 2001; Shaver et al., 2005; Wildman et al., 2003
7-03	Up_4U (Diquafosol, INS365)	0.06 - 0.210 (↓4x[a] / ↓3-4x[b])	0.130 - 0.20 (↓2-4x)[b]	1.16 - 24.8 (↓50-200x)[c]	inactive (n.d.)[c,d]	Diquafosol tetrasodium (Diquas®) **approved in Japan, Korea, Vietnam, and Thailand** as 3 % ophthalmic solution for dry eye disease Boranophosphate analogues patented	US020060287271A1, 2006; Ivanov, Fricks et al., 2007; Keating, 2015; Ko et al., 2007; Lau et al., 2014; Maruoka et al., 2011; Pendergast et al., 2001; Shaver et al., 2005

Table 7 (*continued*)

7-04	Up_3U	1.31 - 22 (n.d.[a] / ↓22-730x[b])	0.87 (1x)[b] >100 (↓>1000x)[b]	0.2 - 0.92 (↓2x)[c]	n.d.	Boranophosphate analogues patented	WO002007002945A2, 2006; US020060287271A1, 2006; Maruoka et al., 2010; Pendergast et al., 2001; Shaver et al., 2005
7-05	Ap_4U	0.35 (↓23x[a] / ↓23x[b])	1.45 (↓21x)[b]	>100 (↓>200x)[c]	n.d.		
7-06	Up_4C	0.45 (n.d.)[a,b] 0.46 (↓3x)[a,b]	0.65 (n.d.)[b] 0.85 (↓12x)[b]	3.5 (n.d.)[c] 12.1 (↓24x)[c]	n.d.	Patented	Shaver et al., 2005; Yerxa et al., 2002; WO001999061012A2,
7-07	Up_4dC (Denufosol, INS37217)	0.22 (n.d.)[a,b] 0.27 (↓18x[a] / ↓18x[b])	0.8 (n.d.)[b] 1.22 (↓17x)[b]	≈70 % at 100 μM 16.0 (↓32x)[c]	n.d.		1999
7-08	N^4-MeO-Cp_3U (MRS2957)	0.17 (n.d.[a] / ↓3x[b])	0.79 (↓9x)[b]	**0.012** (↑25x)[c]	K_i = 5.18 (↓8x[c] /↓2x[d])	Commercially available	Kiselev et al., 2015; Maruoka et al., 2010
7-09	4-S-Up_4U	0.04 (↓3x)[a,b]	0.17 (↓2x)[b]	190 (↓380x)[c]	n.d.	Patented	WO001998034942A2, 1998; Shaver et al., 2005
7-10	P^1,P^4-di(3,N^4-ethenocytidine 5'-)tetraphosphate	0.46 (n.d.)[a,b]	19.8 (n.d.)[b]	inactive (n.d.)[c]	n.d.		
7-11	Monobenzylacetal-Up_3U (INS48823)	inactive (n.d.)[a,b]	inactive (n.d.)[b]	≈0.125 (1x)[c] 0.140 (↑4x)[c]	n.d.	Patented	WO002007002945A2, 2006; Ginsburg-Shmuel et al., 2012; Jayasekara et al., 2013; Korcok et al., 2005; Maruoka et al., 2010
7-12	α,β-Methylene-Up_3U	>10 (n.d.[a] / ↓>170x[b])	inactive at 10 μM (↓110x)[b]	1.30 (↓4x)[c]	n.d.	Patented	WO002007002945A2, 2006; Maruoka et al., 2010

[a]relative to ATP; [b]relative to UTP; [c]relative to UDP; [d]relative to UDP-glucose.

from UTP), AR-C118925 acts as a competitive antagonist (Rafehi, Burbiel, et al., 2017). In the absence of an X-ray structure, a homology model supported by mutagenesis data was recently developed that shows a likely binding pose and interactions of AR-C118925 with residues in the putative orthosteric site (Rafehi et al., 2017). This is so far the only reported $P2Y_2R$ homology model that was developed based on the recently published $P2Y_1R$ and $P2Y_{12}R$ X-ray structures (Zhang et al., 2014; Zhang et al., 2014; Zhang et al., 2015), while previous models were based on the distantly related bovine rhodopsin.

In addition to the relatively high degree of selectivity for the $P2Y_2R$, AR-C118925 (**10-01**, Fig. 10 and Table 10) has a number of desirable physicochemical and pharmacokinetic properties, including high solubility in phosphate-buffered saline and a very high metabolic stability in human and mouse liver microsomes. It has reasonable rat *in vivo* pharmacokinetic properties, with intravenous clearance of 75 mL/min/kg, V_{ss} of 4.34 L/kg and a half-life of 2.12 h. AR-C118925 (**10-01**) was shown to inhibit CYP2C8 but not eight other cytochrome P450 subtypes (including CYP3A4) at 1 μM. However, its bioavailability following oral administration is very low (Conroy, Kindon, Kellam, & Stocks, 2016; Kindon et al., 2017; Rafehi, Burbiel, et al., 2017). The compound, developed by AstraZeneca, was assessed in pre-clinical trials as a topical skin treatment for chronic psoriasis, but was found to be ineffacious (Conroy et al., 2016; Kindon, Meghani, & Thom, 1998). One reason for this might be its high polarity, which may prevent it from penetrating into deeper layers of the skin. AR-C118925 is nevertheless a valuable pharmacological tool to study $P2Y_2R$ function and has been utilized in several *in vivo* and *in vitro* studies (Cosentino et al., 2012; Hochhauser et al., 2013; Kemp et al., 2004; Magni, Merli, Verderio, Abbracchio, & Ceruti, 2015; Önnheim et al., 2014; Wang et al., 2015).

2.2.3. Suramin and Reactive Blue 2

AR-C118925 (**10-01**, Fig. 10 and Table 10) became available through commercial sources only recently and was therefore inaccessible for most research groups. Thus, the antagonists used most widely to study $P2Y_2R$ function were suramin (**10-04**, Fig. 10 and Table 10) and the dye Reactive Blue 2 (RB-2, **10-07**, Fig. 10 and Table 10). RB-2 is a large (molecular weight of 840 g/mol), polysulfonated, and polyaromatic anthraquinone derivative with an IC_{50} value in the micromolar range at the $P2Y_2R$ (Baqi et al., 2010; Rafehi, Neumann, et al., 2017; Weyler et al., 2008). However, smaller derivatives that retain the anthraquinone core are similarly potent at the $P2Y_2R$ (Weyler et al., 2008). RB-2 is more potent at the $P2Y_4R$ than at the $P2Y_2R$; it will consequently be dealt with in more detail in Section 3.2.2.

Suramin (**10-04**, Fig. 10 and Table 10) was developed by the pharmaceutical company Bayer in 1920 as an anti-parasitic drug and is still in use for the treatment of sleeping sickness caused by trypanosomes (see reviews by Singh Grewal, Pandita, Bhardwaj, & Lather, 2016; Voogd, Vansterkenburg, Wilting, & Janssen, 1993). With a molecular

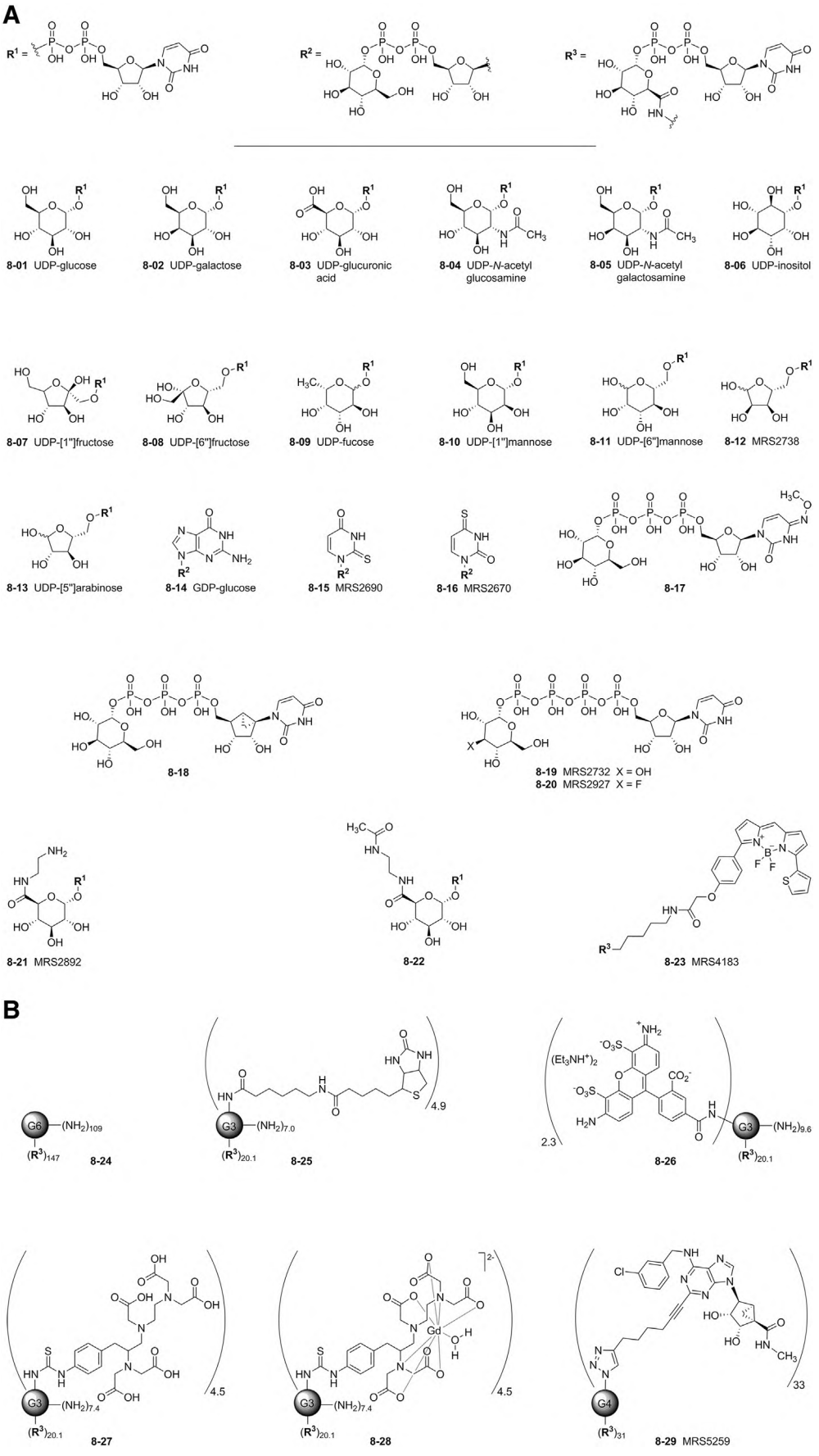


Fig. 8. (A) Structures of nucleotide-sugar agonists at uracil nucleotide-activated P2YRs (for data, refer to Table 8). (B) Structures of UDP-glucuronic acid dendrimer conjugates. G3 and G6 represent 3 and 6 generations of dendrimers, respectively (refer to Table 8 for data).

Table 8
Physiological and synthetic nucleotide-sugar conjugates as agonists for the uracil-activated P2YRs. Shown are the EC_{50} or, in some cases, K_i and K_B values in μM, unless stated otherwise. The correlation factor by which the potency is n-fold higher (↑nx) or lower (↓nx) compared to that of the endogenous agonist in the same test system is given in brackets. Particularly interesting values are shown in bold.

Nucleotide sugars							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
8-01	UDP-glucose	10 (n.d.)[a,b]	n.d.	16 (↓30)[c] **Rat:** >1000 (↓>440x)[c]	0.0022 - 0.400 (1x-↓15x)[c] K_i = 0.020 - 2.23 (↑2x-↓4x)[c] **Rat:** 0.026 (n.d.)[c] 0.189 - 5.98 (1x)[c] **Mouse:** 0.0028 - 0.022 (↑2x)[c] **Chimpanzee:** 0.0044 - 0.0106 (1x-↓2x)[c] K_i = 0.023 (↓2x)[c]	UDP-β-[1'']glucose half as potent at $P2Y_{14}R$ UDP-[6'']glucose of similar potency Fluorinated deoxy-derivatives published	Carter et al., 2009; Chambers et al., 2000; Das et al., 2009; Das et al., 2010; Freeman et al., 2001; Fricks et al., 2009; Gao et al., 2010; Hamel et al., 2011; Ivanov, Fricks et al., 2007; US020090148850A1, 2008; Kiselev et al., 2015; Ko et al., 2007; Ko et al., 2009; Lazarowski & Harden, 1994; Scrivens & Dickenson, 2005
8-02	UDP-galactose	n.d.	n.d.	n.d.	0.0028 - 0.671 (1x-↓32x[c] / 1x-↓4x[d]) partial agonist **Rat:** 0.077 (n.d.[c] / ↓3x[d]) **Mouse:** 0.0028 - 0.063 (↓4x[c] / 1x-↓6x[d]) **Chimpanzee:** 0.0056 - 0.0272 (↓2-4x[c] / 1x-↓3x[d])		Freeman et al., 2001; Hamel et al., 2011; Ivanov, Fricks et al., 2007; US020090148850A1, 2008; Ko et al., 2007; Ko et al., 2009; Scrivens & Dickenson, 2005

(*continued on next page*)

Table 8 (*continued*)

Nucleotide sugars							
No.	**Name**	**EC_{50} in µM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
8-03	UDP-glucuronic acid	n.d.	n.d.	n.d.	0.006 - 0.576 (↓2-82x[c] / 1x-↓6x[d]) partial agonist K_i = 5.3 (↓8x[c] / ↓2x[d]) **Rat:** 0.102 (n.d.[c] / ↓4x[d]) **Mouse:** 0.0096 - 0.0915 (↓2-5x[c] / ↓3-8x[d]) **Chimpanzee:** 0.0054 - 0.0762 (1-↓11x[c] / ↓2-7x[d])	Derivatives of less potency published as well Dendrimer analogues also published	Chambers et al., 2000; Das et al., 2009; Freeman et al., 2001; Fricks et al., 2009; Hamel et al., 2011; Ivanov, Fricks et al., 2007; US20090148850A1, 2008; Kiselev et al., 2015; Ko et al., 2007; Ko et al., 2009; Scrivens & Dickenson, 2005
8-04	UDP-*N*-acetylglucosamine	n.d.	n.d.	n.d.	0.0417 - 4.38 (↓14-180x[c] / ↓2-19x[d]) partial agonist **Rat:** 0.170 (n.d.[c] / ↓7x[d]) **Mouse:** 0.0257 - 0.101 (↓3x[c] / ↓5-9x[d]) **Chimpanzee:** 0.079 - 0.149 (↓17-22x[c] / ↓12-16x[d])		Chambers et al., 2000; Freeman et al., 2001; Fricks et al., 2009; Hamel et al., 2011; Ivanov, Fricks et al., 2007; US20090148850A1, 2008; Ko et al., 2007; Ko et al., 2009; Scrivens & Dickenson, 2005
8-05	UDP-*N*-acetylgalactosamine	n.d.	n.d.	n.d.	0.810 (n.d.[c] / ↓2x[d])		Ko et al., 2007; Ko et al., 2009
8-06	UDP-inositol	n.d.	n.d.	n.d.	1.88 (n.d.[c] / ↓5x[d])		Ko et al., 2007; Ko et al., 2009
8-07	UDP-[1'']fructose	n.d.	n.d.	n.d.	0.880 (n.d.[c] / ↓3x[d])		Ko et al., 2007; Ko et al., 2009
8-08	UDP-[6'']fructose	inactive at 10 µM (n.d.)[a,b]	n.d.	n.d.	0.323 (n.d.[c] / 1x[d])		Ko et al., 2009

Table 8 (*continued*)

Nucleotide sugars							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
8-09	UDP-fucose	n.d.	n.d.	n.d.	0.562 (n.d.[c] / ↓2x[d])		Ko et al., 2009
8-10	UDP-[1'']mannose	n.d.	n.d.	n.d.	0.910 (n.d.[c] / ↓3x[d])		Ko et al., 2007; Ko et al., 2009
8-11	UDP-[6'']mannose	n.d.	n.d.	n.d.	0.658 (n.d.[c] / ↓3x[d])		Ko et al., 2007; Ko et al., 2009
8-12	UDP-[5'']ribose (MRS2738)	n.d.	n.d.	n.d.	0.238 (n.d.[c] / 1x[d])		Ko et al., 2009
8-13	UDP-[5'']arabinose	n.d.	n.d.	n.d.	0.460 (n.d.[c] / ↓2x[d])		Ko et al., 2009
8-14	GDP-glucose	n.d.	n.d.	n.d.	0.141 (↓59x[c] / ↓64x[d])		Hamel et al., 2011
8-15	2-S-UDP-[1'']glucose (MRS2690)	inactive at 10 μM (n.d.)[a,b]	n.d.	>10 (↓>19x)[c]	**0.011** (↑15x[c] / ↑36x[d]) **0.049** (n.d.[c] / ↑7x[d]) K_i = 0.34 (↑2x[c] / ↑7x[d]) **Rat:** 0.0081 - 0.538 (↑10x[c] / ↑11-23x[d])	Commercially available	Das et al., 2010; Gao et al., 2010; Kiselev et al., 2015; Ko et al., 2007; Ko et al., 2009
8-16	4-S-UDP-[1'']glucose (MRS2670)	n.d.	n.d.	n.d.	0.29 (n.d.[c] / 1x[d])		Ko et al., 2007
8-17	N^4-MeO-CTP-glucose	>10 (n.d.[a] / ↓>170x[b])	inactive at 10 μM (↓>110x)[b]	0.18 (↑2x)[c]	n.d.		Maruoka et al., 2010
8-18	(*S*)-methanocarba-UTP-glucose	inactive at 10 μM (n.d.[a] / ↓>170x[b])	inactive at 10 μM (↓>110x)[b]	2.47 (↓8x)[c]	n.d.		Maruoka et al., 2010
8-19	Up_4-[1'']glucose (MRS2732)	0.30 (n.d.[a] / ↓5x[b])	2.06 (↓23x)[b]	7.83 (↓26x)[c]	n.d.	6-fold selective for $P2Y_2R$	Ko et al., 2008
8-20	Up_4-[1'']3'-deoxy-3'-fluoroglucose (MRS2927)	0.710 (n.d.[a] / ↓13x[b])	**0.062** (1x)[b]	0.950 (n.d.)[c]	n.d.	11-fold selective for $P2Y_4R$	Maruoka et al., 2011
8-21	UDP-glucuronic acid-ethylenediamine (MRS2892)	n.d.	n.d.	n.d.	2.59 (n.d.[c] / ↓10x[d])	Amino-functionalized $P2Y_{14}R$ agonist	Das et al., 2009
8-22	UDP-glucuronic acid-(*N*-acetyl-ethylenediamine)	n.d.	n.d.	n.d.	0.496 (n.d.[c] / ↓2x[d])		Das et al., 2009

(*continued on next page*)

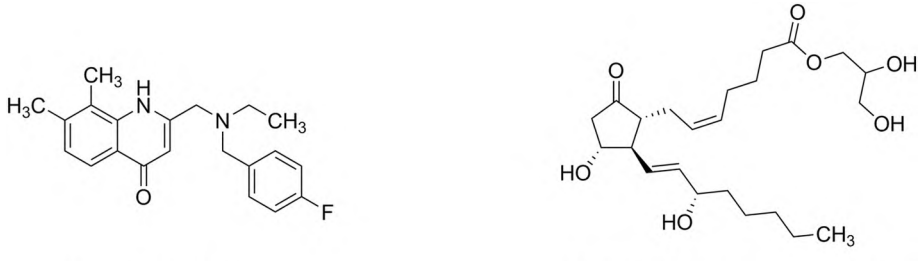
Table 8 (*continued*)

Nucleotide sugars							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
8-23	MRS4183	n.d.	n.d.	n.d.	**0.00096** (↑170x[c] / ↑270x[d])	Analogue with shorter linker published (EC_{50} = 0.091 μM at $P2Y_{14}R$)	Kiselev et al., 2015
8-24	G6-(UDP-glucuronic acid)$_{147}$	n.d.	n.d.	n.d.	**0.0008** (n.d.[c] / ↑330x[d])	Dendrimer-conjugate	Das et al., 2009
8-25	G3-(UDP-glucuronic	n.d.	n.d.	n.d.	0.0034 (n.d.[c] / ↑77x[d])	Dendrimer-	Das et al., 2009
	acid)$_{20.1}$-(biotin)$_{4.9}$					conjugate coupled to biotin for avidin complexation	
8-26	G3-(UDP-glucuronic acid)$_{20.1}$-(Alexa Fluor® 488)$_{2.3}$	n.d.	n.d.	n.d.	0.0398 (n.d.[c] / ↑7x[d])	Dendrimer-conjugate coupled to fluorophore	Das et al., 2009
8-27	G3-(UDP-glucuronic acid)$_{20.1}$-(DTPA)$_{4.5}$	n.d.	n.d.	n.d.	0.0041 (n.d.[c] / ↑64x[d])	Dendrimer-conjugate coupled to metal-chelating group	Das et al., 2009
8-28	G3-(UDP-glucuronic acid)$_{20.1}$-(DTPA-Gd(III))$_{4.5}$	n.d.	n.d.	n.d.	0.421 (n.d.[c] / ↓2x[d])	Dendrimer-conjugate coupled to metal-chelating group and complexed with Gadolinium	Das et al., 2009
8-29	MRS5259	1.39 (n.d.)[a,b]	n.d.	n.d.	0.00224 (n.d.)[c,d]	UDP-glucuronic acid coupled to A_3AR agonist (MRS3558) *via* PAMAM dendrimer Patented	WO2011068978A1, Zoho; Tosh et al., 2010

[a]relative to ATP; [b]relative to UTP; [c]relative to UDP; [d]relative to UDP-glucose.

weight of 1297 g/mol, it is a comparatively large, polyaromatic, and polysulfonated naphthalene derivative. Suramin has a symmetrical structure with a urea group at the center. It caused a parallel rightward shift of UTP dose-response curves at the human $P2Y_2R$. The corresponding Schild plot yielded a slope of 1.57 and an apparent pA_2 value of 4.32 (48 μM) (Charlton et al., 1996a). Suramin can therefore be classified as a very weak competitive $P2Y_2R$ antagonist.

Both RB-2 and suramin are partially selective antagonists but also inhibit ectonucleotidases. In light of insufficient alternatives, both have been in use as pharmacological tools for P2R characterization for



9-01 Compound 89

9-02 Prostaglandin E_2 glyceryl ester

Fig. 9. Structures of non-nucleotide agonists at uracil nucleotide-activated P2YRs (for data, refer to Table 9).

a long time. However, the negative charge on both RB-2 and suramin that prevents the penetration of cell membranes as well as the low degree of potency and, in particular, of selectivity are serious limitations to their usefulness.

2.2.4. Flavonoids

Flavonoids are secondary plant metabolites found in fruits, vegetables, bark, and flowers of many higher plants, including those that are used in traditional herbal medicine. Over 5000 different flavonoids with diverse pharmacological properties are known. A series of 40 flavonoids was assessed for $P2Y_2R$ antagonism. Tangeretin (**10-02**, Fig. 10 and Table 10), isolated from a tangerine fruit peel extract (*Citrus reticulata ssp.*, Rutaceae), was found to be the most potent of these, with an IC_{50} value of 12 μM at the mouse $P2Y_2R$ (Kaulich, Streicher, Mayer, Müller, & Müller, 2003). The structure-activity relationships (SARs) were found to be rather complex, but flavone derivatives were generally more potent than flavanones, indicating that a flat aromatic ring system was preferred. The maximal receptor activation of agonist (UTP) concentration-response curves was reduced with increasing concentrations of tangeretin, while the EC_{50} value of UTP remained unaffected. These compounds, therefore, appeared to act as non-competitive, allosteric antagonists (Kaulich et al., 2003).

2.2.5. Acyclic nucleotide derivatives

Acyclic derivatives of uracil nucleotides, in which the ribose ring is replaced by aliphatic residues, were published as $P2Y_2R$ antagonists (Sauer, El-Tayeb, Kaulich, & Müller, 2009). The phosphate groups were replaced by phosphonates to enhance chemical and metabolic stability. The most potent $P2Y_2R$ antagonist was diphosphoric 5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)pentylphosphonic anhydride (**10-05**, Fig. 10 and Table 10), which had an IC_{50} of 92 μM at the mouse $P2Y_2R$ (Sauer et al., 2009). Thus, their suitability as pharmacological tools appears to be limited at present due to their low potency, but further optimization may improve their properties.

3. The $P2Y_4$ receptor

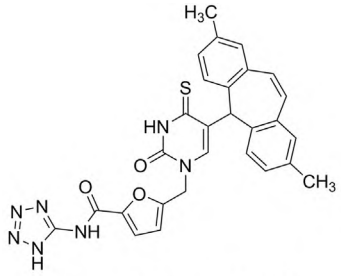
The existence of specific pyrimidinergic receptors activated by uracil nucleotides but not adenine nucleotides was postulated several years prior to the actual cloning of the first uracil nucleotide-selective receptor, the $P2Y_4R$, in 1995 (Communi, Pirotton, Parmentier, & Boeynaems, 1995; Nguyen et al., 1995; Seifert & Schultz, 1989). The $P2Y_4R$ was discovered with the aid of degenerate oligonucleotide primers. These were designed and synthesized based on the most conserved segments of the

Table 9
Non-nucleotide agonists for the uracil-activated P2YRs. Shown are the EC_{50} values in μM, unless stated otherwise. The correlation factor by which the potency is n-fold higher (↑nx) or lower (↓nx) compared to that of the endogenous agonist in the same test system is given in brackets. Particularly interesting values are shown in bold.

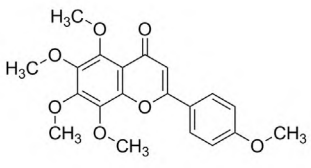
Non-nucleotide agonists							
No.	**Name**	**EC_{50} in μM** (potency higher (↑) or lower (↓) relative to endogenous agonist)				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
9-01	Compound 89	10.5 (↓≈500x[a] / n.d.[b]) 53.9 (n.d.)[a,b] **Mouse:** 2.7 (↓≈15x[a] / n.d.[b])	inactive at 30 μM (↓>600x)[b]	inactive at 30 μM (↓>600x)[c]	n.d.	Ago-allosteric agonist with 66 % (human $P2Y_2R$) and 51 % (mouse $P2Y_2R$) efficacy calculated by extrapolation	Sakuma et al., 2017
9-02	Prostaglandin E_2 glyceryl ester	n.d.	n.d.	**0.2 - 1.2 pM** (↑6000-65,000x)[c] **Mouse:** **0.8 pM** (↑48,000x)[c]	n.d.		Brüser et al., 2017

[a]relative to ATP; [b]relative to UTP; [c]relative to UDP.

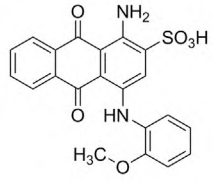
chick $P2Y_1R$ and the mouse $P2Y_2R$ sequences (Communi et al., 1995). Polymerase chain reactions on human genomic DNA using these primers led to the localization of a 712-base pair sequence, which was subsequently used as a probe to screen a human genomic DNA library. The $P2Y_4R$ was eventually characterized as an intronless 1095-base pair open reading frame sequence (Communi et al., 1995). The receptor is both structurally and pharmacologically closely related to the $P2Y_2R$. Accordingly, it also signals primarily *via* $G\alpha_{q/11}$. The residues His262, Arg265, Lys289, and Arg292, that are believed to be involved in nucleotide binding, are conserved between these two receptors (Communi et al., 1995). In fact, the charged amino acids Arg265 and Arg292 were proposed to be directly responsible for the binding of the negatively charged phosphate groups of nucleotides. Mutating Lys289 to arginine in the human $P2Y_4R$ resulted in a shift in agonist preferences from UTP to UDP (Communi et al., 1995). A $P2Y_4R$ homology model was developed based on the human $P2Y_1R$ X-ray structure, and molecular



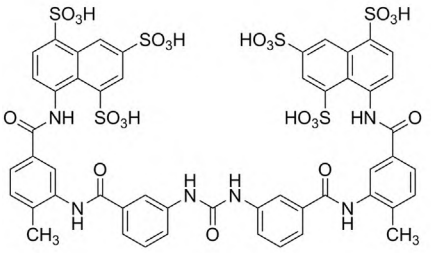
10-01 AR-C118925



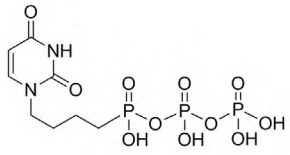
10-02 Tangeretin



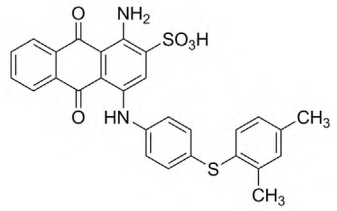
10-03 PSB-716



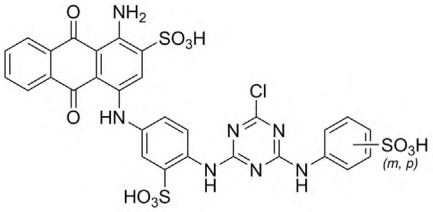
10-04 Suramin



10-05



10-06 PSB-16133



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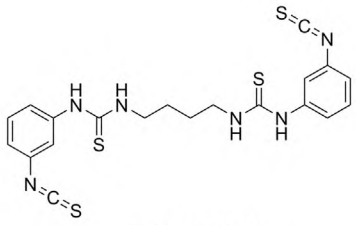
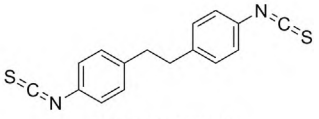


10-08 MRS2578



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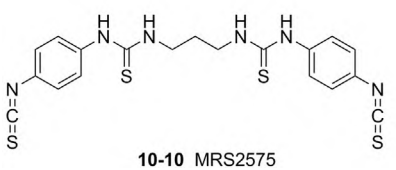
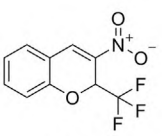
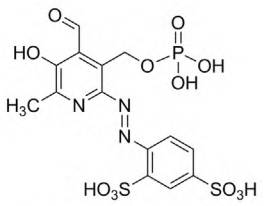


10-10 MRS2575



10-11



10-12 PPADS

Fig. 10. Structures of antagonists at uracil nucleotide-activated P2YRs (for data, refer to Table 10).

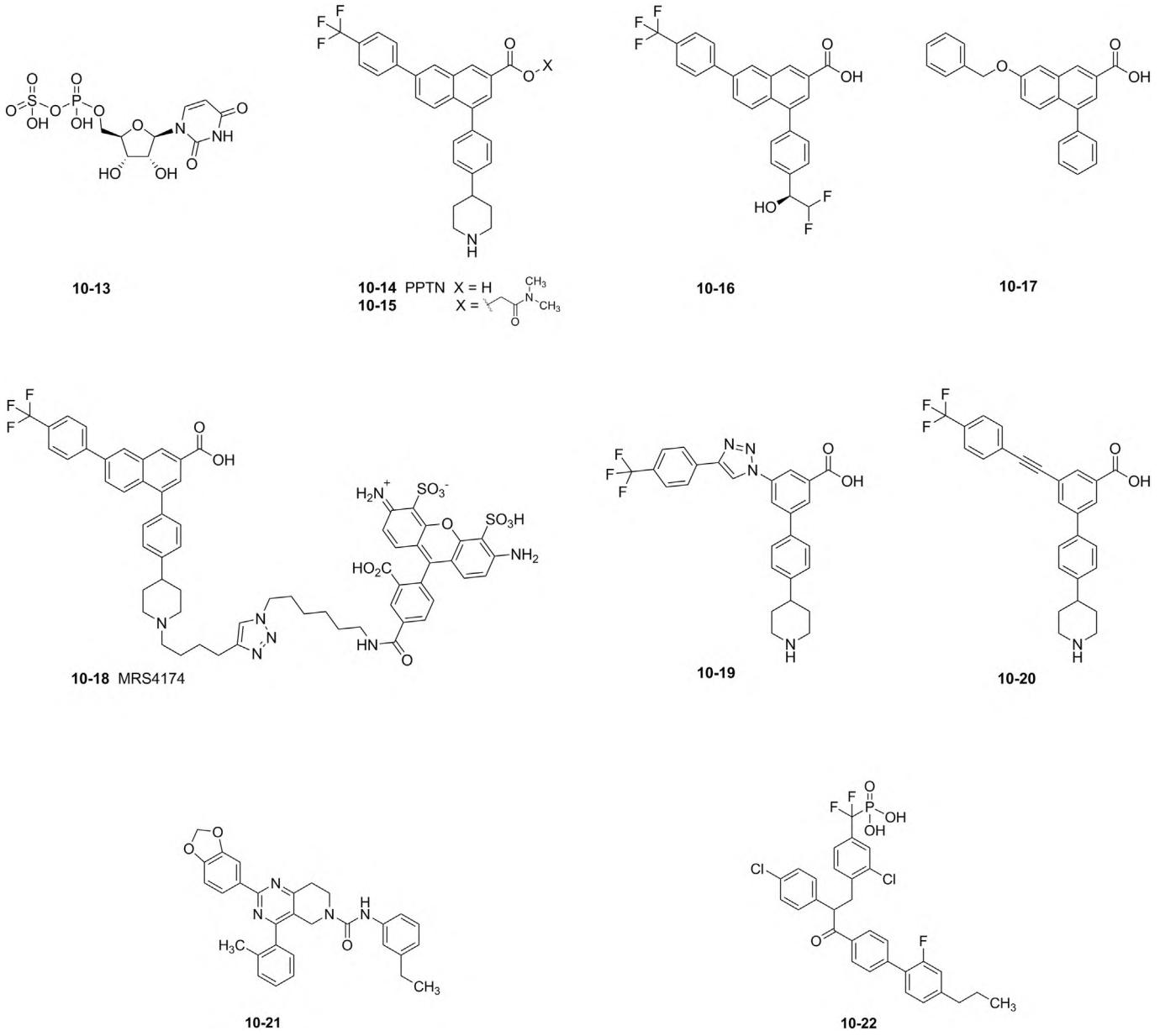


Fig. 10 (*continued*).

docking studies were performed (Rafehi et al., 2017; Zhang et al., 2015). According to these, Arg265 interacts with the uracil base, Arg292 with the α- and β-phosphate groups, and Lys289 with the γ-phosphate group. His262 is in close proximity (approx. 3 Å) to the uracil base and interaction is likely (Rafehi, Malik, et al., 2017). No *N*-glycosylation consensus sequence is found in the putative exofacial region of the human $P2Y_4R$, which is rare but not unique in GPCRs (Communi et al., 1995). In contrast, the rat ortholog contains an Asn in position 179 and a Thr in position 181 that creates a consensus *N*-linked glycosylation site (Herold, Qi, Harden, & Nicholas, 2004).

$P2Y_4R$ expression appears to be more restricted than of the $P2Y_2R$; it is mostly found (at mRNA level) in the GI tract and to a lower extent in the CNS, lungs, heart, prostate, skin, adipose tissue, skeletal muscle, spleen and lymphocytes (Table 1) (Moore et al., 2001; Uhlén et al., 2015). A possible physiological function of the $P2Y_4R$ is to mediate Cl^- secretion in the gastrointestinal tract (DuBose, Wolff, Qi, Naruszewicz, & Nicholas, 2013; Ghanem et al., 2005; Matos, Robaye, Boeynaems, Beauwens, & Leipziger, 2005). Another potentially important role is in the cochlea, where it regulates Na^+ homeostasis in the endolymph. It does this by inhibiting epithelial Na^+ channels of Reissner's membrane, and by K^+ secretion across strial marginal cell epithelial cells in the apical membrane (Kim et al., 2010; Marcus et al., 2005). Furthermore, the $P2Y_4R$ appears to be important for postnatal cardiac development, since $P2Y_4R$-null mice showed microcardia (Horckmans et al., 2012).

Relatively few compounds that target the $P2Y_4R$ have been developed in comparison to the other pyrimidinergic P2YR subtypes. An overview of the ligands and pharmacological tools currently available for the $P2Y_4R$ is provided in Tables 2 to 10. Those of greatest potential are further discussed below.

3.1. $P2Y_4$ receptor agonists

3.1.1. Therapeutic potential of $P2Y_4$ receptor agonists

$P2Y_4Rs$ were shown to be involved in Cl^- secretion in the intestinal epithelium (Ghanem et al., 2005). The Cl^- transport across the epithelium of both the jejunum and the colon was found to be abolished in

Table 10
Antagonists for the uracil-activated P2YRs. Shown are the IC_{50} or, in some cases, pA_2, K_i and K_B values in μM. Particularly interesting values are shown in bold

Antagonists							
No.	**Name**	**IC_{50} in μM**				**Comments**	**References**
		$P2Y_2$	**$P2Y_4$**	**$P2Y_6$**	**$P2Y_{14}$**		
10-01	AR-C118925	**0.0574** - 1	37.1	30.4	>3	Patented Smaller derivative AR-C126313 has pA_2 of 6.9	Kemp et al., 2004; Kindon et al., 2017; WO0019990025O1A1, 1998; Rafehi, Burbiel et al., 2017
10-02	Tangeretin	n.d. **Mouse:** 12	n.d.	n.d.	n.d.	Non-competitive antagonist	Kaulich et al., 2003
10-03	PSB-716	9.82 **Mouse:** 9	n.d.	n.d.	n.d.	Derivative of RB-2	Hillmann et al., 2009; Rafehi, Malik et al., 2017; Weyler et al., 2008
10-04	Suramin	48 pA_2 = 4.32 **Rat:** 8.9 competitive antagonist with pA_2 = 5.40 77 % inhibition at 100 μM **Mouse:** 31	inactive at 300 μM **Rat:** 1027 10 % inhibition at 100 μM **Mouse:** inactive at 100 μM	27 % inhibition at 100 μM **Rat:** competitive antagonist	n.d.		Bogdanov et al., 1998; Charlton et al., 1996b; Chen et al., 1996; Communi, Parmentier et al., 1996; Kaulich et al., 2003; Lazarowski & Harden, 1994; Müller, 2002; Robaye et al., 1997; Suarez-Huerta et al., 2001; Wildman et al., 2003
10-05	Diphosphoric 5-(2,4-dioxo-3,4-dihydro pyrimidin-1(2*H*)-yl) pentylphosphonic anhydride	n.d. **Mouse:** 92	n.d.	n.d.	n.d.		Sauer et al., 2009
10-06	PSB-16133	8.54 allosteric antagonist	**0.233**	12.5	n.d.	**37-fold selective for $P2Y_4R$** Derivative of RB-2	Rafehi, Malik et al., 2017

Table 10 (*continued*)

10-07	Reactive Blue 2 (RB-2)	1.85 22.1	0.625 (purified) 1.14 9.79 33 % inhibition at 100 µM	31 (87 % inhibition at 100 µM)	n.d.	Lead structure for development of **10-03** & **10-06**	Baqi et al., 2010; Bogdanov et al., 1998; Communi, Motte et al., 1996; Kaulich et al., 2003; Kim et al., 2010; Lazarowski & Harden, 1994; Rafehi, Malik et al., 2017; Rafehi, Neumann et al., 2017;
		Rat: >10,000 **Mouse:** 5.0 5.5	**Rat:** 18.5 21.1 competitive antagonist with pA_2 = 6.43 **Mouse:** 47 competitive antagonist **Gerbil:** 4.9	**Rat:** 4.34 non-competitive antagonist			Robaye et al., 1997; Suarez-Huerta et al., 2001; Weyler et al., 2008; Wildman et al., 2003
2-01	ATP	agonist (see Table 2)	competitive antagonist with pA_2 = 6.15 **Rat:** agonist (see Table 2)	almost inactive as agonist (see Table 2)	agonist (see Table 2)		Communi, Motte et al., 1996; Kennedy et al., 2000; Nguyen et al., 1995
4-17	3'-*O*-(4-benzoyl)-benzoyl-ATP (BzATP)	agonist (see Table 4)	159	n.d.	n.d.	ATP analogue used as photoaffinity label	Erb et al., 1993; Lin et al., 1993; Wildman et al., 2003
10-08	MRS2578	inactive at 10 µM	inactive at 10 µM	**0.037** **Rat:** 0.098	no binding at 10 µM	Non-competitive, insurmountable antagonist	Kiselev et al., 2015; Mamedova et al., 2004
10-09	MRS2567	inactive at 10 µM	inactive at 10 µM	0.126 **Rat:** 0.101	n.d.	**10-08** is commercially	

(*continued on next page*)

Table 10 (*continued*)

10-10	MRS2575	inactive at 10 μM	inactive at 10 μM	0.155 **Rat:** inactive	n.d.	available	
10-11	TIM-38	inactive at 50 μM	inactive at 50 μM	4.3	n.d.		Ito et al., 2017
10-12	PPADS	inactive **Rat:** >10,000 **Dog:** inactive	15 (73 % inhibition at 100 μM) >100 **Rat:** 25 - >1000 non-competitive antagonist **Mouse:** 45 non-competitive antagonist	69 % inhibition at 100 μM	n.d.		Bogdanov et al., 1998; Brown, Tanna, & Boarder, 1995; Charlton et al., 1996b, 1996a; Communi, Motte et al., 1996; Lambrecht, 1996; Müller, 2002; Robaye et al., 1997; Suarez-Huerta et al., 2001; Vigne et al., 1996; Wildman et al., 2003; Zambon et al., 2000
10-13	Uridylyl phosphosulfate	inactive	inactive	112	n.d.		Meltzer et al., 2015
10-14	PPTN	inactive at 1 μM	inactive at 1 μM	inactive at 1 μM	**0.00043** **0.006** K_i = **0.0003** K_i = **0.0019** K_B = **0.000434** **Chimpanzee:** 0.0022 K_i = 0.0019	Competitive antagonist **>2300-fold selective for $P2Y_{14}R$** Low oral bioavailability due to zwitterionic structure but ester-prodrug published Patented	Barrett et al., 2013; WO2009070873A1, 2008; Junker et al., 2016; Kiselev et al., 2014; Kiselev et al., 2015; Robichaud et al., 2011
10-15	Ester-prodrug of PPTN	n.d.	n.d.	n.d.	n.d.		Robichaud et al., 2011

Table 10 (*continued*)

10-16	4,7-Disubstituted naphthoic acid derivative	n.d.	n.d.	n.d.	n.d. **Mouse:** 0.008 **Chimpanzee:** 0.001 K_i = 0.004	Competitive antagonist 67 % oral bioavailability in mice, low intrinsic clearance (1.6 ml/min/kg) but > 99% bound to human serum albumin Patented	WO2009070873A1, 2008; Gauthier et al., 2011; Robichaud et al., 2011
10-17	4,7-Disubstituted naphthoic acid derivative	n.d.	n.d.	n.d.	n.d. **Mouse:** 3.5 **Chimpanzee:** 3.5 K_i = 0.16	Reversible, competitive antagonist	Gauthier et al., 2011
10-18	MRS4174	n.d.	n.d.	n.d.	K_i = **0.000080**	Analogue of PPTN coupled to a fluorophore Precursors published	Kiselev et al., 2014
10-19	Triazole analogue of PPTN	inactive at 10 μM	inactive at 10 μM	inactive at 10 μM	0.032		Junker et al., 2016
10-20	Alkyne analogue of PPTN	inactive at 10 μM	inactive at 10 μM	n.d.	5.69		Junker et al., 2016
10-21	3,4-Methylenedioxyphenyl derivative with dihydropyridopyrimidine core	n.d.	n.d.	n.d.	n.d. **Mouse:** 0.010 **Chimpanzee:** 0.081 0.0885	Non-competitive antagonist SARs and Patented	Guay et al., 2011; WO002009000087A1, 2008

(*continued on next page*)

Table 10 (*continued*)

10-22	Compound A (phosphonate derivative)	n.d.	n.d.	n.d.	2.3 8.58 K_i = 1.20 **Chimpanzee:** K_i = 1.52		Hamel et al., 2011; US020090148850A1, 2008
5-08	TMPS	inactive	inactive	partial agonist (see Table 5)	antagonist	AMPS, UMPS, and CMPS are $P2Y_{14}R$ agonists	Gendaszewska-Darmach et al., 2016; Gendaszewska-Darmach & Szustak, 2016

$P2Y_4R$-null mice, which showed an otherwise normal behavior, growth, and reproduction (Ghanem et al., 2005; Robaye et al., 2003). It was proposed that the $P2Y_4R$ is responsible for Cl^- secretion in response to UTP in both the small and large intestines, and, together with the $P2Y_2R$, at the basolateral side of the jejunum (Ghanem et al., 2005). Cystic fibrosis is a disorder that results from a mutation in the Cl^- ion channel CFTR. Thus, it was hypothesized that stimulation of the $P2Y_4R$ could compensate for the defective CFTR in the intestine (in analogy to $P2Y_2R$ activation in the airway epithelium). This is supported by observations in mice homozygous for the ΔF508 mutation of CFTR, the most common cause for cystic fibrosis. The jejunum of these mice was responsive to UTP, although the magnitude of the response was smaller than in wild-type mice (Ghanem et al., 2005). $P2Y_4R$ agonists are therefore considered to be potential drugs for patients suffering from cystic fibrosis. $P2Y_4Rs$ were also found to be involved in the growth and migration of cardiac endothelial cells, and in the secretion of platelet-derived growth factor B (Horckmans et al., 2012). $P2Y_4R$ agonists may modulate angiogenesis, cardiac remodeling, and post-ischemic revascularization. Moreover, activation of the $P2Y_4R$ in rat microglia was observed to promote pinocytosis of soluble β-amyloid, the major constituent of amyloid plaques observed in the brains of patients suffering from Alzheimer's disease (Li et al., 2013). Consequently, $P2Y_4R$ agonists may serve as therapies for this debilitating and as yet incurable disorder.

On the other hand, $P2Y_4R$ antagonism has also been suggested to be potentially beneficial in Alzheimer's disease treatment (see Section 3.2.1): The $P2Y_4R$ contributes to the release of amyloid precursor protein, which can be converted to β-amyloid that forms the characteristic plaques (Tran, 2011). This indicates a complex involvement of the $P2Y_4R$ and demonstrates the necessity of suitable, brain-permeable pharmacological tools for target validation studies.

3.1.2. Physiological ligands of the $P2Y_4$ receptor

The endogenous agonist of the $P2Y_4R$ is UTP (**2-08**, compound **08** of Fig. 2 and Table 2) (Nicholas et al., 1996). Other nucleotide triphosphates, such as guanosine 5′-triphosphate (GTP, **2-03**, Fig. 2 and Table 2) and inosine 5′-triphosphate (ITP, **2-05**, Fig. 2 and Table 2), are less potent than UTP at the human $P2Y_4R$ receptor (Communi, Motte, Boeynaems, & Pirotton, 1996; Kennedy, Qi, Herold, Harden, & Nicholas, 2000; Nguyen et al., 1995). ATP (**2-01**, Fig. 2 and Table 2) was observed to competitively antagonize the human $P2Y_4R$ with moderate potency (Communi, Motte, et al., 1996; Kennedy et al., 2000). ADP (**2-02**, Fig. 2 and Table 2) is inactive at the human $P2Y_4R$. UDP (**2-09**, Fig. 2 and Table 2) exhibited EC_{50} values in the mid-micromolar range but was found inactive when UTP contamination and enzymatic conversion to UTP was excluded (Communi et al., 1995; Communi, Motte, et al., 1996; Nguyen et al., 1995; Nicholas et al., 1996). Up_4U (**7-03**, Fig. 7 and Table 7) is also a physiological $P2Y_4R$ agonist with an EC_{50} value in the high nanomolar range (Maruoka et al., 2011; Pendergast et al., 2001; Shaver et al., 2005). Diuridine polyphosphates with different numbers of phosphate groups are significantly less potent. Ap_4A (**7-01**, Fig. 7 and Table 7) is inactive (Kennedy et al., 2000; Patel et al., 2001; Shaver et al., 2005). The agonist profile of the rat $P2Y_4R$ is strikingly different from that of the human ortholog and more closely resembles that of the $P2Y_2R$ (Bogdanov, Wildman, Clements, King, & Burnstock, 1998). It is thus likely that some of the effects attributed to the functionally defined "P_{2U} receptor" in native rodent-derived cells were not (solely) mediated by the $P2Y_2R$ but possibly by the rodent $P2Y_4R$ as well. At the rat $P2Y_4R$, ATP acts as an agonist with similar potency as UTP, Ap_4A, and ITP (see Tables 2 and 7) (Bogdanov et al., 1998; Kennedy et al., 2000). UDP, ADP, ATPγS (**5-03**, Fig. 5 and Table 5), and 2-methylthio-ATP (2-MeSATP, **3-37**, Fig. 3 and Table 3) were described as partial agonists at the rat receptor (Bogdanov et al., 1998). The sequence identity between the human and the rat orthologs is 83 % in total, and 90 % in the transmembrane regions and extracellular loops (Bogdanov et al., 1998; Kennedy et al., 2000). Human/rat $P2Y_4R$ chimera studies showed that whether ATP behaves as an agonist or an antagonist on the $P2Y_4R$ was mainly dependent on the second extracellular loop, and to a lesser extent on the *N*-terminus (Herold et al., 2004). Mutating three residues within the second extracellular loop of the human receptor to the corresponding residues of the rat ortholog (S177N, V183I, and R190L) imparted ATP sensitivity onto the human receptor (Herold et al., 2004).

3.1.3. Synthetic nucleotide derivatives

The $P2Y_4R$ is often expressed in the same tissues as the more abundant $P2Y_2R$ and, as for example in epithelial cells of the intestine, sometimes even in the same cells. As a result, it is challenging to distinguish pharmacologically between these two structurally related receptor subtypes, especially given the similarities in their pharmacological profiles. While extensive efforts have led to the development of several selective agonists for the $P2Y_2R$, the availability of $P2Y_4R$-selective agonists has been more limited. The currently most selective compounds described in the literature are the CTP derivatives N^4-(phenylpropoxy)-CTP (MRS4062, **3-15**, Fig. 3 and Table 3) and N^4-(phenylethoxy)-CTP (**3-16**, Fig. 3 and Table 3) (Maruoka et al., 2011). The commercially available MRS4062 was twice as potent as UTP and displayed 27- and

32-fold selectivity for the $P2Y_4R$ over the $P2Y_2R$ and $P2Y_6R$, respectively. Compound **3-16** displayed a potency in the mid-nanomolar range, similar to UTP, and was 16-fold selective for the $P2Y_4R$ versus the other two subtypes (Maruoka et al., 2011). These two $P2Y_4R$ agonists are part of a series of synthetic compounds that were initially designed with the intention to develop improved $P2Y_2R$ agonists through combining different substitutions of the pyrimidine moiety and the phosphate groups (Maruoka et al., 2011).

Among the first reported $P2Y_4R$ agonists with good selectivity (>20-fold versus the $P2Y_2R$ and $P2Y_6R$) was an analogue of uridine 5′-monophosphate (UMP) with the 2-keto group replaced by an amino-function. 2-Amino-UMP (iso-CMP, **3-07**, Fig. 3 and Table 3), showed micromolar potency (EC_{50} of 4.98 μM) (El-Tayeb et al., 2011). It is worth mentioning that the 2-amino substitution conferred agonist activity at the $P2Y_4R$ to the otherwise inactive UMP. Certain substitutions in the uracil moiety of UTP, including 2-thio- (**3-01**, Fig. 3 and Table 3), 3-methyl- (**3-09**), 5-bromo- (**3-23**), 5-iodo- (**3-25**), and 5-methyl- (**3-29**), were tolerated but caused a reduction in potency (Communi, Parmentier, & Boeynaems, 1996; El-Tayeb et al., 2006; Jacobson et al., 2006; Nguyen et al., 1995; Nicholas et al., 1996). Several compounds with substitutions in 6-position were found to be completely inactive (Ginsburg-Shmuel et al., 2010). In contrast, thio-substitution in the 4-position did not cause a decrease in potency but appeared to be favorable instead. An EC_{50} value of 23 nM was reported for 4-thio-UTP (**3-12**, Fig. 3 and Table 3), which was thus 3-fold more potent than UTP at the $P2Y_4R$ (but unselective versus the $P2Y_2R$) (Jacobson et al., 2006). As was observed with the $P2Y_2R$, even larger substituents in the 4-position were well-tolerated. The fluorescent probe MRS4162 (**3-40**, Fig. 3 and Table 3), discussed previously in Section 2.1.5., and its analogues with varying substitutions in the 4-position all showed potencies in the nanomolar range at the $P2Y_4R$ (Jayasekara et al., 2014). The fluorescent probe MRS4162 and its precursor MRS4063 (**3-41**, Fig. 3 and Table 3) that can be extended, e.g. by click tethering, are versatile pharmacological tools. The fact that they did not only show activity at the $P2Y_2R$ but also at the $P2Y_4R$ expands the possibilities for further characterization of this receptor. A UTP analogue with a 2′-azido-2′-deoxy substitution at the ribose ring (**4-06**, Fig. 4 and Table 4; EC_{50} of 1.1 μM) was selective for the $P2Y_4R$, albeit only 5-fold versus the $P2Y_2R$ (Jacobson et al., 2006). Not only substitutions at the ribose ring were tolerated by the $P2Y_4R$, but also replacement of the ribose by a methanocarba (bicyclo[3.1.0]hexane) ring, which includes a ribose fixed in the (*N*)-conformation, without significant loss in potency (Jacobson et al., 2006; Kim et al., 2002; for more information on methanocarba compounds and their syntheses, see review article by Tosh & Jacobson, 2013). Related methanocarba analogues of adenosine were patented (Jacobson & Marquez, 2001). (*N*)-Methanocarba substitution is also tolerated by the $P2Y_2R$, which is another example of the large overlap of the pharmacological profiles of these two receptors (Kim et al., 2002). Another similarity to the $P2Y_2R$ is that a thio-substitution at the γ-phosphate group is tolerated while α-thiophosphate is unfavorable (Jacobson et al., 2006).

Substitutions of one uridine moiety of Up_4U (**7-03**, Fig. 7 and Table 7) with various sugars as well as aliphatic or aromatic alcohol moieties led to the development of Up_4-[1″]3′-deoxy-3′-fluoroglucose (MRS2927, **8-20**, Fig. 8 and Table 8). It showed similar potency as UTP in the mid-nanomolar range, 11-fold selectivity over the $P2Y_2R$, and 15-fold selectivity versus the $P2Y_6R$. The δ-phenyl phosphoesters but not the δ-glucosylphosphoesters appeared to be enzymatically more stable than UTP (Maruoka et al., 2011).

3.2. $P2Y_4$ receptor antagonists

3.2.1. Therapeutic potential of $P2Y_4$ receptor antagonists

As $P2Y_4R$ activation promotes epithelial Cl^- secretion in the intestine (refer to Section 3.1.1), blockade of this receptor could be beneficial for treating diarrhea. However, contrary to this, the $P2Y_4R$ was found to inhibit the contraction of mouse ileum longitudinal muscle (Zizzo, Mastropaolo, Grahlert, Mule, & Serio, 2012). Antagonists may induce contractions and have therefore also been proposed for treating constipation. It was further observed that the $P2Y_4R$ (as well as the related $P2Y_2R$) is involved in the production and release of amyloid precursor protein in rat cortical astrocytes (Tran, 2011). Amyloid precursor protein can be cleaved to β-amyloid, and aggregates have been associated with Alzheimer's disease. $P2Y_4R$ blockade may reduce the amount of amyloid precursor protein and, as a consequence, that of β-amyloid as well. It is thus postulated that $P2Y_4R$ antagonists could prevent the formation of amyloid plaques. However, in rat microglia, $P2Y_4R$ activation promoted pinocytosis of β-amyloid (Li et al., 2013). Therefore, the $P2Y_4R$ appears to be a complex target in relation to neurodegenerative diseases, and better pharmacological tools would be required to further elucidate its role. Another possible indication for $P2Y_4R$ antagonists might be myocardial infarction. The $P2Y_4R$ appears to be an important mediator of the inflammatory response following cardiac ischemia. $P2Y_4R$-null mice displayed smaller infarcts in the left descending artery ligation model as well as reduced neutrophil infiltration and fibrosis, indicating that antagonists may be beneficial (Horckmans et al., 2015). The $P2Y_4R$ (as well as the $P2Y_2R$) was found to be significantly overexpressed in different colon cancer cell lines, and antagonizing these two receptors may be beneficial for the treatment of certain cancers (Delbro, Nylund, & Nordgren, 2005; Nylund, Hultman, Nordgren, & Delbro, 2007). Moreover, $P2Y_4R$ expression was shown to be upregulated under hypoglycemic conditions and linked to neuronal cell death. $P2Y_4R$ blockade could thus have neuroprotective effects in diabetes (Cavaliere et al., 2004).

3.2.2. Anthraquinone derivatives

Potent and selective antagonists for the $P2Y_4R$ are scarce. In light of insufficient alternatives, RB-2 (**10-07**, compound **07** in Fig. 10 and Table 10) has been the primary antagonist used for studying $P2Y_4Rs$. The antagonistic potential of RB-2 was already discovered in 1979, when the field of purinergic signaling was still in its early days. In fact, pharmacological studies involving RB-2 assisted in the differentiation and characterization of purinergic receptors (Kerr & Krantis, 1979).

RB-2 exhibited an IC_{50} value of 1.14 μM at the human $P2Y_4R$ and was approximately 20-fold selective versus the $P2Y_2R$ subtype, at which it also acted as a weak antagonist (Rafehi, Malik, et al., 2017; Rafehi, Neumann, et al., 2017). It is a dye that can be obtained from different commercial sources. Commercially available RB-2 is a mixture of isomers with differing identity and purity (Glänzel, Bültmann, Starke, & Frahm, 2003). This causes difficulties in obtaining reliable pharmacological results. For example, a certain batch obtained from Sigma-Aldrich contained only 54 % of RB-2, 12 % of its dichlorotriazinyl precursor, and 34 % of inorganic salts (NaCl and/or Na_3PO_4) (Rafehi, Malik, et al., 2017). As to be expected, purification of the commercially obtained compound by reversed phase-18 flash column chromatography yielded a pure product with increased potency at the $P2Y_4R$ (IC_{50} value of 0.625 μM) (Rafehi, Malik, et al., 2017). A recently published review article provides comprehensive information on RB-2 and related anthraquinone derivatives, including a discussion on their safety and potential toxicity (Malik & Müller, 2016).

RB-2 (**10-07**, Fig. 10 and Table 10) has served as a lead structure for the extensive development of antagonists for different P2 receptors. Examples include the potent and selective competitive $P2Y_{12}R$ antagonist 1-amino-4-[4-phenylamino-3-sulfophenylamino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate (PSB-0739; A_2 value of 0.158 nM) and disodium 1-amino-4-[3-(4,6-dichloro[1,3,5]triazine-2-ylamino)-4-sulfophenylamino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate (PSB-1011; IC_{50} of 79 nM), a competitive inhibitor of the rat P2X2 receptor (Baqi et al., 2011; Baqi, Atzler, Köse, Glänzel, & Müller, 2009; Hoffmann et al., 2009). Although several P2 receptors are blocked by RB-2 derivatives, SARs are significantly different for each receptor (Malik & Müller, 2016).

Recently, the synthesis of a series of $P2Y_4R$ antagonists that are smaller-size analogues of RB-2 was reported (Rafehi, Malik, et al., 2017). While the anthraquinone scaffold was retained, the three aromatic rings in the 4-position of the anthraquinone core of RB-2 were reduced to one or two, and the substituents were varied. This was generally well-tolerated by the $P2Y_4R$. The most potent compound of this series, 1-amino-4-[4-(2,4-dimethylphenylthio)]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate (PSB-16133, **10-06**, Fig. 10 and Table 10), showed an IC_{50} value of 233 nM (Rafehi, Malik, et al., 2017). While its improvement in potency compared to RB-2 was moderate (5-fold), its selectivity towards other P2YR subtypes was significantly enhanced: PSB-16133 is 37-fold and 54-fold selective for the $P2Y_4R$ versus the $P2Y_2$ and $P2Y_6$ receptors, respectively. In comparison, the selectivity of RB-2 for the $P2Y_4R$ is 19-fold and merely 2-fold versus the $P2Y_2R$ and $P2Y_6R$, respectively (Rafehi, Malik, et al., 2017; Rafehi, Neumann, et al., 2017). Based on pharmacological assessments and molecular docking to a homology model of the $P2Y_4R$, the anthraquinone derivatives were described as non-competitive antagonists binding to a site located close to the extracellular surface above the putative orthosteric binding site (Rafehi, Malik, et al., 2017).

4. The $P2Y_6$ receptor

The rat $P2Y_6R$ was first recombinantly expressed in 1995 using cDNA isolated from a rat aortic smooth muscle cell library (Chang, Hanaoka, Kumada, & Takuwa, 1995). Cloning of the human ortholog succeeded a year later following a search for further P2YR subtypes by screening of a human placenta cDNA library using a $P2Y_4R$ probe (Communi, Parmentier, & Boeynaems, 1996). $P2Y_6R$ mRNA is strongly expressed in the spleen, placenta, and kidney. It was also detected at lower levels in the CNS, lung, heart, GI tract, adipose tissue, bone, and other parts of the immune system (Table 1) (Moore et al., 2001; Uhlén et al., 2015).

4.1. $P2Y_6$ receptor agonists

4.1.1. Therapeutic potential of $P2Y_6$ receptor agonists

A possible physiological function of the $P2Y_6R$ may be, similar to the $P2Y_2R$ and $P2Y_4R$, to mediate electrolyte transport in the airway epithelium and the colon (Dulong, Bernard, & Ehrenfeld, 2007; Köttgen et al., 2003; Schreiber & Kunzelmann, 2005). The $P2Y_6R$ may therefore be another possible target for cystic fibrosis. Furthermore, $P2Y_6R$ knockout mice revealed an involvement in the contraction and endothelium-dependent relaxation of the aorta (Bar et al., 2008). Thus, the $P2Y_6R$ could additionally become a target for controlling blood pressure. The receptor was further found to be involved in insulin secretion and is thereby of potential interest for the treatment of diabetes (Balasubramanian, Ruiz de Azua, Wess, & Jacobson, 2010; Ohtani, Suzuki, Jacobson, & Oka, 2008; Parandeh, Abaraviciene, Amisten, Erlinge, & Salehi, 2008). In analogy to $P2Y_2R$ agonists, $P2Y_6R$ activation could become a new strategy for treating ocular hypertension and glaucoma. Topical application of the $P2Y_6R$ agonist UDP led to a reduction in intraocular pressure in mice and rabbits, and $P2Y_6R$ knockout mice showed hypertensive glaucoma-like optic neuropathy (Jacobson & Civan, 2016; Markovskaya et al., 2008; Shinozaki et al., 2017). Furthermore, $P2Y_6R$ activation was observed to have an anti-apoptotic function in different cell types by preventing the activation of caspases 3 and 8 following tumor necrosis factor-α exposure (Haas, Ginsburg-Shmuel, Fischer, & Reiser, 2014; Kim et al., 2003; Kim et al., 2003). This effect was also observed in mouse C2C12 skeletal muscle cells, and $P2Y_6R$ agonism protected against skeletal muscle ischemia/reperfusion injury in mouse hindleg muscle. Thus, $P2Y_6R$ agonists could be useful in skeletal muscle injuries (Mamedova, Wang, Besada, Liang, & Jacobson, 2008). The $P2Y_6R$ was further shown to protect cells from γ-rays by promoting cellular repair responses (Ide, Nishimaki, Tsukimoto, & Kojima, 2014). Anti-proliferative effects on gastric cancer cells were associated with $P2Y_6R$ activation (Wan et al., 2017). Moreover, $P2Y_6R$ activation induced recruitment of monocytes and macrophages in response to bacterial invasion. It also triggered an antiviral immune response following infection with vesicular stomatitis virus. These mechanisms could be employed in the therapy of infectious diseases (Li et al., 2014; Zhang et al., 2011). Patients suffering from neurodegenerative diseases may also benefit from $P2Y_6R$ agonists. This is based on the observation that the $P2Y_6R$ functions as a sensor for phagocytosis on microglia. $P2Y_6R$ agonists could thus promote the clearance of dead cells or harmful debris in the brain (Inoue, Koizumi, Kataoka, Tozaki-Saitoh, & Tsuda, 2009; Koizumi et al., 2007). Altogether, it is evident that $P2Y_6R$ agonists (Tables 2 to 10) are in high demand for a wide range of research fields and clinical applications.

4.1.2. Physiological mononucleotides

The endogenous agonist for the $P2Y_6R$ is UDP (**2-09**, compound **09** in Fig. 2 and Table 2) (Communi, Parmentier, & Boeynaems, 1996). However, since it also activates the $P2Y_{14}R$, it is not selective (Carter et al., 2009). UDP can be phosphorylated to UTP by nucleoside diphosphokinase, and commercial preparations of UDP may contain impurities of UTP, which should be taken into consideration in pharmacological experiments (Lazarowski et al., 2000; Nicholas et al., 1996). Other physiological mononucleotides, e.g. UTP (**2-08**), GDP (**2-04**), and thymidine 5′-diphosphate (TDP, **2-14**), can also activate the $P2Y_6R$ at high concentrations (Table 2). Uracil nucleotides are preferred over adenine nucleotides, and diphosphates are more potent than triphosphates. Accordingly, ATP (**2-01**, Fig. 2 and Table 2) is almost inactive (Communi, Parmentier, & Boeynaems, 1996; Nicholas et al., 1996).

4.1.3. Prostaglandin E_2 glyceryl ester

Very recently, prostaglandin E_2 glyceryl ester (**9-02**, Fig. 9 and Table 9) was proposed as another physiological $P2Y_6R$ agonist with (sub-)picomolar potency (Brüser et al., 2017). Such a high potency is rarely seen with endogenous ligands but appears physiologically reasonable, as **9-02** is produced in low concentrations by cyclooxygenase 2 and disappears rapidly due to hydrolysis. It had previously been shown to induce calcium mobilization and activate protein kinase C as well as extracellular signal-regulated kinase 1/2 (Brüser et al., 2017). To identify the receptor responsible for these effects, mRNA encoding for GPCRs from different cell lines was sequenced. The GPCR expression profiles of cell lines responsive to **9-02** were compared with those that could not be stimulated by **9-02**. Six GPCRs were found to be expressed exclusively in the responsive cell lines, and thus were considered as potential candidates for a putative prostaglandin E_2 glyceryl ester receptor. The $P2Y_6R$ was eventually discovered as the target of **9-02** using calcium mobilization, IP_3, and ERK1/2 phosphorylation assays in recombinant and native cells (Brüser et al., 2017). Based on a $P2Y_6R$ homology model and mutagenesis studies, the binding pocket of **9-02** was proposed to partially overlap with that of UDP. Several mutations resulted in a loss of response to **9-02** but not to UDP, and *vice versa*. This indicated a direct activation of the $P2Y_6R$ by **9-02** as opposed to a paracrine or autocrine mechanism in which **9-02** would induce UDP release leading to $P2Y_6R$ activation (Brüser et al., 2017).

It would not be unusual for the $P2Y_6R$ to be activated by both nucleotides and lipids in light of the fact that the phylogenetically related cysteinyl leukotriene receptors 1 and 2 (CysLT1 and CysLT2) respond to lipids (cysteinyl leukotrienes) as well as to the nucleotide UDP (Costanzi et al., 2004; Mellor et al., 2003; Mellor, Maekawa, Austen, & Boyce, 2001). So far, **9-02** has not been assessed at other P2YR subtypes. Should these recent findings be confirmed, the non-nucleotide lipid agonist with extraordinarily high potency would likely inspire new compound or perhaps even drug development efforts for the $P2Y_6R$ and the other P2YR subtypes. The low stability of prostaglandin E_2 derivatives under physiological conditions, however, constitutes a major challenge (Kozak et al., 2001).

4.1.4. Dinucleotides and analogues

The larger dinucleotide tetraphosphates (Fig. 7 and Table 7) are less potent at the $P2Y_6R$ compared to the nucleotide triphosphate-preferring receptors $P2Y_2$ and $P2Y_4$. Up_4U (**7-03**) has a potency in the micromolar range, while Ap_4A (**7-01**) showed only low activity at 100 μM (Maruoka et al., 2011; Pendergast et al., 2001; Shaver et al., 2005). Up_3U (**7-04**) is of higher potency (EC_{50} of 200-920 nM) and similarly potent as UDP (Maruoka et al., 2010; Pendergast et al., 2001). This matches the pattern observed for the triphosphate nucleotide-preferring P2YR subtypes, where dinucleotide tetraphosphates mimick the activity of the corresponding triphosphates (Brunschweiger & Müller, 2006; Pendergast et al., 2001).

P^1-(Uridine-5′-)-P^3-(N^4-methoxycytidine-5′-)-triphosphate (N^4-(MeO)-Cp_3U, MRS2957, **7-08**, Fig. 7 and Table 7) was 25-fold more potent than UDP and exhibited 14-fold and 66-fold selectivity versus the $P2Y_2R$ and $P2Y_4R$, respectively (Maruoka et al., 2010). The stable analogue of Up_3U, P^1-((2-benzyl-1,3-dioxolo-4-yl)uridine-5′-)-P^3-(uridine-5′-)-triphosphate (INS48823, monobenzylacetal-Up_3U, **7-11**, Fig. 7 and Table 7), is another $P2Y_6R$-selective agonist with an EC_{50} value of 125 nM and no appreciable activity at the $P2Y_2R$ and $P2Y_4R$ (Korcok, Raimundo, Du, Sims, & Dixon, 2005).

4.1.5. Boranophosphate analogues of nucleotides

Among the most promising $P2Y_6R$ agonists are boranophosphate analogues of UDP (**2-09**). The R_p isomer of 5-MeO-uridine 5′-*O*-(α-boranodiphosphate) (**6-12**, Fig. 6 and Table 6) showed an EC_{50} value of 8 nM, and was thus 19-fold more potent than UDP (Ginsburg-Shmuel et al., 2012). It is the most potent $P2Y_6R$ agonist described to date. Since it exhibited no activity at the $P2Y_2R$ and $P2Y_4R$, it is also the most selective one. Furthermore, it was found to be very stable in acidic conditions (e.g. in gastric juice) as well as in blood serum (Ginsburg-Shmuel et al., 2012). However, the negative charges of this compound could be an impediment to its usefulness *in vivo*, as is the case for all nucleotide analogues, which are therefore not orally bioavailable. Compound **6-12** was found to protect 1321N1 astrocytoma cells transfected with the human $P2Y_6R$ from apoptosis induced by tumor necrosis factor-α; it did so more prominently than UDP (Haas et al., 2014). The agonist was patented and its therapeutic potential is under further investigation (Fischer, Pintor, Elyahu, & Ginsburg-Shmuel, 2011). Compound **6-12** is an analogue of 5-MeO-UDP (**3-31**), which was developed two years earlier through a data mining analysis of binding interactions in 44 protein-uridine nucleoside or nucleotide complexes (Ginsburg-Shmuel et al., 2010). Although dinucleotide derivatives were less active than mononucleotides, the introduction of an α-borano-group enhanced their potency (Ginsburg-Shmuel et al., 2012; also see patent by Fischer & Nahum, 2006). Interestingly, the $P2Y_2R$ does not appear to tolerate the presence of an α-borano-substitution; α-borano-substituted ATP and 5-MeO-UTP are less potent at the $P2Y_2R$ than their non-substituted analogues, which explains their selectivity for the $P2Y_6R$ over the $P2Y_2R$ (Ginsburg-Shmuel et al., 2012; Tulapurkar, Laubinger, Nahum, Fischer, & Reiser, 2004).

4.1.6. Phosphorothioate derivatives of nucleotides

Unsurprisingly, uridine 5′-(β-thio)-diphosphate (UDPβS, **5-06**, Fig. 5 and Table 5) potently (EC_{50} of 28 nM, similar to UDP) activates the $P2Y_6R$. This is analogous to UTPγS (**5-05**, Fig. 5 and Table 5) being an agonist at the $P2Y_2R$ and the $P2Y_4R$, and ATPγS (**5-03**, Fig. 5 and Table 5) at the $P2Y_2R$ (Hou et al., 2002; Lazarowski et al., 1995; Lazarowski et al., 1996; Nicholas et al., 1996). UDPβS was equally potent at the $P2Y_{14}R$, which is not astonishing in light of the fact that UDP functions as a potent $P2Y_{14}R$ agonist as well (Carter et al., 2009). Although UMP (**2-10**, Fig. 2 and Table 2) was inactive at the $P2Y_6R$, uridine 5′-*O*-monophosphorothioate (UMPS, **5-07**, Fig. 5 and Table 5) showed some activity (62 % activation at 10 μM, relative to the response induced by 1 μM UDP). Its structural analogue thymidine 5′-*O*-monophosphorothioate (TMPS, **5-08**, Fig. 5 and Table 5) was described as a selective partial agonist at the $P2Y_6R$. At 1 mM concentration, it elicited 50 % receptor activation relative to the effect observed with 1 μM UDP (Gendaszewska-Darmach & Szustak, 2016). At the $P2Y_{14}R$, TMPS appeared to have antagonistic effects (Gendaszewska-Darmach, Weglowska, Walczak-Drzewiecka, & Karas, 2016). The terminal thiophosphate group increases stability towards different ectonucleotidases (Gendaszewska-Darmach et al., 2016; Gendaszewska-Darmach & Szustak, 2016).

4.1.7. Nucleobase-modified nucleotide analogues

Large substituents in the *N3*-position of the uracil base were well tolerated by the $P2Y_6R$, which distinguishes it from the $P2Y_2R$ and the $P2Y_4R$ (El-Tayeb et al., 2006). As a result, 3-phenacyl-UDP (PSB-0474, **3-11**, Fig. 3 and Table 3) was 570-fold selective for the $P2Y_6R$ versus the $P2Y_2R$ and the $P2Y_4R$. With an EC_{50} of 70 nM, it is among the most potent $P2Y_6R$ agonists described to date, and has been made commercially available (El-Tayeb et al., 2006). Also interesting are the following cytidine 5′-diphosphate (CDP) derivatives with mid-nanomolar potencies (Fig. 3 and Table 3): N^4-(benzyloxy)-CDP (MRS2964, **3-17**; 82- and 44-fold selectivity versus the $P2Y_2R$ and $P2Y_4R$, respectively) and N^4-(MeO)-CDP (**3-19**; 51-, 92-, and 47-fold selectivity versus the $P2Y_2$, $P2Y_4$, and $P2Y_{14}$ receptors). An analogue of **3-19**, α,β-methylene-N^4-MeO-CDP (**6-13**, Fig. 6 and Table 6), exhibited an EC_{50} value of 678 nM and 15-fold selectivity (Maruoka et al., 2010). Different nucleobase modifications led to further potent and selective $P2Y_6R$ agonists, including 5-MeO-UDP (**3-31**, Fig. 3 and Table 3; EC_{50} of 80 nM, 250-fold selectivity) and 5-iodo-UDP (MRS2693, **3-26**, Fig. 3 and Table 3; EC_{50} of 15 nM) (Besada et al., 2006; Ginsburg-Shmuel et al., 2010). Substitutions in the 6-position appeared to be unfavorable for the $P2Y_6R$, as was the case for the $P2Y_2R$ and $P2Y_4R$ (Ginsburg-Shmuel et al., 2010). Although different substitutions at the uracil base were tolerated by the $P2Y_6R$, complete replacement by other nucleobases was not (Robaye, Boeynaems, & Communi, 1997).

4.1.8. Ribose-modified nucleotide analogues

Replacement of the ribose moiety by a constrained methanocarba (bicyclo[3.1.0]hexane) ring was tolerated by the $P2Y_6R$, but only if the methanocarba ring was in the (*S*)-configuration (Kim et al., 2002). This is in contrast to the other G_q-coupled human P2YRs, all of which show a preference for the (*N*)-configuration (Costanzi et al., 2005; Kim et al., 2012). The rigid methanocarba analogues of UDP in the (*S*)-configuration, (*S*)-methanocarba-UDP (MRS2795, **4-22**, Fig. 4 and Table 4) and 2′-deoxy-(*S*)-methanocarba-UDP (**4-23**), both displayed a 7-fold higher potency than the flexible UDP and 2′-deoxy-UDP, respectively (Costanzi et al., 2005; Maruoka et al., 2010). Interestingly, **4-23** had been developed following a rational design using molecular modeling (Costanzi et al., 2005). The hydroxyl group in the 2′-position of the ribose ring was found to be important; the 2′-deoxy derivatives of UDP (**4-02**) and (*S*)-methanocarba-UDP (**4-23**) were 5-fold less potent than their corresponding 2′-hydroxy-substituted analogues (**2-09**) and **4-22** (Maruoka et al., 2010; Meltzer et al., 2015). Similar to the $P2Y_2R$ and the $P2Y_4R$, 2′-amino-2′-deoxy and 2′-azido-2′-deoxy substitutions at the ribose moiety caused a reduction in potency at the $P2Y_6R$ (Besada et al., 2006; Jacobson et al., 2006).

4.1.9. Fluorescence-labelled $P2Y_6$ receptor agonists

The BODIPY® 630/650 conjugate MRS4162 (**3-40**, Fig. 3 and Table 3) that was mentioned in Section 2.1.5. can be used to characterize not only the $P2Y_2$ and the $P2Y_4R$ but for the $P2Y_6R$ as well. At the $P2Y_6R$, it displayed an EC_{50} value of 23 nM (Jayasekara et al., 2014). MRS4129 (**3-42**, Fig. 3 and Table 3) is another tool compound, which is coupled to a different fluorescent dye: Alexa Fluor® 488. It showed an EC_{50} value of 9 nM at the $P2Y_6R$ and, in contrast to **3-40**, is highly selective versus the $P2Y_2R$ (280-fold) and the $P2Y_4R$ (>1100-fold) (Jayasekara et al., 2013). Both compounds are cytosine nucleotide derivatives with

alkyloxyimino substitutions in the N^4-position. MRS4162 (**3**-**40**) contains three phosphate groups but MRS4129 (**3**-**42**) is a diphosphate (Jayasekara et al., 2013; Jayasekara et al., 2014).

4.2. $P2Y_6$ receptor antagonists

4.2.1. Therapeutic potential of $P2Y_6$ receptor antagonists

$P2Y_6R$ activation was shown to prolong the survival of osteoclasts by activating NF-κB, a key transcription factor regulating osteoclastogenesis. It was also found to stimulate the formation of osteoclasts from precursor cells (Korcok et al., 2005; Orriss et al., 2011). Antagonists of the $P2Y_6R$ may therefore find use in the treatment of inflammatory bone diseases, such as rheumatoid arthritis and periodontitis (Table 1). $P2Y_6R$ antagonism could also be beneficial in treating other excessive inflammatory reactions, including inflammatory bowel disease, gout, allergic airway inflammation, atherosclerosis, and CNS disorders (Cox et al., 2005; Garcia et al., 2014; Grbic et al., 2012; Grbic, Degagne, Langlois, Dupuis, & Gendron, 2008; Guns, Hendrickx, van Assche, Fransen, & Bult, 2010; Hao, Liang, Chow, Cheung, & Ko, 2014; Khine et al., 2006; Kim et al., 2011; Morioka et al., 2013; Müller et al., 2017; Nakano et al., 2017; Riegel et al., 2011; Shi et al., 2016; Sil et al., 2017; Somers, Hammet, Trute, Southey, & Venter, 1998; Stachon et al., 2014; Uratsuji et al., 2012; Vieira et al., 2011; Warny et al., 2001). Pro-nociceptive effects were attributed to the $P2Y_6R$ in a rat model of inflammatory pain; $P2Y_6R$ antagonists could thus be useful for pain therapy (Barragán-Iglesias et al., 2015). The $P2Y_6R$ was also shown to mediate ATP release from the urothelium, leading to increased bladder sensation and detrusor overactivity in patients with benign prostatic hyperplasia. $P2Y_6R$ antagonism was thus proposed as a novel therapeutic strategy to control persistent storage symptoms in patients with bladder outflow obstruction (Silva, Ferreirinha, Magalhães-Cardoso, Silva-Ramos, & Correia-de-Sá, 2015). Since the $P2Y_6R$ was found to induce interleukin-8 production and epithelial barrier dysfunction following exposure to *Clostridium difficile* toxins, it may further become a target for the treatment of nosocomial diarrhea (Hansen et al., 2013). Moreover, $P2Y_6R$ antagonists may be useful for treating obesity, as $P2Y_6R$ activation in orexigenic agouti-related peptide-expressing neurons in the arcuate nucleus of the hypothalamus was shown to promote feeding in mice. Accordingly, $P2Y_6R$ deficiency in these neurons led to a reduced food intake and improved systemic insulin sensitivity (Steculorum et al., 2015; Steculorum et al., 2017). $P2Y_6R$ mRNA was upregulated in breast cancer cells and its activation facilitated breast cancer metastasis both *in vitro* and *in vivo*. $P2Y_6R$ activation further prolonged survival of different cell lines (Azimi et al., 2016; Gendaszewska-Darmach & Szustak, 2016; Ma et al., 2016). $P2Y_6R$ antagonists could, similar to $P2Y_2R$ antagonists, be useful for cancer therapy. The $P2Y_6R$ appears to be involved in cardiac development, as is the $P2Y_4R$, but with seemingly opposite effects. $P2Y_6R$-null mice showed macrocardia and amplified pathological cardiac hypertrophy (Clouet et al., 2016; Horckmans et al., 2012). It was thus postulated that $P2Y_6R$ antagonists could potentially be used in the therapy of these pathological conditions in humans. Furthermore, blockade of the $P2Y_6R$ was proposed as a strategy to prevent pressure overload-induced cardiac fibrosis (Nishida et al., 2008). $P2Y_6R$ mRNA expression was found to be upregulated in response to the inflammatory mediator interleukin-1β in the rat aorta, where it promotes vascular smooth muscle growth and differentiation (Hou et al., 2002). It is therefore possible that the $P2Y_6R$ contributes to atherosclerosis and the formation of neointima. As the $P2Y_6R$ is also involved in the contraction of human cerebral arteries, antagonists may be useful for preventing cerebral vasospasms (Malmsjö, Hou, Pendergast, Erlinge, & Edvinsson, 2003). Moreover, the $P2Y_6R$ was associated with oxidative stress in neurons and neural degeneration (Qian, Xu, Yang, & Xiao, 2017). $P2Y_6R$ antagonism could thus be beneficial for the treatment of neurodegernative disorders, including Parkinson's disease.

4.2.2. Diisothiocyanate derivatives

The first selective $P2Y_6R$ antagonists described in the literature were from a series of symmetrical aryl diisothiocyanate derivatives (Mamedova, Joshi, Gao, von Kügelgen, & Jacobson, 2004). They were developed following the observation that 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid disodium salt and its dihydro derivative were capable of inhibiting UDP-induced rat $P2Y_6R$ activation at micromolar concentrations. Substitution of one of the two isothiocyanate groups caused a loss in activity, indicating that both isothiocyanate functions were essential (Mamedova et al., 2004). The most potent and selective compounds of this series are MRS2578 (**10**-**08**, compound **08** in Table 10 and Fig. 10), MRS2567 (**10**-**09**), and MRS2575 (**10**-**10**). They showed IC_{50} values of 37 nM, 126 nM, and 155 nM, respectively, at the human $P2Y_6R$. At the related $P2Y_1R$, $P2Y_2R$, $P2Y_4R$, and $P2Y_{11}R$, all three compounds were inactive at 10 μM; the only exception was a slightly higher potency of **10**-**08** at the $P2Y_1R$. Compound **10**-**09** had similar effects on the human and the rat $P2Y_6Rs$, while **10**-**08** was slightly less potent at the rat ortholog (IC_{50} of 98 nM) and **10**-**10** was inactive at the rat receptor (Mamedova et al., 2004). These aryl diisothiocyanate derivatives were described as insurmountable antagonists, as they were unable to induce a parallel shift of UDP concentration–response curves. Due to the absence of radioligands for the $P2Y_6R$, selected derivatives of this compound series that showed activity at the $P2Y_1R$ were assessed in $P2Y_1R$ radioligand binding assays. They were incapable of displacing the radioligand from its binding site on the $P2Y_1R$ and thus were proposed to be non-competitive antagonists (Mamedova et al., 2004). Owing to their hydrophobic nature, the diisothiocyanate derivatives display low solubility in aqueous buffer, which limits their usefulness as pharmacological tools. Disadvantages include their reactivity towards nucleophiles and their instability in aqueous medium. They are likely to bind covalently, and thus irreversibly, to the $P2Y_6R$ (Jacobson, Ivanov, de Castro, Harden, & Ko, 2009). Compound **10**-**08** is commercially available from several sources and currently the most widely used $P2Y_6R$ antagonist. However, it may not be suitable for *in vivo* applications.

4.2.3. Other $P2Y_6$ receptor antagonists

In order to obtain alternatives to the diisothiocyanate derivatives, it was attempted to develop nucleotide-based $P2Y_6R$ antagonists by reducing the efficacy of UDP through different substitutions at the nucleobase, the ribose moiety, and the phosphate chain (Meltzer et al., 2015). These efforts yielded comprehensive SAR data and an antagonist – uridylyl phosphosulfate (**10**-**13**, Fig. 10 and Table 10) – that displayed modest potency ($P2Y_6R$ IC_{50} of 112 μM) but selectivity versus $P2Y_2$ and $P2Y_4Rs$.

The three non-specific P2 receptor antagonists suramin (**10**-**04**), RB-2 (**10**-**07**), and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, **10**-**12**) are weakly active at the $P2Y_6R$ (see Fig. 10 and Table 10). At 100 μM, RB-2 achieved 87 % inhibition (IC_{50} of 31 μM), PPADS 69 %, and suramin only 27 % inhibition of the effect mediated by 2 μM UDP (Robaye et al., 1997). RB-2 may behave as a competitive antagonist, as increasing concentrations caused a parallel shift of UDP concentration-response curves without affecting the upper plateau (Robaye et al., 1997).

5. The $P2Y_{14}$ receptor

The $P2Y_{14}R$ was discovered in 1994 following a systematic approach to identify uncharacterized human genes through predicting their coding sequences in an immature human myeloid cell line (Nomura et al., 1994). It was initially referred to as KIAA0001 or GPR105 until its deorphanization in the year 2000. The endogenous agonist was found *via* the screening of a library of 700 known and putative GPCR ligands (Chambers et al., 2000). The $P2Y_{14}R$ sequence contains several features that are commonly found in GPCRs. These include a DRY motif between transmembrane domain 3 and the second intracellular

loop as well as consensus sites for asparagine-linked glycosylation on extracellular sequences and for phosphorylation by the protein kinases A and C on the third intracellular loop (Chambers et al., 2000). Unlike the other uracil-activated P2YRs, the $P2Y_{14}R$ signals mainly through $G_{i/0}$, thereby inhibiting cAMP production by adenylate cyclase. It was also found to activate extracellular signal-regulated kinase 1/2 (Carter et al., 2009; Fricks, Carter, Lazarowski, & Harden, 2009; Hamel et al., 2011; Scrivens & Dickenson, 2006). The protein sequence of the $P2Y_{14}R$ is 18-45 % identical to that of the other P2YRs, and it shares greatest sequence homology with the $P2Y_{12}R$ and the $P2Y_{13}R$ (particularly in the transmembrane regions) (Abbracchio et al., 2006). The rat ortholog of the $P2Y_{14}R$, also known as VTR15-20, is 80 % and the mouse ortholog 83 % identical to the human receptor. The sequence identity shared between the rat and mouse receptors is 89 % (Freeman et al., 2001; Fricks et al., 2008). The $P2Y_{14}R$ appears to be ubiquitously expressed and was detected at protein level in most tissues and organs (Table 1) (Moore et al., 2001; Uhlén et al., 2015). The physiological function of the $P2Y_{14}R$ remains to be confirmed, but experimental observations and its prominent expression in immune cells suggest an involvement in inflammation and immune responses (Azroyan et al., 2015; Barrett et al., 2013; Charlton, Williams, Fogliano, Sweetnam, & Duman, 1997; Lazarowski & Harden, 2015; Li et al., 2016).

5.1. $P2Y_{14}$ receptor agonists

5.1.1. Therapeutic potential of $P2Y_{14}$ receptor agonists

The $P2Y_{14}R$, together with other P2YRs, was shown to be expressed on human plasmacytoid dendritic cells. Activation through extracellular nucleotides inhibited the release of interferon-α, possibly to avoid excessive tissue damage or the induction of autoimmunity. It was thus postulated that $P2Y_{14}R$ agonists could be useful in treating autoimmune diseases (Shin et al., 2008). $P2Y_{14}R$ knockout mice showed impaired insulin secretion, and agonists might be useful for treating diabetes (Meister et al., 2014).

5.1.2. Physiological agonists

The main function of UDP-glucose (**8**-**01**, compound **01** in Fig. 8 and Table 8) was commonly believed to serve as a glycosyl donor in the enzymatic biosynthesis of carbohydrates. Later, it was discovered to be released from cells as a signaling molecule and to function as an agonist at the $P2Y_{14}R$ (Carter et al., 2009; Chambers et al., 2000; Hamel et al., 2011; Lazarowski & Harden, 2015; Lazarowski, Shea, Boucher, & Harden, 2003). Other sugar derivatives of UDP (Fig. 8 and Table 8), including UDP-galactose (**8**-**02**), UDP-glucuronic acid (**8**-**03**), and UDP-*N*-acetylglucosamine (**8**-**04**), can also activate the $P2Y_{14}R$, albeit less potently (Chambers et al., 2000; Hamel et al., 2011; Ko et al., 2009; Ko, Fricks, Ivanov, Harden, & Jacobson, 2007). Some of these nucleotide sugars have also been described as partial agonists, but reported discrepancies could be due to different receptor expression levels (Fujioka & Omori, 2012; Ko et al., 2007; Scrivens & Dickenson, 2005). The pharmacological profile of UDP-glucose, UDP-galactose, UDP-glucuronic acid, and UDP-*N*-acetylglucosamine is similar for the rat and mouse orthologs (Freeman et al., 2001). In contrast, the glucose conjugates of CDP, ADP, and guanosine 5′-diphosphate (GDP) were inactive (Chambers et al., 2000; Ko et al., 2007). The responsiveness to nucleotide sugars is a characteristic but not an exclusive feature of the $P2Y_{14}R$, as UDP-glucose was also shown to activate the $P2Y_2R$ and $P2Y_6R$ at mid-micromolar concentrations (Das et al., 2010; Ko et al., 2009).

UDP (**2**-**09**, Fig. 2 and Table 2) was initially found inactive at the $P2Y_{14}R$ in a yeast transcription reporter assay, in an assay using transient expression with a promiscuous $G\alpha_{16}$ protein, and also in [^{35}S]GTPγS binding assays. It was also previously described as a competitive antagonist or as a partial agonist at the human $P2Y_{14}R$ (and an agonist at the rat ortholog) when using a chimeric $G\alpha_{q/i}$ protein that couples G_i-activating receptors to phospholipase C_β and inositol lipid hydrolysis (Ault & Broach, 2006; Chambers et al., 2000; Fricks et al., 2008). However, these test systems were later found unsuitable, and UDP was subsequently confirmed as a full $P2Y_{14}R$ agonist in a more natural assay system: when measuring $P2Y_{14}R$-mediated adenylate cyclase inhibition in three stably transfected cell lines, UDP was consistently shown to be even slightly more potent than UDP-glucose and to exhibit similar efficacy (Carter et al., 2009; Hamel et al., 2011; Harden, Sesma, Fricks, & Lazarowski, 2010). Other mononucleotides and different dinucleotides (e.g. $Ap_{3/4/5/6}A$, UP_4U, Up_2U, and Cp_2C) were less potent or inactive at the $P2Y_{14}R$. This is a distinction from the other P2YRs and further emphasizes the unique pharmacological profile of the $P2Y_{14}R$ (Carter et al., 2009; Chambers et al., 2000; Ko et al., 2007).

5.1.3. Synthetic nucleotide analogues

A thio substitution in the 2-position of the uracil moiety caused an improvement in potency at the $P2Y_{14}R$: 2-thio-UDP (**3**-**02**, Fig. 3 and Table 3) was 37- to 83-fold more potent than UDP (Carter et al., 2009; Das et al., 2010). However, this substitution is also well tolerated by the $P2Y_6R$. Although being 230-fold selective for the $P2Y_{14}R$ over the $P2Y_6R$, **3**-**02** activates the $P2Y_6R$ at nanomolar concentrations. It is thus not ideal for discerning these two receptors experimentally (Das et al., 2010; El-Tayeb et al., 2006; Jacobson et al., 2006). The benefits of a 2-thio substitution could be combined with other modifications at the phosphate groups of UDP to obtain compounds with (sub) nanomolar potency and significantly enhanced selectivity versus the $P2Y_6R$ (Das et al., 2010). Among the best $P2Y_{14}R$ agonists is α,β-methylene-2-thio-UDP (MRS2905, **6**-**08**, Fig. 6 and Table 6). It showed an EC_{50} value of 0.92 nM at the $P2Y_{14}R$, which makes this compound 170-fold more potent than UDP and 2000-fold selective versus the $P2Y_6R$. In contrast, an analogue without substitution in the 2-position, α,β-methylene-UDP (**5**-**13**, Fig. 5 and Table 5), was merely 15-fold more potent at the $P2Y_{14}R$ than UDP and 31-fold selective versus the $P2Y_6R$. The derivative α,β-difluoromethylene-UDP (MRS2802, **5**-**15**, Fig. 5 and Table 5) may also be used to pharmacologically distinguish the $P2Y_{14}R$ (EC_{50} of 63 nM) from the $P2Y_6R$ (inactive at 10 μM) (Das et al., 2010). Due to the replacement of the diphosphate by a phosphonate, it is expected to be metabolically stable and therefore useful for *in vivo* studies.

5.1.4. Synthetic nucleotide-sugar derivatives

A simple 2-thio substitution of UDP-glucose led to a 36-fold improvement in potency at the $P2Y_{14}R$. Unlike 2-thio-UDP (**3**-**02**, Fig. 3 and Table 3), 2-thio-UDP-glucose (MRS2690, **8**-**15**, Fig. 8 and Table 8) was inactive at the related $P2Y_6R$ (Das et al., 2010). The selectivity versus the $P2Y_2R$ was also improved through this substitution: MRS2690 was inactive at the $P2Y_2R$ at 10 μM. In contrast, UDP-glucose (**8**-**01**, Fig. 8 and Table 8) showed half-maximal $P2Y_2R$ activation at the same concentration (Ko et al., 2009). A thio substitution in the 4-position was either not tolerated at all (4-thio-Up_2-OMe was inactive), or it had no significant effect on the potency (UDP-glucose and 4-thio-UDP-glucose (MRS2670, **8**-**16**, Fig. 8 and Table 8) were equipotent) (Das et al., 2010; Ko et al., 2007). Different substitutions in 5-position or complete replacement of the uracil moiety by adenine, guanine, and cytosine resulted in inactive compounds (Ko et al., 2007).

The glucose moiety of UDP-glucose was proposed to form H-bonding interactions with charged residues in the putative ligand binding pocket. Yet, this region appears to be the part at which substitutions are best tolerated by the $P2Y_{14}R$. The glucose moiety of UDP-glucose was replaced by different sugars in a systematic approach to investigate the tolerance of the $P2Y_{14}R$ to modifications at this site (Ko et al., 2009). Most analogues were found active (e.g. compounds **8**-**05** to **8**-**13**, Fig. 8 and Table 8), further indicating a large potential for substitutions at the glucose. However, their potencies were mostly lower or similar to that of UDP-glucose, and it has not yet been possible to exploit the steric freedom at the glucose moiety to enhance the potency (Ko et al., 2009).

UDP could further be substituted by smaller ester groups at the terminal phosphate. Potencies in the nanomolar and low micromolar range were achieved with a series of UDP-β-esters, which was further enhanced if combined with a 2-thio substitution. For example, Up_2-OMe (not shown) exhibited an EC_{50} value of 2.73 μM, while its 2-thio analogue **6**-**17** (Fig. 6 and Table 6; EC_{50} of 0.056 μM) was 50-fold more potent (Das et al., 2010). The ethoxy (MRS2906, **6**-**18**), propoxy (MRS2907, **6**-**19**), and *tert*-butyl (**6**-**20**) derivatives of 2-thio-Up_2 as well as **6**-**21** were similarly of mid-nanomolar potency (Fig. 6 and Table 6). They were either inactive at the $P2Y_6R$ (**6**-**17**, **6**-**18**, **6**-**21**), or 64-fold (**6**-**20**) and 230-fold (**6**-**19**) selective for the $P2Y_{14}R$. Larger branched β-alkyl esters (e.g. cyclohexyl) or β-aryloxy esters were less potent (Carter et al., 2009; Das et al., 2010). The chemical and metabolic stability of these phosphoric acid ester derivatives has not been investigated but may be limited.

5.1.5. Modifications at the ribose moiety

Replacement of the flexible ribose moiety with a constrained methanocarba (bicyclo[3.1.0]hexane) ring led to inactivity at the $P2Y_{14}R$ (Das et al., 2010). This is a disparity from the other uracil-activated P2YRs, where a ring constraint in the preferred position (*North* for the $P2Y_2R$ and $P2Y_4R$, *South* for the $P2Y_6R$) was tolerated (Kim et al., 2002; Maruoka et al., 2010). In fact, the $P2Y_{14}R$ appears to be highly restrictive with regard to modifications at the ribose. Several different substitutions in the 2′- and 3′-position, some of which enhanced the potency or selectivity for the $P2Y_2R$ and $P2Y_4R$, caused a complete loss of activity at the $P2Y_{14}R$ (Ko et al., 2007).

5.1.6. Nucleotide-dendrimer conjugates

Dendrimers are branched, polymeric macromolecules that often form a spherical three-dimensional shape and are typically symmetrical around the core. They can serve as nano-carriers for drug delivery. UDP-glucuronic (**8**-**03**, Fig. 8 and Table 8) acid as well as its ethylenediamine analogue (MRS2892, **8**-**21**, Fig. 8 and Table 8) were covalently coupled to polyamidoamine (PAMAM) dendrimers. These consist of several bifurcating layers of methyl acrylate and ethylenediamine (Das et al., 2009). A PAMAM conjugate containing four bound UDP-glucuronic acid moieties was 2-fold more potent (EC_{50} of 159 nM) than UDP-glucuronic acid alone (EC_{50} of 370 nM). If 20 UDP-glucuronic acid molecules were bound, the PAMAM conjugate was 150-fold more potent (EC_{50} of 2.4 nM) than UDP-glucuronic acid alone, which corresponds to an 8-fold increase in potency per dendrimer-bound UDP-glucuronic acid moiety compared to monomeric UDP-glucuronic acid. The larger PAMAM conjugate **8**-**24** (Fig. 8 and Table 8) that contained 147 bound UDP-glucuronic acid moieties was 460-fold more potent (EC_{50} of 0.8 nM) than UDP-glucuronic acid, which still corresponds to a 3-fold increase in potency per UDP-glucuronic acid moiety.

Molecular docking of a UDP-glucuronic acid dendrimer to a $P2Y_{14}R$ homology model suggested that the dendrimer branches extend far beyond the dimensions of the receptor. They could possibly be available for multivalent binding to receptor aggregates. It was proposed that these multivalent conjugates span several $P2Y_{14}R$ binding sites simultaneously in dimers and other higher-order receptor aggregates (Das et al., 2009).

In an attempt to target heteromeric receptor assemblies, a bifunctional hybrid dendrimer conjugate (MRS5259, **8**-**29**, Fig. 8 and Table 8) was developed and patented (Jacobson & Tosh, 2010; Tosh et al., 2010). MRS5259 features the selective A_3AR agonist MRS3558 coupled to amide-linked UDP-glucuronic acid *via* a PAMAM dendrimer structure. Both the $P2Y_{14}R$ and the A_3AR are involved in immune functions and possibly expressed on the same cells. Thus, coactivation of these two receptors with one hybrid compound could be advantageous over separate receptor agonists for therapeutic use. Linking UDP-glucuronic acid to the A_3AR agonist in a PAMAM dendrimer did not alter the potency at the $P2Y_{14}R$ in comparison to monofunctional dendrimer analogues. However, the potency and selectivity at the A_3AR was reduced (Tosh et al., 2010).

Moreover, different prosthetic groups were coupled to dendrimer-nucleotide conjugates to create tools for *in vivo* detection and characterization of the $P2Y_{14}R$. These include biotin for avidin complexation (**8**-**25**), the fluorophore Alexa Fluor® 488 (**8**-**26**), and the metal-chelating group diethylenetriaminepentaacetic acid (DTPA; **8**-**27** and **8**-**28**) that might be useful for magnetic resonance imaging (Fig. 8 and Table 8) (Das et al., 2009).

5.1.7. Fluorescence-labelled agonists

SAR studies revealed that UDP-glucuronic acid (**8**-**03**, Fig. 8 and Table 8) could be linked to larger groups at the C6 carbon atom of the hexose ring without loss in potency (Kiselev et al., 2015). It was therefore used as a precursor for chain extensions to develop fluorescent probes. The diaminoalkyl-linked BODIPY® conjugate MRS4183 (**8**-**23**, Fig. 8 and Table 8) and an analogue with a shorter linker were first docked into a $P2Y_{14}R$ homology model and subsequently synthesized and assessed (Kiselev et al., 2015; Trujillo, Paoletta, Kiselev, & Jacobson, 2015). According to the model, the pharmacophore binds inside the deep orthosteric binding pocket while the fluorophore is located at the extracellular surface. The pharmacophore and the fluorophore are connected by a linker consisting of 14 atoms in MRS4183. The 7-atom linker of the shorter analogue was insufficient to enable optimal binding of the fluorophore on the outside of the receptor (Kiselev et al., 2015). MRS4183 exhibited an EC_{50} value of 0.96 nM and was thus 50-fold more potent than UDP-glucuronic acid, while the shorter analogue was 100-fold less active (EC_{50} of 91 nM). MRS4183 showed high specific binding to the $P2Y_{14}R$ in a flow cytometry assay with an apparent binding constant ($K_{d\ app}$) of 21.4 nM and a half-life of 23.9 min for the binding of 50 nM. It was successfully used to detect $P2Y_{14}R$-expressing Chinese hamster ovary cells using confocal microscopy (Kiselev et al., 2015).

5.1.8. Radioligands

A tritiated form of UDP-glucose, uridine diphospho-D-[6-^{3}H]glucose, was used as a radioligand for the $P2Y_{14}R$. It showed an affinity of 8.1 nM (Chambers et al., 2000). However, the detected specific binding was essentially identical in non-transfected and $P2Y_{14}R$-transfected HEK293 cells, 1321N1 astrocytoma cells, and Chinese hamster ovary cells. The compound is thus unsuitable as a radioligand for $P2Y_{14}R$ assays (Chambers et al., 2000; Hamel et al., 2011). In contrast, a radioligand binding assay with [^{3}H]UDP could be established. It was used to characterize UDP-glucose (**8**-**01**, Fig. 8 and Table 8), UDP (**2**-**09**, Fig. 2 and Table 2), UMP (**2**-**10**, Fig. 2 and Table 2), and a non-nucleotide antagonist (**10**-**22**, Fig. 10 and Table 10) in competition binding assays using HEK cells transiently-transfected with the $P2Y_{14}R$ (Hamel et al., 2011). A K_d value of 16.8 nM was determined for [^{3}H]UDP in saturation binding experiments. [^{3}H]UDP was found to be stable under the assay conditions of radioligand binding for at least one hour, as detected by C18 reverse-phase high-performance liquid chromatography. In contrast, [^{3}H]UTP was rapidly dephosphorylated to UDP. However, in the presence of 25 % human plasma, [^{3}H]UDP was also broken down, yielding uridine as the final product (Hamel et al., 2011).

5.2. $P2Y_{14}$ receptor antagonists

5.2.1. Therapeutic potential of $P2Y_{14}$ receptor antagonists

The $P2Y_{14}R$ is prominently expressed in different cells of the immune system. Furthermore, $P2Y_{14}R$ activation was shown to promote chemotaxis and recruitment of neutrophils and macrophages, as well as the release of proinflammatory cytokines, chemokines, and mast cell mediators (Amison et al., 2017; Arase et al., 2009; Barrett et al., 2013; Ferreira et al., 2017; Gao, Ding, & Jacobson, 2010; Gao, Wei, Jayasekara, & Jacobson, 2013; Gendaszewska-Darmach et al., 2016; Jokela et al., 2014; Li et al., 2016; Müller et al., 2005; Sesma et al.,

2012; Sesma et al., 2016; Xu et al., 2012). $P2Y_{14}R$ expression was found to be upregulated in rat brain and spleen following challenge with lipopolysaccharide. It was also upregulated in spinal microglia following peripheral nerve injury, where it contributes to mechanical pain hypersensitivity (Kobayashi, Yamanaka, Yanamoto, Okubo, & Noguchi, 2012; Moore et al., 2003). Nucleotide-sugar conjugates are resistant to hydrolysis by classical ectonucleotidases (such as CD39). As a result, UDP-glucose is found in high concentrations in the extracellular tissue surrounding airway epithelial cells as well as in lung secretions of cystic fibrosis patients. All of these experimental observations indicated that UDP-glucose functions as an extracellular mediator of inflammation by exerting its actions *via* the $P2Y_{14}R$ (Barrett et al., 2013; Sesma et al., 2016). Antagonists may thus find use in treating excessive inflammatory reactions, including asthma, as had also been postulated for other P2YRs. The concept of diagnosing renal inflammation through measuring the concentration of UDP-glucose in, for example, urine samples and treatment with a $P2Y_{14}R$ antagonist has been patented (Breton, Brown, Azroyan, Cortez-Retamozo, & Pittet, 2014). A reduced resistance to insulin was observed in obese $P2Y_{14}R$ knockout mice. Antagonists could thus be of interest for treating type II diabetes (Xu et al., 2012). Moreover, UDP-sugars were shown to stimulate osteoclastogenesis, and $P2Y_{14}R$ downregulation by RNA interference inhibited osteoclast formation. Antagonists might thus also be useful for treating bone disorders, such as osteoporosis (Lee, Park, & Lee, 2013).

5.2.2. Naphthoic acid derivatives as competitive $P2Y_{14}$ receptor antagonists

High-throughput screening led to the discovery of 4,7-disubstituted 2-naphthoic acid **10-17** (compound **17** in Fig. 10 and Table 10) to be a weak competitive $P2Y_{14}R$ antagonist (IC_{50} of 3.5 μM at both the mouse and chimpanzee $P2Y_{14}Rs$, K_i of 0.16 μM at chimpanzee $P2Y_{14}R$) (Gauthier et al., 2011). In an attempt to optimize its potency and pharmacokinetic properties, different substitutions in the 3-, 4- and 7-position were explored. The most potent compound (**10-16**, Fig. 10 and Table 10) displayed IC_{50} values of 8 nM and 1 nM at the mouse and chimpanzee $P2Y_{14}Rs$, respectively (Belley et al., 2008; Gauthier et al., 2011). It showed a bioavailability of 67 % following oral administration and a low intrinsic clearance of 1.6 ml/min/kg in mice (Gauthier et al., 2011). In the presence of 5 % human serum albumin, more than 99 % of the compound was bound, which may have a negative impact on *in vivo* activity (Robichaud et al., 2011). The K_i value at the chimpanzee $P2Y_{14}R$ was reduced to 1.29 μM in the presence of 2 % human serum albumin while it was 4 nM in the absence of human serum albumin, which corresponds to a 320-fold difference (Gauthier et al., 2011; Robichaud et al., 2011). Consequently, 4-(4-(piperidin-4-yl)-phenyl)-7-(4-(trifluoromethyl)phenyl)-2-naphthoic acid (PPTN, **10-14**, Fig. 10 and Table 10) was developed. It showed less protein binding and K_i values at the chimpanzee $P2Y_{14}R$ of 1.9 nM and 35 nM in the absence and presence of 2 % human serum albumin (Belley et al., 2008; Robichaud et al., 2011). PPTN exhibited a K_B value calculated from Schild analysis of 0.434 nM and a K_i value of 0.3 nM at the human $P2Y_{14}R$. It was completely inactive at all other P2YR subtypes at a concentration of 1 μM, and is thus a highly selective $P2Y_{14}R$ antagonist (Barrett et al., 2013; Kiselev et al., 2014). A clear parallel rightward shift towards higher concentrations was observed for concentration-effect curves of UDP-glucose in the presence of increasing concentrations of PPTN, which suggests competitive antagonism. However, the zwitterionic PPTN displayed a very low oral bioavailability. To overcome this issue, an ester prodrug was developed with superior pharmacokinetic properties and efficient conversion to the active drug *in vivo* (Robichaud et al., 2011). The potency of PPTN could be preserved by chain extension at the piperidine moiety, as predicted by homology modeling and confirmed through the synthesis and assessment of a series of chain-elongated alkynyl and amino derivatives of PPTN (Kiselev et al., 2014). The most potent derivative, MRS4174 (**10-18**, Fig. 10 and Table 10), displayed a K_i value of 80 pM, while those of most other compounds in this series were in the low nanomolar range. MRS4174 is an analogue of PPTN coupled to the fluorophore Alexa Fluor® 488. It is astonishing that the addition of the large fluorophore did not cause a reduction in potency, but in fact led to a 160-fold increase. In contrast, a BODIPY® conjugate was significantly less active (K_i >100 nM). The potent fluorescent antagonist MRS4174 was employed as a tracer in flow cytometry binding assays, where it displayed high affinity and low non-specific binding (Kiselev et al., 2014). Its synthesis could subsequently be improved to yield sufficient quantity for use in routine assays (Junker et al., 2016). It will be a useful probe for detecting the $P2Y_{14}R$ in different experimental settings and can be an alternative to the fluorescent $P2Y_{14}R$ agonist MRS4183 (**8-23**, Fig. 8 and Table 8) (Kiselev et al., 2015). The fact that both fluorescent probes, MRS4174 (**10-18**) and MRS4183 (**8-23**), were designed using a $P2Y_{14}R$ homology model based on the $P2Y_{12}R$ X-ray structure emphasizes the value of computer modeling for medicinal chemistry, especially after the publication of several P2YR X-ray structures (Zhang et al., 2015; Zhang, Zhang, Gao, Paoletta, et al., 2014; Zhang, Zhang, Gao, Zhang, et al., 2014).

5.2.3. Analogues of PPTN

The hydrophobic naphthalene ring of PPTN (**10-14**, Fig. 10 and Table 10) is responsible for the low solubility of the compound. It was thus attempted to replace the naphthalene structure with a bioisostere to improve its physicochemical properties (Junker et al., 2016). Docking studies and molecular dynamics simulations on a $P2Y_{14}R$ homology model led to the design of alkyne and triazole derivatives. The alkyne derivative **10-20** (Fig. 10 and Table 10; IC_{50} value of 5.69 μM) was 950-fold less potent than PPTN. The corresponding triazole derivative **10-19** (Fig. 10 and Table 10) was more potent (IC_{50} of 32 nM, 5-fold less potent than PPTN) and therefore used as a lead structure for further optimization. However, an improvement in potency has not been achieved; the IC_{50} values were in between 72 nM and 481 nM (Junker et al., 2016). Despite the 5-fold lower potency of **10-19**, it may nevertheless be preferably used in biological experiments if its physicochemical properties are indeed superior. No data with regard to this have been published so far, but computer calculations predicted a higher solubility and lower plasma protein binding (Junker et al., 2016).

PPTN and **10-19** were assessed in the Psychoactive Drug Screening Program for off-target activities (Besnard et al., 2012). PPTN displayed micromolar K_i values at the dopamine D_3 and the δ-opioid receptors, and **10-19** showed affinity for the α_{2A} and α_{2C} adrenergic receptors. A nanomolar K_i value (170 nM) was observed for **10-19** only at the H_1 histamine receptor. PPTN and **10-19** were inactive at all other receptors, ion channels, and transporters assessed in the standard diverse screen of the Psychoactive Drug Screening Program (Besnard et al., 2012; Junker et al., 2016). Thus, they showed only few off-target interactions (Junker et al., 2016).

5.2.4. Dihydropyridopyrimidines – non-competitive $P2Y_{14}$ receptor antagonists

High-throughput screening at Merck Frosst (Canada) led to the discovery of another non-nucleotide $P2Y_{14}R$ antagonist with a dihydropyridopyrimidine core. It showed micromolar potency at the mouse $P2Y_{14}R$ (Guay et al., 2011). This hit compound formed the basis for comprehensive SAR studies that resulted in several derivatives with potencies in the nano- to micromolar range at the mouse and chimpanzee receptors (Guay et al., 2008). The 3,4-methylenedioxyphenyl derivative **10-21** (Fig. 10 and Table 10) was among the most promising antagonists, exhibiting IC_{50} values of 10 nM and 81 nM on the mouse and chimpanzee $P2Y_{14}Rs$, respectively (Guay et al., 2008; Guay et al., 2011). It was found to be readily bioavailable following oral administration and provided sufficient exposure in mice to be a suitable tool for *in vivo* studies. The compound did not displace [^{3}H]UDP in filtration binding assays and was thus proposed to be a non-competitive antagonist. Although several analogues showed slightly higher potencies, they were exempted from further

considerations due to binding to the human Ether-à-go-go-Related Gene (hERG) channel that may cause serious side-effects (Guay et al., 2011).

5.2.5. Phosphonates

One of the first non-nucleotide $P2Y_{14}R$ antagonists, **10-22** (Fig. 10 and Table 10), was discovered through the screening of a compound library that consisted of 608 phosphonates (Hamel et al., 2011). Compound **10-22** exhibited an IC_{50} value of 2.3 μM in calcium mobilization assays and a K_i value of 1.28 μM in competition binding assays with [^{3}H]UDP. It was suggested that the activity of **10-22** could be improved by altering its structure in a manner to more closely resemble a diphosphate compound, but no such attempts have been reported so far (Hamel et al., 2011).

6. Conclusions

6.1. Agonists

The P2YR family of GPCRs is of considerable therapeutic interest. Consequently, extensive research efforts have been directed towards the design of ligands suitable for target validation studies and drug development. A relatively large number of agonists has been developed in the past years as a result, and the most useful ones with respect to potency, selectivity, and physicochemical properties are collected in Fig. 11.

Among the most potent and selective $P2Y_2R$ agonists is 2′-amino-2′-deoxy-2-thio-UTP (MRS2698, **6-01** compound **01** in Fig. 6 and Table 6). It shows an EC_{50} value in the low nanomolar range, is 6-fold more potent than the endogenous agonist UTP, and is at least 300-fold selective versus the $P2Y_4R$ and $P2Y_6R$. Highly selective (>6600-fold) $P2Y_2R$ agonists were also developed by replacing the nucleobase of nucleotide triphosphates with 'unnatural' bicyclic aromatic residues (e.g. **3-35**). Owing to its enhanced ectonucleotidase stability, 4-thio-β,γ-difluoromethylene-UTP (PSB-1114, **6-05**) is of great interest as well, despite its 8-fold lower potency than UTP. Dinucleotides are also more resistant to enzymatic hydrolysis as compared to mononucleotides, but are generally less potent (except at the $P2Y_6R$), and lack selectivity in many cases. Eye drops (Diquas®) containing Up_4U (3 % solution of the tetrasodium salt, diquafosol, INS365, **7-03**) was approved in Japan and other Asian countries as a treatment for dry eye syndrome. All of these compounds except **3-35** can be obtained from commercial distributors. Very recently, a 4(1*H*)-quinoline derivative (**9-01**) was described as a non-nucleotide ago-allosteric agonist with micromolar potency at the $P2Y_2R$ and no detectable response at most other P2YRs. It may serve as a lead structure for the development of more potent non-nucleotide $P2Y_2R$ agonists.

For the $P2Y_4R$, fewer ligands have been described so far. Among the most potent and selective agonists is the commercially available N^4-(phenylpropoxy)-CTP (MRS4062, **3-15**), which is twice as potent as the endogenous agonist UTP and 27-fold selective.

The development of agonists for the $P2Y_6R$ has been far more successful and yielded several potent and highly selective compounds. The R_p isomer of 5-MeO-uridine 5′-O-(α-boranodiphosphate) (**6-12**) is one of the most potent (EC_{50} of 8 nM) and selective (>12,000-fold) $P2Y_6R$ agonists known to date. It was reported to be relatively stable towards degradation by ectonucleotidases. Very recently, prostaglandin E_2 glyceryl ester (**9-02**) was described as an extraordinarily potent (EC_{50} in the low picomolar range) physiological $P2Y_6R$ agonist. Should this be confirmed by other laboratories, it will enable new possibilities for compound/drug design, which, however, will be challenging due to its instability under physiological conditions and its lipid character. Other useful $P2Y_6R$ agonists include 3-phenacyl-UDP (PSB-0474, **3-11**; 570-fold selective), N^4-(benzyloxy)-CDP (MRS2964, **3-17**; 44-fold

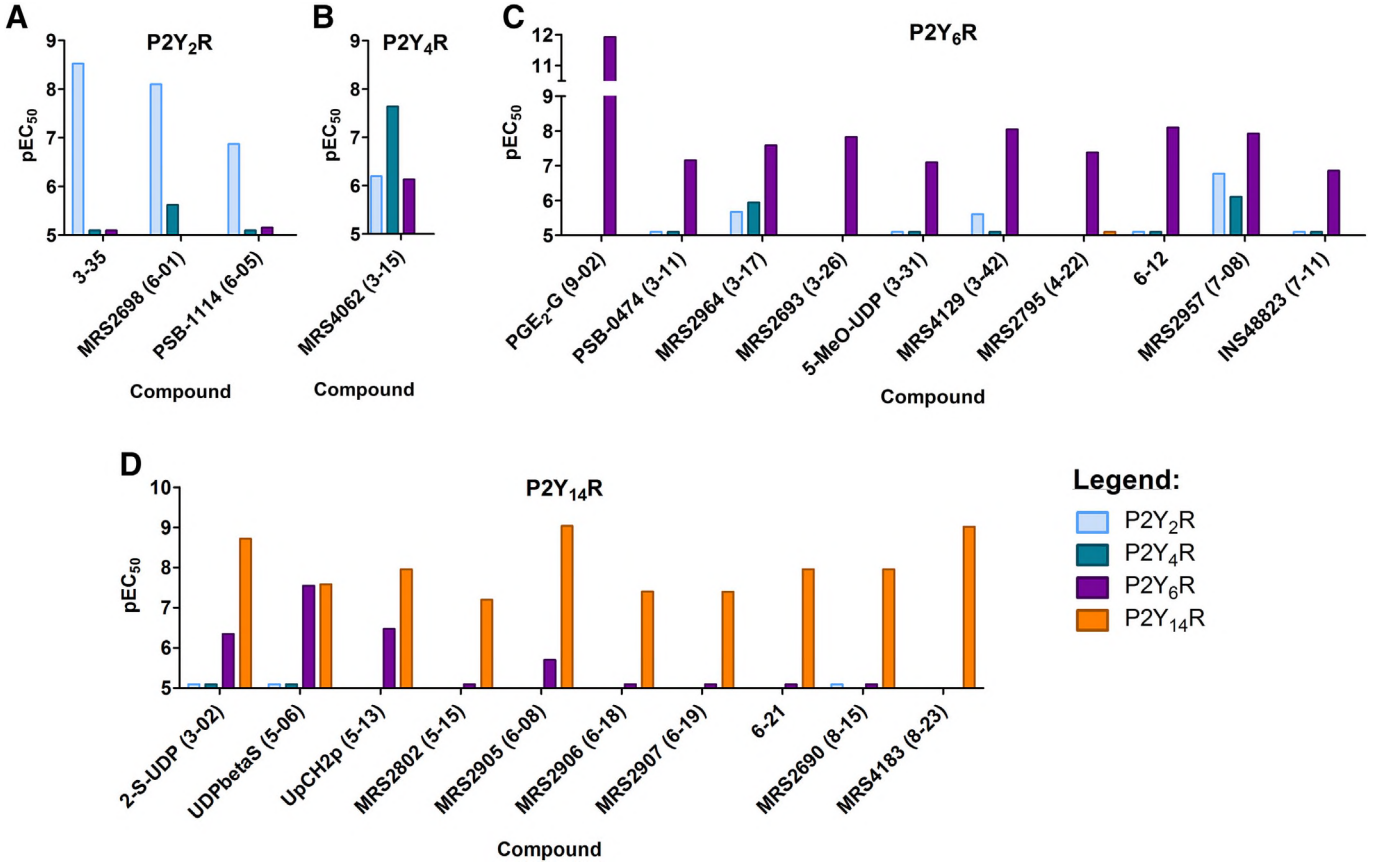


Fig. 11. The most potent and selective agonists for the human (A) $P2Y_2R$, (B) $P2Y_4R$, (C) $P2Y_6R$, and (D) $P2Y_{14}R$. No data is available in cases where no bar chart is indicated.

selective), 5-MeO-UDP (**3**-**31**), 5-iodo-UDP (MRS2693, **3**-**26**), (*S*)-methanocarba-UDP (MRS2795, **4**-**22**), as well as the dinucleotides monobenzylacetal-Up_3U (INS48823, **7**-**11**) and N^4-MeO-Cp_3U (MRS2957, **7**-**08**). The selective partial agonist TMPS (**5**-**08**) may also be of value.

The pharmacological profile of the $P2Y_{14}R$ is more distinct. Unlike the other three uracil nucleotide-activated receptors, it is potently activated by UDP-sugars. UDP (**2**-**09**) is another physiological agonist of the $P2Y_{14}R$, overlapping with the $P2Y_6R$. Thus, a challenge was to confer selectivity for the $P2Y_{14}R$ over the $P2Y_6R$. A 2-thio substitution of the uracil base was highly favorable for the $P2Y_{14}R$. As a result, α,β-methylene-2-thio-UDP (MRS2905, **6**-**08**), 2-thio-UDP-glucose (MRS2690, **8**-**15**), and the 2-thio-UDP-β-esters **6**-**17**, **6**-**18**, and **6**-**21** are highly potent and selective $P2Y_{14}R$ agonists. They were inactive at the $P2Y_6R$. In addition, 2-thio-UDP (**3**-**02**) was found to be 230-fold selective for the $P2Y_{14}R$ but could activate the $P2Y_6R$ at high nanomolar concentrations. Alternatively, α,β-difluoromethylene-UDP (MRS2802, **5**-**15**) showed >160-fold selectivity for the $P2Y_{14}R$ over the $P2Y_6R$. PAMAM dendrimers add further diversity to the selection of $P2Y_{14}R$ tools. They were connected to the $P2Y_{14}R$ agonist UDP-glucuronic acid (**8**-**03**) in addition to different prosthetic groups, such as an A_3AR agonist, a fluorophore, biotin for avidin complexation, and the metal chelator DTPA for use in magnetic resonance imaging. The fluorescent BODIPY® conjugate MRS4183 (**8**-**23**) and the radioligand [^{3}H]UDP enable further characterization of this receptor. Despite being the most recently described P2YR subtype, ligand development efforts for the $P2Y_{14}R$ have been most successful.

In addition to the compounds described above, the fluorescent probes MRS4129 (**3**-**42**; selective for the $P2Y_6R$) and MRS4162 (**3**-**40**; potent agonist at the $P2Y_2R$, $P2Y_4R$, and $P2Y_6R$) serve as practical tools for detecting and quantifying these receptors in living cells.

6.2. Antagonists

The availability of antagonists for the four uracil nucleotide-activated P2YR subtypes is more limited. The thiouracil derivative AR-C118925 (**10**-**01**, compound **01** in Fig. 10 and Table 10; 50-fold selectivity) for the $P2Y_2R$, and the anthraquinone derivative PSB-16133 (**10**-**06**) as 37-fold selective $P2Y_4R$ antagonist, are superior to the non-selective, moderately-potent standard antagonists suramin (**10**-**04**), RB-2 (**10**-**07**), and PPADS (**10**-**12**) for these two receptors. Among the most frequently used $P2Y_6R$ antagonists is the non-competitive, insurmountable diisothiocyanate derivative MRS2578 (**10**-**08**) with mid-nanomolar potency and at least 270-fold selectivity. However, the hydrophobic structure of MRS2578 and its reactivity towards nucleophiles and its instability in aqueous media constitute severe limitations. MRS2578 binds covalently, and thus irreversibly, to the $P2Y_6R$. The 2-naphthoic acid derivative PPTN (**10**-**14**) is a competitive $P2Y_{14}R$ antagonist with (sub-)nanomolar potency and 2300-fold selectivity. However, it showed low aqueous solubility and poor oral bioavailability. An ester prodrug (**10**-**15**) was subsequently developed for *in vivo* studies. Several potent derivatives were also synthesized, including **10**-**16**, **10**-**19**, and the fluorescent $P2Y_{14}R$ antagonist MRS4174 (**10**-**18**) that exhibited a sub-nanomolar K_i value at the $P2Y_{14}R$. Moreover, dihydropyridopyrimidines were reported to function as non-competitive $P2Y_{14}R$ antagonists, with **10**-**21** showing nanomolar potency and high oral bioavailability. A summary of the most potent and selective antagonists is shown in Fig. 12.

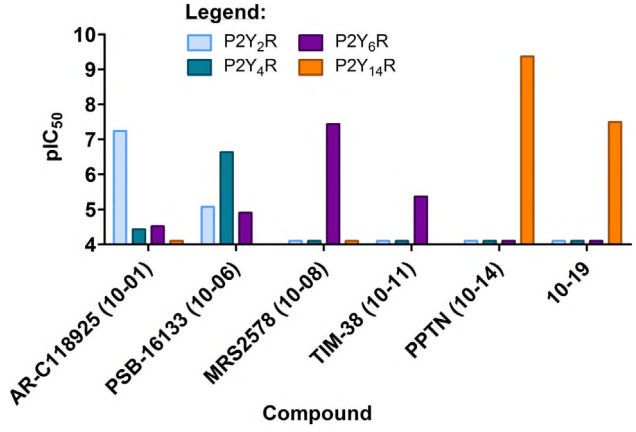


Fig. 12. The most potent and selective P2YR antagonists. No data is available in cases where no bar chart is indicated.

6.3. Outlook

Several agonists showing potencies in the low nanomolar range and good selectivity profiles could be developed for the $P2Y_2R$, $P2Y_6R$, and $P2Y_{14}R$, while agonists for the $P2Y_4R$ remain scarce. With very few exceptions, the described agonists are all nucleotide derivatives. These have generally poor oral bioavailability due to the negative charges at physiological pH, lack brain penetration, and may be subject to degradation by ectonucleotidases, although the stability could be improved for some synthetic analogues. Only few antagonists have been reported. Although most of them are not derived from nucleotides, they still have disadvantages in many cases, such as a low water solubility, high plasma protein binding, or insufficient selectivity. Compounds with higher potency in the low nanomolar range are required for the development of radioligands that would advance further receptor characterization. In conclusion, future efforts are necessary towards the development of (non-nucleotide) agonists and improved antagonists for the uracil nucleotide-activated P2YRs, and the $P2Y_4R$ in particular.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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