Published in final edited form as:

Neuropharmacology. 2016 May ; 104: 31–49. doi:10.1016/j.neuropharm.2015.12.001.

Medicinal Chemistry of Adenosine, P2Y and P2X Receptors

Kenneth A. Jacobson^{a,*} and Christa E. Müller^b

^aMolecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 20892 Bethesda, USA

^bPharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, Bonn, Germany

Abstract

Pharmacological tool compounds are now available to define action at the adenosine (ARs), P2Y and P2X receptors. We present a selection of the most commonly used agents to study purines in the nervous system. Some of these compounds, including A₁ and A₃ AR agonists, P2Y₁R and P2Y₁₂R antagonists, and P2X₃, P2X₄ and P2X₇ antagonists, are potentially of clinical use in treatment of disorders of the nervous system, such as chronic pain, neurodegeneration and brain injury. Agonists of the A_{2A}AR and P2Y₂R are already used clinically, P2Y₁₂R antagonists are widely used antithrombotics and an antagonist of the A_{2A}AR is approved in Japan for treating Parkinson's disease. The selectivity defined for some of the previously introduced compounds has been revised with updated pharmacological characterization, for example, various AR agonists and antagonists were deemed A₁AR or A₃AR selective based on human data, but species differences indicated a reduction in selectivity ratios in other species. Also, many of the P2R ligands still lack bioavailability due to charged groups or hydrolytic (either enzymatic or chemical) instability. X-ray crystallographic structures of AR and P2YRs have shifted the mode of ligand discovery to structure-based approaches rather than previous empirical approaches. The X-ray structures can be utilized either for in silico screening of chemically diverse libraries for the discovery of novel ligands or for enhancement of the properties of known ligands by chemical modification. Although X-ray structures of the zebrafish P2X₄R have been reported, there is scant structural information about ligand recognition in these trimeric ion channels. In summary, there are definitive, selective agonists and antagonists for all of the ARs and some of the P2YRs; while the pharmacology of P2XRs is still in nascent stages. The therapeutic potential of selectively modulating these receptors is continuing to gain interest in such fields as cancer, inflammation, pain, diabetes, ischemic protection and many other conditions. Reported potencies refer to the human receptors unless otherwise noted. Additional affinity data can be found in recent review papers (Müller and Jacobson, 2011; Jacobson et al., 2015; Coddou et al., 2011a).

Graphical Abstract

*Address correspondence to: Bldg. 8A, Rm. B1A-19, Bethesda, MD 20892-0810 USA, Tel.: 1-301-496-9024, Fax: 1-301-480-8422. kennethj@helix.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

ATP; nucleosides; nucleotides; GPCR; ion channel; agonists; antagonists

1. Introduction

The effects of extracellular purines and pyrimidines at their receptors in the central and peripheral nervous systems have been under intense research scrutiny. Tools that can be used in this effort, in addition to genetic knock-out or knock-down of receptor expression include a vast collection of directly acting agonists and antagonists, allosteric modulators of the receptors, and indirect modulators that affect the level of endogenous agonists present. This review will concentrate on selective agonists and antagonists of the adenosine receptors (ARs), P2Y receptors (P2YRs) and P2X receptors (P2XRs), and in particular compounds that are readily available to the research community. The principle endogenous agonists are adenosine for the ARs and ATP for the P2XRs, while at the P2YRs a variety of adenine and uracil nucleotides have been shown to be native activators. These native P2YR agonists include ATP, ADP, UTP, UDP, UDP-sugars and some dinucleoside polyphosphates. Some compounds that might be even more selective than the ones discussed here might be under development, but they are not treated in the present work in detail. Reported potencies refer to the human receptors unless otherwise noted.

2. AR modulators

Numerous selective agonists of the four subtypes of ARs (A₁, A_{2A}, A_{2B} and A₃ ARs, Table 1) and their precursors have been used in studies of the nervous system (Chen *et al.*, 2013), and a selection of the many ligand analogues, both directly acting agonists (2 – 21), antagonists (31 – 63), and indirect modulators (26 – 31), is presented here (Figures 1 and 2). Adenosine itself **1** is a native, nonselective AR agonist that is short-lived in the body; while its metabolite inosine **2**, following the action of adenosine deaminase, weakly activates the A₃AR (Gao *et al.*, 2011). A hybrid molecule, abbreviated NECI, resembling both inosine

and the potent nonselective agonist NECA (adenosine 5'-N-ethyluronamide) was shown to have enhanced affinity at the A₃AR (van Galen *et al.*, 1994). Extracellular adenosine is produced indirectly from ATP **4** via AMP **3** by the sequential action of ectonucleotidases. Thus, release of ATP and other nucleotides under stress conditions generally results in increased AR activation. Nucleotides such as ATP **4** are generally inactive at ARs, although AR agonist effects have been ascribed to AMP **3**, either as an intact molecule or as a ready precursor for locally produced adenosine through the action of ecto-5'-nucleotidase (Bhattarai *et al.*, 2015). Other studies indicate that direct AR activation by AMP itself would not occur at sub- μ M concentrations (van Galen *et al.*, 1994). Regadenoson (CVT-3146), a selective agonist of the A_{2A}AR used to induce stress in cardiac imaging, and istradefylline (KW-6002), a xanthine antagonist of the A_{2A}AR is approved in Japan for treating Parkinson's disease.

2.1. AR agonists

AR agonists, particularly those selective for A₁AR or A₃AR, have shown neuroprotective effects in stroke and other models (Zylka *et al.*, 2011; Fishman *et al.*, 2012; Rivera-Oliver and Díaz-Ríos, 2014). However, the precursor nucleotide molecules such as ATP can be damaging in neuroprotective models by activating P2Rs, and it is more commonly observed that P2Y or P2X antagonists are more protective than P2 agonists. The native agonist adenosine **1** is short lived in vivo, and therefore it has only limited use in models of neuroprotection. However, most of the synthetic analogues of adenosine shown in Figure 1 are more stable biologically and suitable for in vivo administration. All of the AR knockout mice have been generated and none are lethal.

AR subtype selective agonists are generally synthetic adenosine derivatives that are long lasting metabolically.

2.1.1. A₁AR agonists—A₁AR agonists tend to be substituted at the N⁶ position with arylalkyl (**5**), cycloalkyl (**6** – **9**), bicycloalkyl (**10**), or aryl (**11**, **12**) substituents (Müller and Jacobson, 2011). The C2 and 5' positions also may be substituted with Cl, **7** and **9**, respectively, or the 5' position with cyclic moieties (**12**). Compound **5** was one of the first AR agonists to be widely used. It was previously defined as a moderately A₁AR selective agonist, but its use in that capacity is discouraged in favor of more selective nucleosides. A₁AR agonists **6a** and **6b** are widely used pharmacological probes with **6b** being more selective for the A₁AR. A cautionary note is that many of the selective A₁AR agonists used routinely such as **6a** and **6b** have considerable activity at the A₃AR. Compound **6b** and agents that increased endogenous adenosine mimicked the acute antinociceptive effect of acupuncture, consistent with the hypothesis that the A₁AR mediates this effect (Goldman *et al.*, 2010). The agonist INO-8875 **7** (also known as Trabodenoson), which is an A₁ agonist in Phase III clinical trials for glaucoma, is also intended for optic neuropathy.

A₁-selective nucleoside derivative **8** failed to show efficacy in a clinical trial for dental pain and was tested in a Phase II trial for postherpetic neuralgia or peripheral nerve injury before being discontinued (Elzein and Zablocki, 2008). A₁-selective nonnucleoside derivative capadenoson (BAY68-4986) **9** is in trials for treatment of persistent atrial fibrillation and is

now available as a research tool (Tendera *et al.*, 2012). Compound **10** is highly selective for the A₁AR and displayed analgesic activity in the formalin test in mice (Luongo *et al.*, 2012, Franchetti *et al.*, 2009). Compound **11** is a peripherally-selective agonist, due to its permanently charged sulfonate group, that is moderately A₁AR selective (70-fold). Compound **12** was tested in a clinical trial for peripheral neuropathic pain but discontinued (Ochoa-Cortes *et al.*, 2014). Compound **13** displayed antinociceptive effects in mice and was suggested to cross the blood brain barrier and act directly through the A₁AR despite the 5'-phosphate group (Korboukh *et al.*, 2012). Partial agonists of the A₁AR (such as the 2'-deoxy analogue of **6**) are effective in chronic neuropathic but not acute pain models and lack some of the cardiovascular side effects of full A₁AR agonists (Schaddelee *et al.*, 2005). Compound **14** is a moderately selective full A₁AR agonist that protects in some seizure models (Tosh *et al.*, 2012b). It is well tolerated in rodents and does not produce the toxicity upon dose escalation that is typical of more widely used A₁AR agonists, likely from cardiovascular effects. Compound **14** was shown to induce anti-depressant effects through an increase of homer1a in the brain (Serchov *et al.*, 2015).

2.1.2. A_{2A}-selective agonists—A_{2A}AR agonists are often substituted at the C2 position with arylalkyl (**15**) or ethynyl (**16**) substituents. Compound **15** is a widely used pharmacological probe for activation of the A_{2A}AR, but its high AR subtype selectivity seen in rat and mouse is reduced at human (h) ARs, and its degree of entry into the brain is low. A single intrathecal injection of **15** or **16** was reported to have a long duration of protection against mechanical allodynia and thermal hyperalgesia in a model of chronic constriction injury (CCI) in mice (Loram *et al.*, 2009). An X-ray structure of the hA_{2A}AR complex with agonist **15** was recently reported (Lebon *et al.*, 2015). Another A_{2A}AR agonist BVT.115959 (structure not disclosed) advanced to clinical trials for pain (Ochoa-Cortes *et al.*, 2014). Compound **17** is a selective A_{2A}AR agonist that was in clinical trials for COPD, which were discontinued due to lack of efficacy. With its large molecular weight and many H-bonding groups, it is not orally bioavailable. However, these molecular features increase the stability of the A_{2A}AR complex, such that it was possible to obtain an X-ray crystallographic structure without extensive stabilizing point mutations (Xu *et al.*, 2011). This was the first X-ray structure of an AR complex with an agonist to be reported. Although not detected in the X-ray structures, the A_{2A}AR can form in situ various functional homo- and hetero- (e.g. with the A₁AR or the D₂ dopamine receptor) di- or multimers (Ferré *et al.*, 2014; Bonaventura *et al.*, 2015; Navarro *et al.*, 2015). The A₁AR can also heterodimerize with the P2Y₁R.

2.1.3 A_{2B}-selective agonists—Compound **18** has been used as a nonnucleoside agonist for selective activation of the A_{2B}AR, but its potency, selectivity and efficacy are less than originally reported (Wessam *et al.*, 2015; Hinz *et al.*, 2014). Thus, it should be used cautiously and in combination with appropriate antagonists. **18** is of the same chemical series (3,5-dicyanopyridines) as selective A₁AR agonist **9**.

2.1.4. A₃-selective agonists—A₃AR agonists (**19** – **24**) are generally adenosine derivatives substituted at the C2, N⁶ and 5' positions with various substituents. Those groups most favorable for A₃AR selectivity include: N⁶ - benzyl (**19**, **20**, **22** – **24**) or small alkyl

(**21**); C2 - H, Cl, alkynyl or arylethynyl. Compounds **19** (also known as Piclidenoson) and **20** are widely used pharmacological probes with **20** being more selective for the A₃AR, and they are in clinical trials for inflammation (rheumatoid arthritis and psoriasis) and primary liver cancer, respectively (Fishman *et al.*, 2012). Compound **21** has been used as a highly selective A₃AR radioligand of high affinity (Klotz *et al.*, 2007). The actions of A₃AR agonists **19** and **24** in chronic neuropathic pain and cerebroprotection have been described (Little *et al.*, 2015). They completely reverse or prevent allodynia and hyperalgesia in the chronic constriction injury (CCI) model but have no effect on acute pain. Compounds **22** – **24** contain a conformationally constrained bicyclic ring in place of ribose with a North conformation (*N*)-methanocarba ring system, which adds to the A₃AR selectivity (Tosh *et al.*, 2012a, 2014, 2015). Compound **23** is a peripherally-selective agonist that is highly A₃AR selective (Paoletta *et al.*, 2013). The pharmacokinetics of A₃AR agonist **24**, administered intraperitoneally in rat, indicates a t_{1/2} of 1.09 h (Tosh *et al.*, 2015), consistent with its duration of action in vivo in the CCI pain model. **24** is also orally active in the CCI model.

2.2. Positive allosteric modulation of AR function

Compounds that indirectly modulate the action or levels of extracellular adenosine include: inhibitors of adenosine deaminase (e.g. Pentostatin, **25**); inhibitors of adenosine kinase (e.g. nucleoside 5-iodotubercidin, **26** and nonnucleoside ABT-702, **27**); and positive allosteric modulators (PAMs, e.g. A₁AR-selective T-26, **28** and TRR469, **29**) (Müller *et al.*, 2012). Compound **27** decreased both chronic and acute pain and acted when administered either peripherally or centrally (Kowaluk *et al.*, 2000). It advanced toward clinical trials but the process was discontinued. Compound **29** suppressed pain in a manner comparable to and additive with morphine in formalin and writhing tests and had an antiallodynic effect in the streptozotocin-induced model of diabetic neuropathic pain (Vincenzi *et al.*, 2014). The structure of **29** was derived from the prototypical enhancer 2-amino-3-benzoylthiophene PD-81,723 (structure not shown). The combination of A₁AR enhancer **29** and agonist **6b** inhibited the release of excitatory amino acid analogue D-aspartate in spinal cord synaptosomes. A positive allosteric modulator of the hA₃AR **30** was shown to enhance the actions of both adenosine and inosine at this subtype (Gao *et al.*, 2011).

2.3. AR antagonists

AR antagonists are shown in Figure 2. Various AR antagonists, in particular those selective for A_{2A}AR, have shown neuroprotective effects in models of Parkinson's and Alzheimer's disease and some other models (Navarro *et al.*, 2015; Rivera-Oliver M and Díaz-Ríos, 2014; Armentero *et al.*, 2011; Chen, 2014). Selective AR antagonists have been used as tool compounds in studies of neurodegeneration and pain. In general, the affinity of selective AR antagonists at the other AR subtypes and species differences should be taken into consideration when selecting a dose (Müller and Jacobson, 2011; Alnouri *et al.*, 2015).

The widely ingested alkylxanthines theophylline **31** (1,3-dimethylxanthine) and caffeine **32** (1,3,7-trimethylxanthine) at physiological concentrations are weak (affinity ~10 µM) nonselective antagonists the ARs, except that the affinity is particularly low at rodent A₃AR. The main caffeine metabolite paraxanthine **33** (1,7-dimethylxanthine) also acts as a central

nervous stimulant and with even higher potency than caffeine at ARs and might be useful for treating hypersomnia associated with neurodegenerative diseases (Okuro *et al.*, 2010). 8-p-Sulphophenyl derivatives **34** and **35** are moderately potent nonselective antagonists of ARs with the added benefits of water solubility and restricted entry to the brain.

2.3.1. A₁-selective antagonists—A₁AR-selective antagonists are derived from nonxanthines, e.g. adenine derivative **36** and pyrrolopyrimidine **37**. An 8-cycloalkylxanthine derivative **38** is the most widely used A₁AR-selective antagonist with nM affinity, but its A₁AR selectivity is greater in rodents than in human. Several 8-bicycloalkylxanthine derivatives **39** and **40** are even more selective for the A₁AR.

2.3.2. A_{2A}-selective antagonists—An 8-styrylxanthine derivative **41** is approved for Parkinson's disease treatment in Japan. The related compound **42** is selective for the A_{2A}AR, but also inhibits monoamino oxidase-2 (MAO-2), two activities that would be beneficial in neurodegenerative diseases. Two A_{2A}AR-selective antagonists, **48** and **51**, are commonly used as A_{2A}AR-selective pharmacological probes of nM affinity. The X-ray structure of A_{2A}AR complex with antagonist **48** was the first antagonist-bound AR structure to be reported (Jaakola *et al.*, 2008), and now a higher resolution structure of 1.8 Å resolution is available (Liu *et al.*, 2012). In the same family as **51** is compound **50**, which was in clinical trials for treatment of Parkinson's disease. A_{2A}AR-selective antagonists **46** and **47** were also in clinical trials for the same condition. The A_{2A}AR affinity of **51** was initially reported in the subnanomolar range, but the K_i value was redetermined to be 4 nM (Müller and Jacobson, 2011). The A_{2A}AR-selective adenine derivative **52**, with a relatively low molecular weight, is effective in Parkinson's disease models. A_{2A}AR-selective antagonists are also of interest in the context of checkpoint blockade for cancer immunotherapy, possibly by coadministration with other anticancer agents (Leone *et al.*, 2015).

2.3.3. A_{2B}-selective antagonists—Compounds **53–58** are A_{2B}AR-selective antagonists (Müller and Jacobson, 2011), and in general the affinity among these xanthine derivatives is greater at the human A_{2B}AR than at the rodent A_{2B}AR. The first A_{2B}AR-selective antagonists to be reported were **53** and **54**. Subsequently, other 1,3-dialkylxanthines with 8-aryl substitution **55–58**, including the highly water-soluble derivative **57**, were found to be A_{2B}AR-selective (Borrmann *et al.*, 2009). Compounds **53** and **54** were introduced by the pharmaceutical industry for possible application to asthma, but neither remains on a clinical path.

2.3.4. A₃-selective antagonists—An effective and moderately selective A₃AR antagonist for use in mouse and rat is **62** (Li *et al.*, 1998; Kreckler *et al.*, 2006). Various other heterocyclic antagonists of the A₃AR, such as MRS1191 **59a**, MRS1334 **59b**, MRS1220 **60**, MRS3008-F20 **61**, PSB-10 **63a** and PSB-11 **63b**, in most cases have much higher affinity at the human than the murine A₃ARs and should be used cautiously in pharmacological experiments utilizing rodents.

2.3.5. Probes for studying ARs—Although radioligand binding assays are most commonly used in the discovery of new ligands, the use of fluorescent AR agonists and antagonists is increasing (Kozma *et al.*, 2013; Stoddart *et al.*, 2015). Other specialized tool compounds for ARs include irreversibly binding agonists and antagonists (Jacobson *et al.*, 1989; Shryock *et al.*, 1998), which have been used to detect receptor reserve.

2.4. Co-crystal structures of the A_{2A}AR with ligands

The only one of the ARs to be probed extensively using X-ray crystallography is the A_{2A}AR. There are currently 14 crystal structures of the human A_{2A}AR, including four structures of the complex with the selective antagonist ZM241385 **48** (Xu *et al.*, 2011; Liu *et al.*, 2012; Lebon *et al.*, 2015; Congreve *et al.*, 2015). The receptor has been crystallized with a various agonists and both high and low affinity antagonists using receptor constructs that are stabilized by mutagenesis at multiple single sites. The X-ray crystal structures are useful for structure-based studies at ARs leading to the design of new ligands and for the modeling of closely related GPCRs, i.e. the other three ARs (Paoletta *et al.*, 2013; Tosh *et al.*, 2012a). For example, two of the A_{2A}AR antagonist-bound crystal structures are results of the application of the receptor structure for the screening and identification of novel triazine-based ligands, one of which has entered a clinical path for treating attention deficit/hyperactivity disorder (Congreve *et al.*, 2015).

3. P2YR modulators

The eight subtypes (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, P2Y₁₂, P2Y₁₃, and P2Y₁₄) of P2YRs are gaining attention in studies of the nervous system (Weisman *et al.*, 2012; Puchałowicz *et al.*, 2014; Brunschweiler *et al.*, 2006). All of the P2YR knockout mice have been generated (and some double P2YR knockouts) and studied except for the P2Y₁₁R, which appears to be absent in the mouse (Table 2). P2YRs have been found to regulate the adaptation of the central nervous system to ischemia, tissue damage, inflammation and chronic neurodegenerative diseases (Burnstock and Verkhratsky, 2012). Various P2YR ligands were studied for their effects in models of neuropathic pain and migraine (Andó *et al.*, 2010; Magni and Ceruti, 2013). This section will focus on the currently available agonists and antagonist for these receptors. Ligand discovery at the P2YRs has lagged behind the discovery of AR ligands, but recently many agonist and antagonist ligands have been reported. Nevertheless, not all of the P2YR subtypes have selective agonists and antagonists; thus, in order to characterize the P2YR subtype(s) involved in a particular phenomenon, pharmacological studies often require sequential use of various agonists (**64 – 85**, Figure 3) and antagonists (**86 – 109**, Figure 4) that may not be definitive for a single P2YR subtype. Some of the compounds are limited in their application due to their enzymatic lability in biological systems and restricted bioavailability. Thus, there remains a need to introduce a wider range of P2YR ligands of diverse structure and pharmacological selectivity. A few P2YR drugs are approved for clinical use. In addition to three P2Y₁₂R antagonists that are widely used antithrombotics, a nonselective agonist of the P2Y₂R is used clinically in Japan for treating dry eye disease.

3.1. Physiological P2YR agonists

The native agonists include: ATP, **4**; ADP, **64**; UTP, **72**; UDP, **78**; UDP-glucose (UDPG), **84**. These nucleotides are generally short-lived in the body, and thus the interconversion (including enzymatically mediated formation and deactivation) of P2YR agonists in situ has been a complicating factor from the outset of studies of P2YRs and purine/pyrimidine receptors in general. Various inhibitors of enzymes that process extracellular nucleotides have been reported recently (Bhattarai et al., 2015; Lee et al., 2015; Chang et al., 2014; Al-Rashida and Iqbal, 2014; Baqi, 2015; Corbelini et al., 2015), according to the strategy that the activation of P2Rs (and ARs) can be modulated effectively by indirect means (Zimmermann et al., 2012).

The receptor subtypes that are preferentially activated by 5'-diphosphates are: P2Y₁, P2Y₁₂ and P2Y₁₃ (ADP); and P2Y₆ (UDP) receptors. The P2Y₁₄R is dually activated by UDP-sugars, such as UDP-glucose (**84**), and also by UDP **78**, although this combination of agonists was not recognized at first as the cloning of this receptor concluded that only UDP-sugars are active (Chambers *et al.*, 2000). Only later, was the ability of UDP to activate the P2Y₁₄R established (Carter *et al.*, 2009a). The receptor subtypes that are preferentially activated by 5'-triphosphates are: P2Y₂ and P2Y₄ and P2Y₁₁. ATP **4** acts at all three subtypes, and furthermore UTP **72** activates the P2Y₂ and P2Y₄Rs. The P2Y₄R has a functional difference between species that causes ATP **4** and its derivatives to act as partial agonists (or antagonists) at the human homologue and full agonists at the rat homologue (Herold *et al.*, 2004). Various naturally occurring dinucleotides also activate P2YRs, i.e. the asymmetric dinucleoside tetraphosphate Up₄A (Jankowski *et al.*, 2005), which is a vasoconstrictor, and a range of symmetric diadenosine polyphosphates Ap_xA (Jankowski *et al.*, 2003).

3.2. P2Y₁R ligands

3.2.1. P2Y₁R agonists—The P2Y₁R is activated by adenine, but not uracil nucleotides. A 2-methylthioadenosine-5'-diphosphate analogue **68** that has a rigid substitution of the ribose ring in the form of a (N)-methanocarba ring system, has been shown to be a potent and selective agonist of the P2Y₁R in comparison to the P2Y₁₂R and P2Y₁₃R (Chhatiwala et al., 2004). This ring maintains a P2Y₁R-preferred conformation, which is responsible for the high potency and selectivity. However, the potent 2-methylthio-substituted agonist **65**, 2-MeSADP (riboside), which is widely used in pharmacological studies, acts at all three subtypes. Naturally occurring dinucleotides **70** and **71** (dinucleoside tetraphosphates) activate the P2Y₁R, but not selectively (Jankowski *et al.*, 2009; Durnin *et al.*, 2014; Yelovitch *et al.*, 2012). For example, Ap₄A **70** also activates the P2Y₂R (former known as P_{2U}; Lazarowski *et al.*, 1995). The biology of such dinucleotides in vasodilation, vasoconstriction and other cardiovascular activities has been explored. The concentration of Ap₄U **71** in human plasma is ~56 nM, which is relevant to activation of P2YRs (Jankowski *et al.*, 2009). Ap₄U also activates the P2Y₂R (Wiedon *et al.*, 2012).

3.2.2. P2Y₁R antagonists—Antagonists of the P2Y₁R have been sought as potential anti-thrombotic agents, although none have been approved for clinical use. P2Y₁R antagonists include both nucleotides, e.g. **86** – **88** (Jacobson *et al.*, 2015), and

nonnucleotides, e.g. **89**, which represents a large group of diarylurea derivatives reported in the past several years. The urea derivative **89**, which is commercially available, has a K_i value of 6 nM at the P2Y₁R (Chao *et al.*, 2013); it and many of its analogues tend to be hydrophobic in nature. On the other hand, very high affinity has been achieved in the hydrophilic series of nucleotides. The principle by which nucleotide derivatives antagonize the P2Y₁R instead of activating it involves formalistically splitting the 5'-diphosphate and attaching a monophosphate group as a phosphoester at the 3'-position, removal of the 2'-hydroxyl group, methylation of the exocyclic amino group and other modifications (Boyer *et al.*, 1996; Jacobson *et al.*, 2015). All of these modifications contribute to the loss of efficacy in this chemical series, and the antagonism of the bisphosphates applies to P2Y₁R homologues species-independently. The most potent nucleotide antagonist of the P2Y₁R **88**, which also contains a potency-enhancing (*N*)-methanocarba ring system, has subnanomolar affinity. Thus, the (*N*)-conformation of the ribose-like ring at the P2Y₁R is a requirement for both nucleotide agonists and antagonists. A corresponding South conformation (*S*)-isomer in the bisphosphate series is greatly reduced in affinity (Jacobson *et al.*, 2015).

While, in general, 5'-diphosphates are more potent and efficacious, ATP **4** acts as a partial agonist or agonist, respectively, at the P2Y₁R and P2Y₁₂R (Waldo and Harden, 2004; Paoletta *et al.*, 2015). ATP- γ -S **67** is also an agonist at various P2YRs, including the P2Y₁R (Waldo and Harden, 2004). The γ -thiophosphate group impedes (Malmjö *et al.*, 2003), but does not completely prevent its enzymatic hydrolysis. A caged form of ATP **69**, which regenerates ATP upon irradiation has been studied and is potentially useful for studies of P2Rs (Amatrudo *et al.*, 2015). Similarly, the 1-(3,4-dimethyloxy-6-nitro-phenyl)ethyl (DMNPE) group was used to block the β -phosphate of agonist **65** to abolish its activity at the P2Y₁R and P2Y₁₂R, and this activity could be recovered upon irradiation (Gao *et al.*, 2008). Such caged compounds are potentially useful for studying rapid neuronal responses to P2Y (or P2X) receptor activation.

P2Y₁R is present on both astrocytes and neurons in the brain. P2Y₁R activation produces anxiolytic effects in the rat elevated plus-maze (Kittner *et al.*, 2004). The P2Y₁R is a possible target for the treatment of traumatic brain injury (Choo *et al.*, 2013). An astrocytic P2Y₁R was found to be hyperactivated in an Alzheimer's disease model in the mouse (Delekate *et al.*, 2014).

3.3. P2Y₂ and P2Y₄ receptor ligands

Both UTP **72** and UTP- γ -S **74** activate the P2Y₂R and P2Y₄R, and the γ -thiophosphate group of **74** increases stability toward ectonucleotidases. However, the γ -thiophosphate group of **74** and **67** introduces chemical lability toward oxidation when left exposed to the air. Compound **75** is a potent and selective P2Y₂R agonist that contains a triphosphate mimic consisting of a β,γ -dihalomethylene bridge. δ -Blocked 5'-tetraphosphate derivative **76** is a selective P2Y₂R agonist with moderate (μ M) potency. Few effective P2Y₂R antagonists and none of nM affinity are readily available. A complex derivative of 4-thiouracil **91** is a P2Y₂R antagonist with μ M potency (Kemp *et al.*, 2004). There are no selective P2Y₄R antagonists, but a number of P2Y₄R agonists were reported, of which **77** is representative. There is a tolerance for steric bulk at P2Y₂R, P2Y₄R and P2Y₆R in the receptor-bound

uracil nucleotides that have an alkyloximino group at the 4-position of the nucleobase (Jacobson *et al.*, 2015). Thus, they are more properly cytosine derivatives; 5'-CTP itself is only weakly active at the rat P2Y₂R and antagonizes the hP2Y₄R (Kennedy *et al.*, 2000). The precise selectivity in this chemical series of 5'-di- and triphosphates among these three P2YR subtypes can be largely modulated by structural changes of the 4-alkyloximino group (Jacobson *et al.*, 2015). Nonselective dinucleotide P2Y₂R/P2Y₄R agonist diquafosol (INS365, Up₄U) was approved in Japan (2010) for dry eye disease. Another nonselective dinucleotide P2Y₂R/P2Y₄R agonist denufosol (INS37217, Up₄dC) has been evaluated in phase III trials for bronchial indications, but was not approved.

3.4. P2Y₆ receptor ligands

Numerous derivatives of UDP, e.g. **78** – **81**, have shown selectivity as agonists of the P2Y₆R. Certain dinucleotides (dinucleoside triphosphates), e.g. **82**, potently activate the P2Y₆R (Shaver *et al.*, 2005). This dinucleotide also contains a 4-alkoxyimino group on one of the two pyrimidine nucleobases and is highly potent, but only moderately selective. Only one compound **92** is currently used as a selective antagonist of the P2Y₆R. Unfortunately, it is poorly soluble in water and of limited chemical stability due to its two isothiocyanate groups, which are essential for its antagonist activity (Mamedova *et al.*, 2004). UDP activates the P2Y₆R in microglial cells to induce phagocytosis (Koizumi *et al.*, 2007). A novel P2Y₆R agonist protects glial cells against apoptosis (Haas *et al.*, 2015). High affinity P2Y₆R fluorescent agonists MRS4129 (selective) and MRS4162 (also potent at P2Y₂R, P2Y₄R) are useful in flow cytometry (Jayasekara *et al.*, 2014).

3.5. P2Y₁₁ receptor ligands

Both ATP and ATP-γ-S **74** activate the P2Y₁₁R, with the latter being more potent, but not selective. Recently, β,γ-dichloromethylene analogues of a 2-substituted derivative of ATP were reported to be agonists of the P2Y₁₁R (Haas *et al.*, 2013). A selective nonnucleotide agonist NF546 **83** of the P2Y₁₁R is available (Meis *et al.*, 2011). Curiously, this derivative of the nonselective P2R antagonist suramin **96** activates, rather than antagonizes the P2Y₁₁R. Other derivatives of highly charged suramin antagonize the P2Y₁₁R: **97** and **98**.

3.6. P2Y₁₂ receptor ligands

A huge number of nucleotide and nonnucleotide antagonists of the P2Y₁₂R have been reported (Paoletta *et al.*, 2015). The reason for this focus on P2Y₁₂R antagonists is their value as antithrombotic agents. They include: β,γ-dihalomethylene bridged 5'-triphosphate mimics (**93**, **94**); uncharged nucleotide like derivative **95** that is now used clinically (ticagrelor); sulfonated anthraquinone derivatives, e.g. **99**; uncharged heterocycles **104** (previously in clinical trials as an antithrombotic) and **108** (commercially available). Ticagrelor **98** is the first competitive P2Y₁₂R antagonist approved as an antithrombotic drug and was the product of a long program in pharma industry to circumvent the need for anionic groups in such antagonists (Springthorpe *et al.*, 2007; Hoffman *et al.*, 2014). The X-ray structure of the P2Y₁₂R complexes with nonnucleotide antagonist **108** and with nucleotide agonist **65** and partial agonist **66** were recently reported (Zhang *et al.*, 2014a, 2014b). These structures showed dramatic changes in conformation of the extracellular

regions, in comparing forms with nonnucleotide or nucleotides bound. The nucleotides-bound structures are greatly contracted in the upper regions surrounding the ligand, with the highly positively charged extracellular loops forming electrostatic coordination with the phosphate groups. Thienopyridine P2Y₁₂R antagonists clopidogrel **100** and prasugrel **102** are widely used antithrombotic agents, which must be preactivated by enzymatic transformation in the liver to active metabolites, **101** and **103**, respectively. The active metabolites have limited chemical stability, but compound **103** is available commercially. Radiolabeled nucleotide agonist **65** is a suitable tracer for binding studies in cells overexpressing the P2Y₁₂R, and it can also serve as an agonist radioligand for the P2Y₁R (Zhang *et al.*, 2014a, 2015). Radiolabeled **93**, known as [³H]PSB-0413, is a selective nucleotide antagonist radioligand for the P2Y₁₂R (Ohlmann *et al.*, 2013).

ADP activates the P2Y₁₂R in microglial cells to induce chemotaxis (Haynes *et al.*, 2006). Thus, it serves as a “find me” signal, while UDP acts as an “eat me” signal. P2Y₁₂R antagonists are a target for control of inflammatory and chronic neuropathic pain (Horváth *et al.*, 2014).

3.7. P2Y₁₃ receptor ligands

Highly selective P2Y₁₃R agonists and antagonists are unknown, but various nucleotide antagonists of the P2Y₁₂R, e.g. **93** and **94**, also activate the P2Y₁₃R. At the P2Y₁₃R, one compound **107** is currently used as a selective antagonist. This diazo derivative of pyridoxal phosphate is closely related to the disulfonate derivatives **105** and **106**, which are useful antagonists at various P2YRs and P2XRs.

3.8. P2Y₁₄ receptor ligands

Nonnucleotide antagonists of the P2Y₁₄R have been reported (Robichaud *et al.*, 2011). One naphthoic acid derivative **109** has been useful in pharmacological studies, although this chemical series was rejected for development by the pharmaceutical industry due to a lack of oral bioavailability. Compound **109** displays high selectivity for the P2Y₁₄R in comparison to all other P2YRs (Barrett *et al.*, 2013). Because of its low absorption, attempts were made to design prodrugs of **109** for in vivo use (Robichaud *et al.*, 2011). Other analogues of compound **109** were reported to have potent antagonist activity at the P2Y₁₄R, including MRS4174, a fluorescent conjugate of AlexaFluor488 (structure not shown, K_i 0.8 nM). MRS4174 is a useful tracer for flow cytometric characterization of binding to the P2Y₁₄R (Kiselev *et al.*, 2014). The SAR of nucleotide derivatives at the P2Y₁₄R has been extensively explored, including the report of a high affinity P2Y₁₄R fluorescent agonist MRS4183 (structure not shown, Kiselev *et al.*, 2015). This fluorescent agonist can be prepared from available starting materials in a single reaction step.

3.9. Co-crystal structures of P2YR with ligands

The X-ray structure of the P2Y₁R complexes with antagonists **88** and **89** were recently reported (Zhang *et al.*, 2015). Unexpectedly, both of these ligands bound at sites distal from the conventional binding region for small molecules deep in the TM helical domain. Nucleotide **88** bound to a more exofacial site in close contact with the extracellular loops, and nonnucleotide **89** bound on the outer surface of the receptor in contact with the

phospholipid bilayer. The locations of the urea derivative caused a reexamination of the assumption that it was a competitive inhibitor. It proved to be allosteric with respect to agonist **65**, which was used as a radioligand in dissociation kinetic experiments in conjunction with site-directed mutagenesis. The binding sites on the P2Y₁R of **88** and **89** were completely distinguishable by mutagenesis studies, consistent with the X-ray structures. Another apparently allosteric P2Y₁R inhibitor is 2,2'-pyridylisatogen tosylate (PIT, **90**, Gao *et al.*, 2004), although this was not shown using mutant P2Y₁Rs, and the site for its binding is undetermined.

Docking of nucleotides in a homology model of the P2Y₁₄R, based on the X-ray structure of the P2Y₁₂R in complex with nucleotide agonist **65**, was consistent with the observed SAR (Trujillo *et al.*, 2015). Also, both forms of X-ray structures of the P2Y₁₂R have been helpful in characterizing the specific protein-ligand interactions for a wide variety of P2Y₁₂R agonists and antagonists (Paoletta *et al.*, 2015). This suggested that the new P2YR X-ray structures are suitable templates for structure-based design of new ligands. The possibility of hetero- and homodimerization of P2YRs has not yet been addressed structurally with X-ray crystallography, but there is pharmacological support for this phenomenon, e.g. in P2Y₁R/P2Y₁₁R heterodimers (Ecke *et al.*, 2008).

4. P2XR modulators

P2XRs are ion channels permeable for Na⁺, K⁺ and Ca²⁺ which are activated by ATP (Coddou *et al.*, 2011a). They show a wide distribution in the body. A variety of orthosteric and allosteric ligands for P2X receptors have been reported, some of which are highly charged molecules and therefore not orally bioavailable, but chemically diverse classes of drug-like P2X antagonists are under development (Gunosewoyo and Kassiou, 2010). The P2XR family in mammals consists of seven different subunits, P2X1–P2X7, that form homo- or heterotrimeric channels (Hausmann *et al.*, 2015). There are currently no ligands that are highly selective for different combinations of P2X subunits. Homomeric P2X5R and P2X6R do not seem functional in humans. P2X2/3R heteromers represent a well established heteromeric P2XR subtype expressed in the dorsal root ganglia. P2XRs are involved in nerve transmission, pain sensation and inflammation (Coddou *et al.*, 2011a; Di Virgilio, 2015; Bele & Fabbretti, 2015; Burnstock, 2015). Prolonged receptor activation can lead to pore formation, and the cells become permeable for large molecules (molecular mass up to ca. 1000 Da). This has been especially observed for the P2X7R (Di Virgilio, 2007). That receptor subtype is also exceptional insofar as it requires high, millimolar ATP concentrations to be activated, while the other subtypes are typically activated at low micromolar or high nanomolar concentrations of ATP (see Table 3).

4.1. X-ray structures, mutagenesis and modeling studies

Two X-ray structures of the zebrafish P2X4R with high resolution were published, one in the closed state, and one with ATP bound (Kawate *et al.*, 2009; Hattori *et al.*, 2012; Grimes & Young, 2015). The previously suggested trimeric structure was confirmed (Nicke *et al.*, 1998). The receptor has a chalice-like structure, and each of the three subunits contains a large rigid ectodomain, two transmembrane domains and intracellular C- and N-termini.

There are three equivalent extracellular ATP-binding sites, in about 40 Å distance from the cell membrane, located at the subunit interfaces of the trimeric receptor. ATP binding induces the opening of the ion channel by bending of the lower part of the receptor thereby expanding the region near the ion channel pore (Hattori et al., 2012). Mutagenesis studies were useful for the identification of key residues important for receptor-ligand interaction, receptor modulation and ion channel function (Hausmann et al., 2015). Subsequent molecular modeling studies have been performed based on experimental data (Dal Ben et al., 2015). It would still be of paramount interest (i) to obtain X-ray structures of the native human P2X4R, and (ii) to identify binding sites of allosteric modulators by determination of X-ray co-crystal structures.

4.2. Allosteric modulation of P2XR by physiological ions, ethanol, and lipids

P2XR function can be allosterically modulated by ions (e.g. Mg^{2+} , Ca^{2+} , Zn^{2+}) (Coddou et al., 2011b; Müller, 2015), steroids (De Roo et al., 2003) and lipids (Bernier et al., 2013), e.g., phosphatidylinositol polyphosphates (e.g., $PI(4,5)P_2$) (Bernier et al., 2008a,b; Mo et al., 2009). The PIPs appear to bind to positively charged amino acids on the cytosolic C-terminal domain. The P2X4R and to some extent also the P2X2R are positively modulated by high concentrations of ethanol (ca. 100 mM) (Yi et al., 2009; Ostrovskaya et al., 2011).

4.3. Orthosteric versus allosteric ligands for P2XR

The binding site of the physiological agonist ATP - the so-called orthosteric binding site - is well conserved in the different P2XR subtypes. It contains five positively charged amino acid residues (one Arg and four Lys) which interact with the triphosphate chain (Chataigneau et al., 2013). All potent agonists known so far that bind to the ATP binding site are negatively charged. Also, many orthosteric antagonists are highly polar compounds which cannot penetrate into the CNS. Therefore, and also because of the fact that numerous ATP binding proteins are found in the body, the development of positive and negative allosteric modulators (PAMs and NAMs) appears to be more promising.

4.4. P2XR agonists

Most of the known P2XR agonists are structurally derived from the physiological agonist ATP (Lambertucci et al., 2015). Agonists with high selectivity for a single subtype are presently not available. ATP (**4**) displays potency in the low micromolar range at all subtypes, except for the P2X7R, which requires millimolar ATP concentrations for activation. 2-Methylthio-ATP (**66**) and γ -thio-ATP (**67**) display a similar profile as ATP, but both compounds are metabolically more stable. α,β -Methylene-ATP (**111**, Figure 5) shows a preference for P2X1 and P2X3Rs with somewhat lower potency at P2X4 and much lower potency at P2X7R, while β,γ -Methylene-ATP (**112**) is most potent at P2X1R and shows only negligible potency at the other subtypes. Benzoyl-ATP (BzATP, **110**) is more potent as compared to ATP with highest potencies at P2X1 and P2X3R subtypes. It should be kept in mind that the activity of allosteric modulators may be probe-dependent, i.e. their potency is likely to depend on the employed agonist (Müller *et al.*, 2012). Therefore, the physiological agonist ATP should preferably be used when characterizing positive or negative allosteric modulators.

4.5. Positive allosteric modulators of P2XRs

Ivermectin (**113**, Figure 5) a macrocyclic lactone used in veterinary medicine as an antiparasitic agent binds with high affinity to glutamate-gated chloride channels in nerve and muscle cells of the parasites and prevents their closure (Omura & Crump, 2004). It is lipophilic, accumulates in cell membranes and penetrates into the CNS. Ivermectin interacts with many different ion channels (Zemkova et al., 2014). It acts as a PAM at P2X4R facilitating the opening and retarding the closing of the channel. Effective concentration are about 100 nM - 3 μ M (Lalo et al., 2007). The exact binding site and mechanism of allosteric enhancement of the P2X4R remain speculative (Müller, 2015). Recently, it was found that ivermectin is also a PAM at the hP2X7R at similar concentrations (3 μ M), but not at rat and mouse P2X7Rs (Nörenberg et al., 2012).

Within several series of antagonists some compounds with positive modulatory activity (PAMs) were discovered. This shows that small modifications can turn an allosteric inhibitor or NAM into a PAM.

MRS2219 (**114**) was found to selectively potentiate ATP-induced responses at recombinant rat P2X1Rs expressed in *Xenopus* oocytes with an EC₅₀ value of 5.9 μ M (Jacobson et al., 1998). Compound **114** was inactive at rat P2X2, P2X3 and P2X4Rs.

Among a series of P2X2 antagonists with an anthraquinone core structure several derivatives were identified that showed positive allosteric modulation. PSB-10129 (**115**) was one of the most potent PAMs leading to a 3-fold maximal increase in the ATP-elicited current with an EC₅₀ value of 489 nM at the hP2X2R (Baqi et al., 2011).

The anthraquinone derivative Cibacron Blue (**116**, Figure 6) acts as a PAM of hP2X3Rs. It showed a 3–7-fold increase in the magnitude and the potency of ATP activating Ca²⁺ influx and transmembrane currents with an EC₅₀ value of 1.4 μ M (Alexander et al., 1999). Compound **116** was also found to be a PAM at the rat P2X4R at concentrations of 3–30 μ M, while it blocked the receptor at high concentrations of 300 μ M (Miller et al., 1998). Thus, the compound is non-selective and may also interact with other P2XR subtypes.

The development of PAMs may be a promising approach for the development of drugs to achieve an increase in P2XR activation.

4.6. Non-selective antagonists

Moderately potent, non-selective P2XR antagonists include suramin (**96**), Reactive Blue 2 (RB-2, **117**), PPADS (**105**), and iso-PPADS (**106**). These compounds are of limited use and should be replaced by more potent and selective antagonists that are now available. The ATP derivative TNP-ATP (**118**) is very potent at P2X1 and P2X3R (low nanomolar IC₅₀ values), and somewhat less potent at P2X2 and P2X4R. It is virtually inactive at P2X7R.

4.7. Effects associated with specific subtypes of P2XRs

Effects, especially those related to CNS activity, are outlined below for P2X1, P2X2, P2X3, P2X4 and P2X7Rs. The effects of P2X5 and P2X6Rs in the nervous system are mostly unexplored; there are no selective ligands for those subtypes.

4.7.1. P2X1R—P2X1Rs are expressed in smooth muscle cells of various organs including arteries, and vas deferens (Mulryan et al., 2000). P2X1 knockout mice were resistant to thromboembolism suggesting the P2X1R to be a potential target for thrombosis and stroke prevention (Hechler & Gachet, 2011). The P2X1R is also expressed in neuronal and glial cells, and a strong upregulation was found after mechanical CNS injury. Inhibition of P2X1Rs resulted in neuroprotective effects after ischemic or toxic CNS injury. Thus, the P2X1R was suggested as a drug target for neuroprotective action (Hausmann et al., 2012). P2X1 antagonists have also been suggested for the treatment of Parkinson's disease since they might reduce alpha-synuclein accumulation (Navarro et al., 2015).

Only few P2X1 antagonists are known so far (Figure 6). These include suramin and derivatives: NF279 (**119**) and NF449 (**120**). Compound **120** is significantly more potent than **119** with subnanomolar potency at P2X1 and very high selectivity. It is therefore the preferred P2X1 antagonist. They appear to be competitive antagonists (Hausmann et al., 2012; El-Ajouz et al., 2012; Soto et al., 1999; Rettinger et al., 2000; Kassack et al., 2004). Their interaction with the receptor has recently been studied by a mutagenesis approach (Farmer et al., 2015).

RO0437626 (RO-1, previously also known as RO116-6446, **121**) is a non-acidic P2X1 antagonist, with moderate potency (IC₅₀ recombinant hP2X1, calcium assay, 3 μM) (Jaime-Figueroa et al., 2005; Ford et al., 2006). It shows selectivity versus hP2X2, hP2X3 and hP2X2/3Rs (IC₅₀ > 100 μM). In a rat model it attenuated bladder contractions at 1 or 10 μmol/kg i.v. (King et al., 2004).

4.7.2. P2X2R—P2X2Rs show a wide distribution in the peripheral and the central nervous system, as well as on many non-neuronal cell. They play a role in sensory transmission and the modulation of synaptic function. P2X2R are slowly desensitized and are the only homomeric P2XR subtype that is potentiated by acids. Potentiation is also observed by Zn²⁺, but other divalent cations inhibit the receptor at high concentrations (Gever et al., 2006). P2X2 subunits form homotrimeric or heterotrimeric channels with P2X3Rs. P2X2R knockout mice displayed a reduced pain response (Cockayne et al., 2005). Potential indications for P2X2 antagonists include pain, and they may also show neuroprotective properties.

Only few selective antagonists for P2X2R have been developed. The anionic standard P2R antagonists PPADS (**105**), RB-2 (**117**), TNP-ATP (**118**) and suramin (**96**) are moderately potent, non-selective P2X2R antagonists. The nucleotide **118** was shown to act as a competitive antagonist while suramin binds to an allosteric site as shown in radioligand binding studies at rat P2X2Rs (Trujillo et al., 2006). The suramin derivative NF770 (**122**, Figure 6) is more potent and selective for P2X2Rs, and evidence was presented that it exhibits a competitive mechanism of action (Wolf et al., 2011). Potent and selective P2X2 antagonists related to the anthraquinone derivative Reactive Blue 2 (**117**) were developed, PSB-10211 (**123**) and PSB-1011 (**124**), which was characterized as a competitive P2X2R inhibitor, although an allosteric mode of action could not completely be excluded (Baqi et al., 2011).

4.7.3. P2X3R—P2X3 subunits may form homomeric or heteromeric (P2X2/3)Rs. They are expressed on nerve cells in the central peripheral nervous system. Particularly high expression is found on small nociceptive sensory and sympathetic neurons. Knock-out mice showed, among other effects, reduced temperature sensation and pain responses (Coddou et al., 2011). P2X3R expression in dorsal root ganglia is upregulated after sciatic nerve ligation in the CCI model. P2X3 knock-out mice are less sensitive to pain stimuli (Jarvis et al., 2003). P2XR antagonists have therefore potential for the treatment of various kinds of pain including neuropathic pain and migraine. They may also be useful for the treatment of epilepsy and sleep disorders.

Many potent, selective and drug-like P2X3R antagonists were developed. Since 2001 more than 50 patents have been filed which claim small molecules as P2X3 and/or P2X2/3R antagonists (Bölcskei & Farkas, 2014; Ford, 2012; Müller, 2010). However, most of the compounds have only been published in the patent literature and only few research reports have appeared so far.

One of the first described selective P2X3 antagonists was A-317491 (**125**, Figure 6), a tricarboxylate, which binds to the ATP site acting as a competitive antagonist (Jarvis et al., 2002). The compound displays low peroral and CNS bioavailability and high plasma-protein binding but may still be useful as a pharmacological tool compound (Sharp et al., 2006).

A series of allosteric antagonists was derived from the drug trimethoprim discovered in a high-throughput screening campaign by Roche (see Müller, 2010). RO-3 (**126**), RO-4 (**127**) and RO-51 (**128**) belong to that series. RO-4 (later named AF-353, **127**) displays IC₅₀ values of 6 nM (hP2X3), 13 nM (rat P2X3) and 25 nM (hP2X2/3), and is selective versus P2X₁, P2X₂, P2X₅, P2X₇ (IC₅₀ > 10 µM) as well as a wide range of other receptors and enzymes. Compound **127** shows peroral bioavailability and penetrates into the brain (Carter et al., 2009b). The compound was obtained in ³H-labeled form and used to label its allosteric binding site (Gever et al., 2010). Compound **127** was further modified resulting in the more potent and polar AF-906 (RO-51, **128**), which showed superior pharmacokinetic properties (Jahangir et al., 2009). AF-906 (**128**) displayed IC₅₀ values of 2 nM (hP2X3) and 5 nM (hP2X2/3). Clinical trials have been performed with another member of the series, AF-219 (structure not disclosed), for chronic, treatment-refractory cough, and the outcome was positive (Ford et al., 2013).

The physiological heptapeptide spinorphin (LVVYPWT, **129**), which inhibits the effect of enkephalin-degrading enzymes resulting in analgesic activity, was reported to act as a potent allosteric antagonist at P2X3Rs. It showed an IC₅₀ value of 8.3 pM in patch clamp studies at the recombinant hP2X3R expressed in *Xenopus* oocytes, and was virtually inactive at the mouse P2X1 and the hP2X7Rs (Jung et al., 2007).

4.7.4. P2X4R—P2X4Rs are widely expressed in the central nervous system and in the periphery, e.g., in microglia, and on endothelial cells. Peripheral nerve injury leads to microglial activation in the spinal cord which results in increased P2X4R levels (Tsuda et al., 2003; Beggs et al., 2012). This process leads to neuropathic pain. P2X4R knockout mice displayed reduced pain and no development of allodynia. P2X4R antagonists are therefore

developed for the treatment of neuropathic pain. Further potential indications include spinal cord injury (de Rivero Vaccari et al., 2012), epilepsy (Ulmann et al., 2013), stroke, multiple sclerosis, and neurodegenerative diseases such as Parkinson's and Alzheimer's (Varma et al., 2009).

The serotonin reuptake inhibitor paroxetine (**130**, Figure 7) was found to act as an allosteric antagonist of P2X4Rs at high concentrations with IC₅₀ values at the rat and the hP2X₄Rs of 2.45 μ M (rat), and 1.87 μ M (human), respectively (Nagata et al., 2009; Müller, 2010). Paroxetine was subsequently investigated in a rat model of neuropathic pain. Intrathecal administration showed an anti-allodynic effect when applied 7 or 14 days after spinal nerve injury. In contrast, the antidepressant amitriptyline which is clinically used for treating neuropathic pain blocked P2X4Rs only weakly (Sim et al., 2010).

The benzodiazepine derivative 5-BDBD (**131**) is a moderately potent (IC₅₀ 0.5 μ M), selective allosteric P2X₄ antagonist (Donnelly-Roberts et al., 2008; Balázs et al., 2013). The compound displays a very low water-solubility and is therefore not easy to handle.

The allosteric P2X₄ antagonist *N*-(benzyloxycarbonyl)phenoxazine (PSB-12054, **132**) exhibited an IC₅₀ of 0.189 μ M at the hP2X₄R, but was less potent at the rat (2.10 μ M) and the mouse P2X₄Rs (1.77 μ M) (Hernandez-Olmos et al., 2012). It showed >50-fold selectivity for the human P2X₄ versus hP2X₂, hP2X₃ and hP2X₇Rs, and >30-fold selectivity versus the hP2X₁R. PSB-12054 is currently one of the most potent antagonists at hP2X₄Rs. The carbamate structure of PSB-12054 was found to be hydrolytically stable. A drawback is its high lipophilicity and moderate water-solubility.

A more water-soluble analogue is PSB-12062 (**133**), the *N*-(*p*-methylphenylsulfonyl)-substituted phenoxazine. It was about similarly potent at human (IC₅₀ 1.38 μ M), rat (0.928 μ M) and mouse P2X₄Rs (1.76 μ M) and showed selectivity versus P2X₁, P2X₃ and P2X₇Rs (Hernandez-Olmos et al., 2012).

A series of carbamazepine derivatives was studied as P2X₄ antagonists (Tian et al., 2014). The most potent derivative was *N,N*-diisopropylcarbamazepine (**134**) displaying an IC₅₀ value at the hP2X₄R of 3.44 μ M. However, the compound was weaker at mouse and rat P2X₄Rs and was not very selective versus hP2X₁ and hP2X₃Rs, whereas it was very selective versus hP2X₂ and hP2X₇Rs.

Recently, a urea derivative, BX-430 (**135**) identified by screening of a compound library as an allosteric antagonist with an IC₅₀ value of 0.54 μ M at the hP2X₄R and selectivity versus the other P2XR subtypes. The compound had no effect on mouse and rat P2X₄Rs (Ase et al., 2015).

Several patents have been filed presenting further P2X₄ antagonists, e.g. by Nippon Chemphar and Kyushu University, Japan.

4.7.5. P2X₇R—The P2X₇R is highly expressed on immune cells, such as macrophages, mast cells, and microglial cells, and can also be found on oligodendrocytes. The receptor is a potential drug target for the treatment of inflammation including neuroinflammatory

diseases, pain (neuropathic, inflammatory, nociceptive and chronic), multiple sclerosis, neurodegenerative disorders, cerebral ischemia, brain and spinal cord injury, depression, anxiety and bipolar disorders (Bartlett et al., 2014; Bartlett & Sluyter, 2014; Gunosewoyo et al., 2009; Navarro et al., 2015; Chrovian et al., 2014). P2X7 ligands may also be effective in the treatment of cancer, including brain cancers (Burnstock & Di Virgilio, 2013). The P2X7 is only activated by high, millimolar ATP concentrations, indicating that it has a high relevance under pathological conditions, and pore formation is typical upon its prolonged activation

In recent years much effort has been invested in the development of selective P2X7R antagonists, which have been reviewed recently (Mehta et al., 2014; Baudalet et al., 2015). Significant species differences were observed for some compounds. Useful P2X7 antagonists for human as well as rodent P2X receptors include A438079 (**136**), A740003 (**137**), A804598 (**138**), A839977 (**139**), AZ1060612 (**140**), AZ11645373 (**141**), and GW791343 (**142**) belonging to different chemical compound classes (see Figure 7). All of the P2X7 antagonists displayed in Figure 7 show potency in the low nanomolar concentration range and high selectivity versus other P2X receptor subtypes.

Several clinical studies have been performed with P2X7R antagonists. Results with AZD9056 (**142**) and CE-224,535 (**145**) (Duplantier et al., 2011) in rheumatoid arthritis were published (Keystone et al., 2012; Stock et al., 2012). Both drugs failed to show a clear benefit for the patients. Related, more lipophilic benzamide derivatives (**143**, **144**) were reported to show brain penetration (Wilkinson et al., 2014). These structures indicate that the P2X7 receptor tolerates very bulky residues, like cycloheptyl, adamantyl and related ring systems. Several other P2X7 antagonists that penetrate well into the brain have recently been described, including GSK1482160 (**146**), which has been prepared in ¹¹C-labeled form to provide a ligand for positron emission tomography (PET) studies (Gao et al., 2015), JNJ-47865567 (**147**) (Letavic et al., 2013; Bhattacharya et al., 2013), JNJ-42253432 (**148**) (Lord et al., 2014) and the triazolopyrazinylmethanone **149** (Rudolph et al., 2015). A novel, useful ³H-labeled antagonist radioligand, [³H]JNJ-54232334 (**150**) has been reported (Lord et al., 2015).

5. Conclusions

Many novel ligands are now available as pharmacological tool compounds to define action at subtypes of the ARs, P2YRs and P2XRs in the nervous system. We have attempted to focus on the most useful agents in this review. The development of SAR at the adenosine receptors, and to a lesser extent at P2Y and P2XRs, has led to therapeutic concepts and experimental agents for treatment of diseases of the nervous system. Some of these compounds, including A₁ and A₃ AR agonists, A_{2A} antagonists, P2Y₁R and P2Y₁₂R antagonists, and P2X₃R, P2X₄R and P2X₇R antagonists, are potentially useful in disorders of the nervous system, such as chronic pain, neurodegeneration and brain injury. Researchers using these compounds should carefully consider the pharmacological properties as reported in primary literature to avoid their improper application. For example, the selectivity defined for some of the AR agonists and antagonists that were deemed selective for the A₁AR or A₃AR has been revised with updated pharmacological characterization. Notably, species

differences occur frequently leading to a reduction in selectivity ratios in certain species. Also, many of the P2R ligands still lack bioavailability due to charged groups or hydrolytic (either enzymatic or chemical) instability.

The ligand development is continuing; thus, there is reason to expect that subtypes that do not yet have definitive agonist or antagonist ligands will have them in coming years. Although very selective ligands already exist for ARs and there are fewer definitive P2XR and P2YR ligands, the therapeutic potential of selectively modulating these receptors is continuing to gain interest in such fields as cancer, inflammation, pain, diabetes, ischemic protection and many other conditions. X-ray crystallographic structures of the AR and P2YR families have shifted the mode of ligand discovery to structure-based approaches rather than previous empirical approaches. The X-ray structures can be utilized either for in silico screening of chemically diverse libraries for novel ligands or for enhancement of the properties of known ligands by chemical modification. Although X-ray structures of the P2X4R have been reported, there is scant structural information about ligand recognition in this class of trimeric ion channels. There are currently no ligands that are selective for different combinations of P2X subunits. In summary, there are definitive selective agonists and antagonists for all of the ARs and some of the P2YRs, but the pharmacology of P2XRs is still in nascent stages.

Abbreviations

AR	adenosine receptor
CCI	chronic constriction injury
CHO	Chinese hamster ovary
CNS	central nervous system
EL	extracellular loop
GPCR	G protein-coupled receptor
HEK	human embryonic kidney
LTD	long-term depression
NAM	negative allosteric modulator
PAM	positive allosteric modulator
TM	transmembrane helix

References

- Alexander K, Niforatos W, Bianchi B, Burgard EC, Lynch KJ, Kowaluk EA, Jarvis MF, van Biesen T. Allosteric modulation and accelerated resensitization of human P2X(3) receptors by cibacron blue. *J Pharmacol Exp Ther*. 1999; 291:1135–1142. [PubMed: 10565834]
- Alnouri MW, Jepards S, Casari A, Schiedel AC, Hinz S, Müller CE. Selectivity is species-dependent: characterization of standard agonists and antagonists at human, rat, and mouse adenosine receptors. *Purinergic Signal*. 2015; 11:389–407. [PubMed: 26126429]

- Al-Rashida M, Iqbal J. Therapeutic potentials of ecto-nucleoside triphosphate diphosphohydrolase, ecto-nucleotide pyrophosphatase/phosphodiesterase, ecto-5'-nucleotidase, and alkaline phosphatase inhibitors. *Med Res Rev.* 2014; 34:703–743. [PubMed: 24115166]
- Amatrudo JM, Olson JP, Agarwal HK, Ellis-Davies GC. Caged compounds for multichromatic optical interrogation of neural systems. *Eur J Neurosci.* 2015; 41:5–16. [PubMed: 25471355]
- Andó RD, Méhész B, Gyires K, Illes P, Sperlág B. A comparative analysis of the activity of ligands acting at P2X and P2Y receptor subtypes in models of neuropathic, acute and inflammatory pain. *Br J Pharmacol.* 2010; 159:1106–1117. [PubMed: 20136836]
- Armentero MT, Pinna A, Ferré S, Lanciego JL, Müller CE, Franco R. Past, present and future of A_{2A} adenosine receptor antagonists in the therapy of Parkinson's disease. *Pharmacol Ther.* 2011; 132:280–299. [PubMed: 21810444]
- Ase AR, Honson NS, Zaghdane H, Pfeifer TA, Séguéla P. Identification and characterization of a selective allosteric antagonist of human P2X₄ receptor channels. *Mol Pharmacol.* 2015; 87:606–616. [PubMed: 25597706]
- Balázs B, Dankó T, Kovács G, Köles L, Hediger MA, Zsembery A. Investigation of the inhibitory effects of the benzodiazepine derivative, 5-BDBD on P2X₄ purinergic receptors by two complementary methods. *Cell Physiol Biochem.* 2013; 32:11–24. [PubMed: 23867750]
- Baqi Y, Hausmann R, Rosefort C, Rettinger J, Schmalzing G, Müller CE. Discovery of potent competitive antagonists and positive modulators of the P2X₂ receptor. *J Med Chem.* 2011; 54:817–830. [PubMed: 21207957]
- Baqi Y. Ecto-Nucleotidase inhibitors: recent developments in drug discovery. *Mini-Rev Med Chem.* 2015; 15:21–33. [PubMed: 25694081]
- Barrett MO, Sesma JI, Ball CB, Jayasekara PS, Jacobson KA, Lazarowski ER, Harden TK. A selective high affinity antagonist of the P2Y₁₄ receptor inhibits UDP-glucose-stimulated chemotaxis of human neutrophils. *Mol Pharmacol.* 2013; 84:41–49. [PubMed: 23592514]
- Bartlett RSL, Stokes L, Sluyter R. The P2X₇ Receptor Channel: Recent Developments and the Use of P2X₇ Antagonists in Models of Disease. *Pharmacol Rev.* 2014; 66:638–675. [PubMed: 24928329]
- Baudelet D, Lipka E, Millte R, Ghinet A. Involvement of the P2X₇ purinergic receptor in inflammation: an update of antagonists series since 2009 and their promising therapeutic potential. *Curr Med Chem.* 2015; 22:713–729. [PubMed: 25515510]
- Beggs S, Trang T, Salter MW. P2X₄R+ microglia drive neuropathic pain. *Nat Neurosci.* 2012; 15:1068–1073. [PubMed: 22837036]
- Bele T, Fabbretti E. P2X receptors, sensory neurons and pain. *Curr Med Chem.* 2015; 22:845–850. [PubMed: 25312207]
- Bernier LP, Ase AR, Tong X, Hamel E, Blais D, Zhao Q, Logothetis DE, Séguéla P. Direct modulation of P2X₁ receptor-channels by the lipid phosphatidylinositol 4,5-bisphosphate. *Mol Pharmacol.* 2008a; 74:785–792. [PubMed: 18523136]
- Bernier LP, Ase AR, Chevallier S, Blais D, Zhao Q, Boué-Grabot E, Logothetis D, Séguéla P. Phosphoinositides regulate P2X₄ ATP-gated channels through direct interactions. *J Neurosci.* 2008b; 28:12938–12945. [PubMed: 19036987]
- Bernier LP, Ase AR, Séguéla P. Post-translational regulation of P2X receptor channels: modulation by phospholipids. *Front Cell Neurosci.* 2013; 7:226. [PubMed: 24324400]
- Bhattacharya A, Wang Q, Ao H, Shoblock JR, Lord B, Aluisio L, Fraser I, Nepomuceno D, Neff RA, Welty N, Lovenberg TW, Bonaventure P, Wickenden AD, Letavic MA. Pharmacological characterization of a novel centrally permeable P2X₇ receptor antagonist: JNJ-47965567. *Br J Pharmacol.* 2013; 170:624–640. [PubMed: 23889535]
- Bhattarai S, Freundlieb M, Pippel J, Meyer A, Abdelrahman A, Fiene A, Lee SY, Zimmermann H, Yegutkin GG, Sträter N, El-Tayeb A, Müller CE. β-Methylene-ADP (AOPCP) derivatives and analogues: development of potent and selective ecto-5'-nucleotidase (CD73) inhibitors. *J Med Chem.* 2015; 58:6248–6263. [PubMed: 26147331]
- Bölcskei H, Farkas B. P2X₃ and P2X_{2/3} receptor antagonists. *Pharm Pat Anal.* 2014; 3:53–64. [PubMed: 24354979]
- Bonaventura J, Navarro G, Casadó-Anguera V, Azdad K, Rea W, Moreno E, Brugarolas M, Mallol JJ, Canela EI, Lluís C, Cortés A, Volkow ND, Schiffmann SN, Ferré S, Casadó V. Allosteric

- interactions between agonists and antagonists within the adenosine A_{2A} receptor-dopamine D₂ receptor heterotetramer. *Proc Natl Acad Sci USA*. 2015; 112:E3609–E3618. [PubMed: 26100888]
- Borrmann T, Hinz S, Bertarelli DC, Li W, Florin NC, Scheiff AB, Müller CE. 1-Alkyl-8-(piperazine-1-sulfonyl)phenylxanthines: development and characterization of adenosine A_{2B} receptor antagonists and a new radioligand with subnanomolar affinity and subtype specificity. *J Med Chem*. 2009; 52:3994–4006. [PubMed: 19569717]
- Boyer JL, Romero-Avila T, Schachter JB, Harden TK. Identification of competitive antagonists of the P2Y₁ receptor. *Mol Pharmacol*. 1996; 50:1323–1329. [PubMed: 8913364]
- Brunschweiler A, Müller CE. P2 receptors activated by uracil nucleotides - an update. *Curr Med Chem*. 2006; 13:289–312. [PubMed: 16475938]
- Burnstock G, Di Virgilio F. Purinergic Signalling and cancer. *Purinergic Signal*. 2013; 9:491–540. [PubMed: 23797685]
- Burnstock, G.; Verkhratsky, A. *Purinergic Signalling and the Nervous System*. Springer-Verlag; Berlin Heidelberg: 2012.
- Carter RL, Fricks IP, Barrett MO, Burianek LE, Zhou Y, Ko H, Das A, Jacobson KA, Lazarowski ER, Harden TK. Quantification of Gi-mediated inhibition of adenylyl cyclase activity reveals that UDP is a potent agonist of the human P2Y₁₄ receptor. *Mol Pharmacol*. 2009a; 76:1341–1348. 2009. [PubMed: 19759354]
- Carter DS, Alam M, Cai H, Dillon MP, Ford AP, Gever JR, Jahangir A, Lin C, Moore AG, Wagner PJ, Zhai Y. Identification and SAR of novel diaminopyrimidines Part 1: The discovery of RO-4, a dual P2X(3)/P2X(2/3) antagonist for the treatment of pain. *Bioorg Med Chem Lett*. 2009b; 19:1628–1631. [PubMed: 19231180]
- Chambers JK, Macdonald LE, Sarau HM, Ames RS, Freeman K, Foley JJ, Zhu Y, McLaughlin MM, Murdock P, McMillan L, et al. A G protein-coupled receptor for UDP-glucose. *J Biol Chem*. 2000; 275:10767–10771. [PubMed: 10753868]
- Chang L, Lee SY, Leonczak P, Rozewski J, De Jonghe S, Hanck T, Müller CE, Herdewijn P. Imidazopyridine- and purine-thioacetamide derivatives: potent inhibitors of nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1). *J Med Chem*. 2014; 57:10080–10100. [PubMed: 25372276]
- Chao H, Turdi H, Herpin TF, Roberge JY, Liu Y, Schnur D, Poss MA, Rehfuss R, Hua J, Wu Q, Price LA, Abell LM, Schumacher WA, Bostwick JS, Steinbacher TE, Stewart AB, Ogletree ML, Huang CS, Chang M, Cacace AM, Arcuri MJ, Celani D, Wexler RR, Lawrence RM. Discovery of 2-(phenoxypyridine)-3-phenylureas as small molecule P2Y₁ antagonists. *J Med Chem*. 2013; 56:1704–1714. [PubMed: 23368907]
- Chataigneau T, Lemoine D, Grutter T. Exploring the ATP-binding site of P2X receptors. *Front Cell Neurosci*. 2013; 7:273. [PubMed: 24415999]
- Chen JF. Adenosine receptor control of cognition in normal and disease. *Int Rev Neurobiol*. 2014; 119:257–307. [PubMed: 25175970]
- Chen JF, Eltzschig HK, Fredholm BB. Adenosine receptors as drug targets — what are the challenges? *Nature Rev Drug Disc*. 2013; 12:265–286.
- Chhatriwala M, Ravi RG, Patel RI, Boyer JL, Jacobson KA, Harden TK. Induction of novel agonist selectivity for the ADP-activated P2Y₁ receptor versus the ADP-activated P2Y₁₂ and P2Y₁₃ receptors by conformational constraint of an ADP analogue. *J Pharm Exp Therap*. 2004; 311:1038–1043.
- Choo AM, Miller WJ, Chen YC, Nibley P, Patel TP, Goletiani C, Morrison B 3rd, Kutzing MK, Firestein BL, Sul JY, Haydon PG, Meaney DF. Antagonism of purinergic signalling improves recovery from traumatic brain injury. *Brain*. 2013; 136:65–80. [PubMed: 23293266]
- Chrovia CC, Rech CJ, Bhattacharya A, Letavic MA. P2X7 antagonists as potential therapeutic agents for the treatment of CNS disorders. *Prog Med Chem*. 2014; 53:65–100. [PubMed: 24418608]
- Cockayne DA, Dunn PM, Zhong Y, Rong W, Hamilton SG, Knight GE, Ruan HZ, Ma B, Yip P, Nunn P, McMahon SB, Burnstock G, Ford AP. P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP. *J Physiol*. 2005; 567:621–639. [PubMed: 15961431]

- Coddou C, Yan Z, Obsil T, Huidobro-Toro JP, Stojilkovic SS. Activation and regulation of purinergic P2X receptor channels. *Pharmacol Rev*. 2011a; 63:641–683. [PubMed: 21737531]
- Coddou C, Stojilkovic SS, Huidobro-Toro JP. Allosteric modulation of ATP-gated P2X receptor channels. *Rev Neurosci*. 2011b; 22:335–354. [PubMed: 21639805]
- Congreve, M.; Doré, AS.; Jazayeri, A.; Nonoo, R. Multifaceted Roles of Crystallography in Modern Drug Discovery. In: Scapin, et al., editors. NATO Science for Peace and Security Series A: Chemistry and Biology. Vol. Chapter 1. Springer Science+Business Media; Dordrecht: 2015.
- Corbelini PF, Figueiró F, das Neves GM, Andrade S, Kawano DF, Oliveira Battastini AM, Eifler-Lima VL. Insights into ecto-5'-nucleotidase as a new target for cancer therapy: a medicinal chemistry study. *Curr Med Chem*. 2015 in press.
- Dal Ben D, Buccioni M, Lambertucci C, Marucci G, Thomas A, Volpini R. Purinergic P2X receptors: structural models and analysis of ligand-target interaction. *Eur J Med Chem*. 2015; 89:561–580. [PubMed: 25462266]
- Delekatte A, Fuchtemeier M, Schumacher T, Ulbrich C, Foddis M, Petzold GC. Metabotropic P2Y₁ receptor signaling mediates astrocytic hyperactivity in vivo in an Alzheimer's disease mouse model. *Nat Commun*. 2014; 5:5422. [PubMed: 25406732]
- De Rivero Vaccari JP, Bastien D, Yurcisin G, Pineau I, Dietrich WD, De Koninck Y, Keane RW, Lacroix S. P2X₄ receptors influence inflammasome activation after spinal cord injury. *J Neurosci*. 2012; 32:3058–3066. [PubMed: 22378878]
- De Roo M, Rodeau J, Schlichter R. Dehydroepiandrosterone potentiates native ionotropic ATP receptors containing the P2X₂ subunit in rat sensory neurones. *J Physiol*. 2003; 552:59–71. [PubMed: 12844512]
- Di Virgilio F. Liaisons dangereuses: P2X₇ and the inflammasome. *Trends Pharmacol Sci*. 2015; 28:465–472. [PubMed: 17692395]
- Donnelly-Roberts D, McGaraughty S, Shieh CC, Honore P, Jarvis MF. Painful purinergic receptors. *J Pharmacol Exp Ther*. 2008; 324:409–415. [PubMed: 18042830]
- Di Virgilio F. P2X receptors and inflammation. *Curr Med Chem*. 2015; 22:866–877. [PubMed: 25524252]
- Duplantier AJ, Dombroski MA, Subramanyam C, Beaulieu AM, Chang S-P, Gabel CA, Jordan C, Kalgutkar AS, Kraus KG, Labasi JM, Mussari C, Perregaux DG, Shepard R, Taylor TJ, Trevena KA, Whitney-Pickett C, Yoon K. Optimization of the physicochemical and pharmacokinetic attributes in a 6-azauracil series of P2X₇ receptor antagonists leading to the discovery of the clinical candidate CE-224,535. *Bioorg Med Chem Lett*. 2011; 21:3708–3711. [PubMed: 21565499]
- Durnin L, Hwang SJ, Kurahashi M, Drumm BT, Ward SM, Sasse KC, et al. Uridine adenosine tetraphosphate is a novel neurogenic P2Y₁ receptor activator in the gut. *Proc Natl Acad Sci USA*. 2014; 111:15821–15826. [PubMed: 25341729]
- Ecke D, Hanck T, Tulapurkar ME, Schäfer R, Kassack M, Stricker R, Reiser G. Hetero-oligomerization of the P2Y₁₁ receptor with the P2Y₁ receptor controls the internalization and ligand selectivity of the P2Y₁₁ receptor. *Biochem J*. 2008; 409:107–116. [PubMed: 17824841]
- El-Ajouz S, Ray D, Allsopp RC, Evans RJ. Molecular basis of selective antagonism of the P2X₁ receptor for ATP by NF449 and suramin: contribution of basic amino acids in the cysteine-rich loop. *Br J Pharmacol*. 2012; 165:390–400. [PubMed: 21671897]
- Elzein E, Zablocki J. A₁ adenosine receptor agonists and their potential therapeutic applications. *Exp Opin Investig Drugs*. 2008; 17:1901–1910.
- Farmer LK, Schmid R, Evans RJ. Use of chimeras, point mutants, and molecular modeling to map the antagonist-binding site of 4,4',4'',4'''-(carbonylbis-(imino-5,1,3-benzenetriylbis(carbonylimino)))tetrakisbenzene-1,3-disulfonic acid (NF449) at P2X₁ receptors for ATP. *J Biol Chem*. 2015; 290:1559–1569. [PubMed: 25425641]
- Ferré S, Casadó V, Devi LA, Filizola M, Jockers R, Lohse MJ, Milligan G, Pin JP, Guitart X. G protein-coupled receptor oligomerization revisited: Functional and pharmacological perspectives. *Pharmacol Rev*. 2014; 66:413–434. [PubMed: 24515647]
- Fishman P, Bar-Yehuda S, Liang BT, Jacobson KA. Pharmacological and therapeutic effects of A₃ adenosine receptor (A₃AR) agonists. *Drug Disc, Today*. 2012; 17:359–366.

- Ford AP, Gever JR, Nunn PA, Zhong Y, Cefalu JS, Dillon MP, Cockayne DA. Purinoceptors as therapeutic targets for lower urinary tract dysfunction. *Br J Pharmacol*. 2006; 147(Suppl 2):132–143.
- Ford AP. In pursuit of P2X3 antagonists: novel therapeutics for chronic pain and afferent sensitization. *Purinergic Signal*. 2012; 8:3–26. [PubMed: 22095157]
- Ford AP, Udem BJ. The therapeutic promise of ATP antagonism at P2X3 receptors in respiratory and urological disorders. *Front Cell Neurosci*. 2013; 7:267.10.3389/fncel.2013.00267 [PubMed: 24391544]
- Franchetti P, Cappellacci L, Vita P, Petrelli R, Lavecchia A, Kachler S, et al. N⁶-cycloalkyl- and N⁶-bicycloalkyl-C5' (C2')-modified adenosine derivatives as high-affinity and selective agonists at the human A₁ adenosine receptor with antinociceptive effects in mice. *J Med Chem*. 2009; 52:2393–2406. [PubMed: 19317449]
- Gao ZG, Mamedova L, Tchilibon S, Gross AS, Jacobson KA. 2,2'-Pyridylisatogen tosylate antagonizes P2Y₁ receptor signaling without affecting nucleotide binding. *Biochem Pharmacol*. 2004; 68:231–237. [PubMed: 15193995]
- Gao ZG, Hechler B, Besada P, Gachet C, Jacobson KA. Caged agonist of P2Y₁ and P2Y₁₂ receptors for light-directed facilitation of platelet aggregation. *Biochem Pharmacol*. 2008; 75:1341–1347. [PubMed: 18199424]
- Gever JR, Cockayne DA, Dillon MP, Burnstock G, Ford AP. Pharmacology of P2X channels. *Pflugers Arch*. 2006; 452:513–537. [PubMed: 16649055]
- Gever JR, Soto R, Henningsen RA, Martin RS, Hackos DH, Panicker S, Rubas W, Oglesby IB, Dillon MP, Milla ME, Burnstock G, Ford AP. AF-353, a novel, potent and orally bioavailable P2X₃/P2X_{2/3} receptor antagonist. *Br J Pharmacol*. 2010; 160:1387–1398. [PubMed: 20590629]
- Gao M, Wang M, Green MA, Hutchins GD, Zheng QH. Synthesis of [3H]GSK1482160 as a new PET agent for targeting P2X7 receptor. *Bioorg Med Chem Lett*. 2015; 25:1965–1970. [PubMed: 25819093]
- Gao ZG, Verzijl D, Zweemer A, Ye K, Göblyös A, IJzerman AP, et al. Functionally biased modulation of A₃ adenosine receptor agonist efficacy and potency by imidazoquinolinamine allosteric enhancers. *Biochem Pharmacol*. 2011; 82:658–668. [PubMed: 21718691]
- Goldman N, Chen M, Fujita T, Xu Q, Peng W, Liu W, et al. Adenosine A₁ receptors mediate local anti-nociceptive effects of acupuncture. *Nat Neurosci*. 2010; 13:883–888. [PubMed: 20512135]
- Gunosewoyo H, Coster MJ, Bennett MR, Kassiou M. Purinergic P2X7 receptor antagonists: Chemistry and fundamentals of biological screening. *Bioorg Med Chem Lett*. 2009; 17:4861–4865.
- Gunosewoyo H, Kassiou M. P2X purinergic receptor ligands: recently patented compounds. *Expert Opin Ther Pat*. 2010; 20:625–646. [PubMed: 20205618]
- Haas M, Ben-Moshe I, Fischer B, Reiser G. *S**p*-2-propylthio-ATP- α -B and *S**p*-2-propylthio-ATP- α -B, β - γ -dichloromethylene are novel potent and specific agonists of the human P2Y₁₁ receptor. *Biochem Pharmacol*. 2013; 86:645–655. [PubMed: 23810430]
- Haas M, Ginsburg-Shmuel T, Fischer B, Reiser G. 5-OMe-uridine-5'-O-(α -boranodiphosphate), a novel nucleotide derivative highly active at the human P2Y₆ receptor protects against death-receptor mediated glial apoptosis. *Neurosci Lett*. 2014; 578:80–84. [PubMed: 24970757]
- Hattori M, Gouaux E. Molecular mechanism of ATP binding and ion channel activation in P2X receptors. *Nature*. 2012; 485:207–212. [PubMed: 22535247]
- Hausmann R, Schmalzing G. P2X1 and P2X2 receptors in the central nervous system as possible drug targets. *CNS Neurol Disord Drug Targets*. 2012; 11:675–686. [PubMed: 22963438]
- Hausmann R, Kless A, Schmalzing G. Key sites for P2X receptor function and multimerization: overview of mutagenesis studies on a structural basis. *Curr Med Chem*. 2015; 22:799–818. [PubMed: 25439586]
- Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, Julius D. The P2Y₁₂ receptor regulates microglial activation by extracellular nucleotides. *Nature Neuroscience*. 2006; 9:1512–1519. [PubMed: 17115040]
- Hechler B, Gachet C. P2 receptors and platelet function. *Purinergic Signal*. 2011; 7:293–303. [PubMed: 21792575]

- Hernandez-Olmos V, Abdelrahman A, El-Tayeb A, Freudendahl D, Weinhausen S, Müller CE. N-substituted phenoxazine and acridone derivatives: structure-activity relationships of potent P2X₄ receptor antagonists. *J Med Chem.* 2012; 55:9576–9588. [PubMed: 23075067]
- Hinz S, Lacher SK, Seibt BF, Müller CE. BAY60–6583 acts as a partial agonist at adenosine A_{2B} receptors. *J Pharmacol Exp Ther.* 2014; 349:427–436. [PubMed: 24633424]
- Herold CL, Qi AD, Harden TK, Nicholas RA. Agonist versus antagonist action of ATP at the P2Y₄ receptor is determined by the second extracellular loop. *J Biol Chem.* 2004; 279:11456–11464. [PubMed: 14670966]
- Hoffmann K, Lutz DA, Straßburger J, Baqi Y, Müller CE, von Kügelgen I. Competitive mode and site of interaction of ticagrelor at the human platelet P2Y₁₂-receptor. *J Thromb Haemost.* 2014; 12:1898–1905. [PubMed: 25186974]
- Horváth G, Göllöncsér F, Csölle C, Király K, Andó RD, Baranyi M, Koványi B, Máté Z, Hoffmann K, Algaier I, Baqi Y, Müller CE, Von Kügelgen I, Sperlágh B. Central P2Y₁₂ receptor blockade alleviates inflammatory and neuropathic pain and cytokine production in rodents. *Neurobiol Dis.* 2014; 70(100):162–178. [PubMed: 24971933]
- Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EYT, Lane JR, IJzerman AP, Stevens RC. The 2.6 angstrom crystal structure of a human A_{2A} adenosine receptor bound to an antagonist. *Science.* 2008; 322:1211–1217. [PubMed: 18832607]
- Jacobson KA, Barone S, Kammula U, Stiles GL. Electrophilic derivatives of purines as irreversible inhibitors of A₁-adenosine receptors. *J Med Chem.* 1989; 32:1043–1051. [PubMed: 2709373]
- Jacobson KA, Kim YC, Wildman SS, Mohanram A, Harden TK, Boyer JL, King BF, Burnstock G. A pyridoxine cyclic phosphate and its 6-azoaryl derivative selectively potentiate and antagonize activation of P2X₁ receptors. *J Med Chem.* 1998; 41:2201–2206. [PubMed: 9632352]
- Jacobson KA, Paoletta S, Katritch V, Wu B, Gao ZG, Zhao Q, Stevens RC, Kiselev E. Nucleotides acting at P2Y receptors: Connecting structure and function. *Mol Pharmacol.* 2015; 88:220–230. [PubMed: 25837834]
- Jahangir A, Alam M, Carter DS, Dillon MP, Bois DJ, Ford AP, Gever JR, Lin C, Wagner PJ, Zhai Y, Zira J. Identification and SAR of novel diaminopyrimidines. Part 2: The discovery of RO-51, a potent and selective, dual P2X(3)/P2X(2/3) antagonist for the treatment of pain. *Bioorg Med Chem Lett.* 2009; 19:1632–1635. [PubMed: 19231178]
- Jankowski J, Jankowski V, Seibt B, Henning L, Zidek W, Schlüter H. Identification of dinucleoside polyphosphates in adrenal glands. *Biochem Biophys Res Commun.* 2003; 304:365–370. [PubMed: 12711324]
- Jankowski V, Tölle M, Vanholder R, Schönfelder G, van der Giet M, Henning L, Schlüter H, Paul M, Zidek W, Jankowski J. Uridine adenosine tetraphosphate: a novel endothelium- derived vasoconstrictive factor. *Nature Med.* 2005; 11:223–227. [PubMed: 15665829]
- Jankowski V, van der Giet M, Mischak H, Morgan M, Zidek W, Jankowski J. Dinucleoside polyphosphates: strong endogenous agonists of the purinergic system. *Br J Pharmacol.* 2009; 157:1142–1153. [PubMed: 19563527]
- Jarvis MF. Contributions of P2X₃ homomeric and heteromeric channels to acute and chronic pain. *Expert Opin Ther Targets.* 2003; 7:513–522. [PubMed: 12885270]
- Jarvis MF, Burgard EC, McGaraughty S, Honore P, Lynch K, Brennan TJ, Subieta A, Van Biesen T, Cartmell J, Bianchi B, Niforatos W, Kage K, Yu H, Mikusa J, Wismer CT, Zhu CZ, Chu K, Lee CH, Steward AO, Polakowski J, Cox BF, Kowaluk E, Williams M, Sullivan J, Faltynek C. A-317491, a novel potent and selective non-nucleotide antagonist of P2X₃ and P2X_{2/3} receptors, reduces chronic inflammatory and neuropathic pain in the rat. *Proc Natl Acad Sci U S A.* 2002; 99:17179–17184. [PubMed: 12482951]
- Jayasekara PS, Barrett MO, Ball CB, Brown KA, Hammes E, Balasubramanian R, Harden TK, Jacobson KA. 4-Alkylxyimino derivatives of uridine-5'-triphosphate: Distal modification of potent agonists as a strategy for molecular probes of P2Y₂, P2Y₄ and P2Y₆ receptors. *J Med Chem.* 2014; 57:3874–3883. [PubMed: 24712832]
- Jung KY, Moon HD, Lee GE, Lim HH, Park CS, Kim YC. Structure-activity relationship studies of spinorphin as a potent and selective human P2X₃ receptor antagonist. *J Med Chem.* 2007; 50:4543–4547. [PubMed: 17676725]

- Kemp PA, Sugar RA, Jackson AD. Nucleotide-mediated mucin secretion from differentiated human bronchial epithelial cells. *Am J Respir Cell Mol Biol*. 2004; 31:446–455. [PubMed: 15231488]
- Jaime-Figueroa S, Greenhouse R, Padilla F, Dillon MP, Gever JR, Ford AP. Discovery and synthesis of a novel and selective drug-like P2X(1) antagonist. *Bioorg Med Chem Lett*. 2005; 15:3292–3295. [PubMed: 15927468]
- Kassack MU, Braun K, Ganso M, Ullmann H, Nickel P, Böing B, Müller G, Lambrecht G. Structure-activity relationships of analogues of NF449 confirm NF449 as the most potent and selective known P2X1 receptor antagonist. *Eur J Med Chem*. 2004; 39:345–357. [PubMed: 15072843]
- Kawate T, Michel JC, Birdsong WT, Gouaux E. Crystal structure of the ATP-gated P2X4 ion channel in the closed state. *Nature*. 2009; 460:592–598. [PubMed: 19641588]
- Kennedy C, Qi AD, Herold CL, Harden TK, Nicholas RA. ATP, an agonist at the rat P2Y₄ receptor, is an antagonist at the human P2Y₄ receptor. *Mol Pharmacol*. 2000; 57:926–931. [PubMed: 10779375]
- Keystone EC, Wang MM, Layton M, Hollis S, McInnes IB. Clinical evaluation of the efficacy of the P2X7 purinergic receptor antagonist AZD9056 on the signs and symptoms of rheumatoid arthritis in patients with active disease despite treatment with methotrexate or sulphasalazine. *Ann Rheum Dis*. 2012; 71:1630–1635. [PubMed: 22966146]
- King BF, Knowles ID, Burnstock G, Ramage AG. Investigation of the effects of P2 purinoceptor ligands on the micturition reflex in female urethane-anaesthetized rats. *Br J Pharmacol*. 2004; 142:519–530. [PubMed: 15148261]
- Kiselev E, Barrett M, Katritch V, Paoletta S, Weitzer CD, Hammes E, Yin AL, Zhao Q, Stevens RC, Harden TK, Jacobson KA. Exploring a 2-naphthoic acid template for the structure-based design of P2Y₁₄ receptor antagonist molecular probes. *ACS Chem Biol*. 2014; 9:2833–2842. [PubMed: 25299434]
- Kiselev E, Balasubramanian R, Uliassi E, Brown KA, Trujillo K, Katritch V, Hammes E, Stevens RC, Harden TK, Jacobson KA. Design, synthesis and pharmacological characterization of a fluorescent agonist of the P2Y₁₄ receptor. *Bioorg Med Chem Lett*. 2015 in press.
- Kittner H, Franke H, Fischer W, Schultheis N, Krügel U, Illes P. Stimulation of P2Y₁ receptors causes anxiolytic effects in the rat elevated plus-maze; implications for the involvement of P2Y₁ receptor mediated nitric oxide production. *Neuropsychopharmacol*. 2003; 28:435–444.
- Klotz KN, Falgner N, Kachler S, Lambertucci C, Vittori S, Volpini R, Cristalli G. [³H]HEMADO--a novel tritiated agonist selective for the human adenosine A₃ receptor. *Eur J Pharmacol*. 2007; 556(1–3):14–8. [PubMed: 17126322]
- Koizumi S, Shigemoto-Mogam Y, Nasu-Tada K, Shinozaki Y, Ohsawa K, Tsuda M, Joshi BV, Jacobson KA, Kohsaka S, Inoue K. UDP acting at P2Y₆ receptors is a novel mediator of microglial phagocytosis. *Nature*. 2007; 446:1091–1095. [PubMed: 17410128]
- Korboukh I, Hull-Ryde EA, Rittiner JE, Randhawa AS, Coleman J, Fitzpatrick BJ, et al. Orally active adenosine A₁ receptor agonists with antinociceptive effects in mice. *J Med Chem*. 2012; 55:6467–6477. [PubMed: 22738238]
- Kowaluk EA, Mikusa J, Wismer CT, Zhu CZ, Schweitzer E, Lynch JJ, et al. ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-d]pyrimidine), a novel orally effective adenosine kinase inhibitor with analgesic and anti-inflammatory properties. II In vivo characterization in the rat. *J Pharmacol Exp Ther*. 2000; 295:1165–1174. [PubMed: 11082454]
- Kozma E, Jayasekara PS, Squarcialupi L, Paoletta S, Moro S, Federico S, Spalluto G, Jacobson KA. Fluorescent ligands for adenosine receptors. *Bioorg Med Chem Lett*. 2013; 23:26–36. [PubMed: 23200243]
- Kreckler LM, Wan TC, Ge ZD, Auchampach JA. Adenosine inhibits tumor necrosis factor- α release from mouse peritoneal macrophages via A_{2A} and A_{2B} but not the A₃ adenosine receptor. *J Pharmacol Exp Ther*. 2006; 317:172–180. [PubMed: 16339914]
- Lambertucci C, Dal Ben D, Buccioni M, Marucci G, Thomas A, Volpini R. Medicinal chemistry of P2X receptors: agonists and orthosteric antagonists. *Curr Med Chem*. 2015; 22:915–928. [PubMed: 25515515]

- Lalo U, Verkhatsky A, Pankratov Y. Ivermectin potentiates ATP-induced ion currents in cortical neurones: evidence for functional expression of P2X4 receptors? *Neurosci Lett*. 2007; 421:158–162. [PubMed: 17566648]
- Lazarowski ER, Watt WC, Stutts MJ, Boucher RC, Harden TK. Pharmacological selectivity of the cloned human P2U-purinoceptor: potent activation by diadenosine tetraphosphate. *Br J Pharmacol*. 1995; 116:1619–1627. [PubMed: 8564228]
- Lebon G, Edwards PC, Leslie AG, Tate CG. Molecular Determinants of CGS21680 Binding to the Human Adenosine A_{2A} Receptor. *Mol Pharmacol*. 2015; 87:907–915. [PubMed: 25762024]
- Lee SY, Fienem A, Li W, Hanck T, Brylev KA, Fedorov VE, Lecka J, Haider A, Pietzsch HJ, Zimmermann H, Sévigny J, Kortz U, Stephan H, Müller CE. Polyoxometalates - potent and selective ecto-nucleotidase inhibitors. *Biochem Pharmacol*. 2015; 93:171–181. [PubMed: 25449596]
- Leone RD, Lo YC, Powell JD. A_{2A}R antagonists: Next generation checkpoint blockade for cancer immunotherapy. *Comput Struct Biotechnol J*. 2015; 13:265–272. [PubMed: 25941561]
- Letavic MA, Lord B, Bischoff F, Hwryluk NA, Pieters S, Sales JZ, Velter AI, Ao H, Bonaventure P, Contreras V, Jiang X, Morton KL, Scott B, Wang Q, Wickenden AD, Carruthers NI, Bhattacharya A. Synthesis and pharmacological characterization of two novel penetrating P2X7 antagonists. *ACS Med Chem Lett*. 2013; 12:419–422. [PubMed: 24900687]
- Li AH, Moro S, Melman N, Ji X-d, Jacobson KA. Structure activity relationships and molecular modeling of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective A₃ adenosine receptor antagonists. *J Med Chem*. 1998; 41:3186–3201. [PubMed: 9703464]
- Little JW, Ford A, Symons-Liguori AM, Chen Z, Janes K, Doyle T, Xie J, Luongo L, Tosh DK, Maione S, Bannister K, Dickenson A, Vanderah TW, Porreca F, Jacobson KA, Salvemini D. Endogenous adenosine A₃ receptor activation selectively alleviates persistent pain states. *Brain*. 2015; 138:28–35. [PubMed: 25414036]
- Liu W, Chun E, Thompson AA, Chubukov P, Xu F, Katritch V, Han GW, Roth CB, Heitman LH, IJzerman AP, Cherezov V, Stevens RC. Structural basis for allosteric regulation of GPCRs by sodium ions. *Science*. 2012; 337:232–236. [PubMed: 22798613]
- Loram LC, Harrison JA, Sloane EM, Hutchinson MR, Sholar P, Taylor FR, et al. Enduring reversal of neuropathic pain by a single intrathecal injection of adenosine 2A receptor agonists: a novel therapy for neuropathic pain. *J Neurosci*. 2009; 29:14015–14025. [PubMed: 19890011]
- Lord B, Aluisio L, Shoblock JR, Neff RA, Varlinskaya EI, Ceusters M, Lovenberg TW, Carruthers N, Bonaventure P, Letavic MA, Deak T, Drinkenburg W, Bhattacharya A. Pharmacology of a novel central nervous system-penetrant P2X7 antagonist JNJ-42253432. *J Pharmacol Exp Ther*. 2014; 351:628–641. [PubMed: 25271258]
- Lord B, Ameriks MK, Qang Q, Forugeaud L, Vliegen M, Verluyten W, Haspeslagh P, Carruthers NI, Lovenberg TW, Bonaventure P, Letavic MA, Bhattacharya A. A novel radioligand for the ATP-gated ion channel P2X7: [³H]JNJ-54232334. *Eur J Pharmacol*. 2015; 765:551–559. [PubMed: 26386289]
- Luongo L, Petrelli R, Gatta L, Giordano C, Guida F, Vita P, Franchetti P, Grifantini M, de Novellis V, Cappellacci L, Malone S. 5'-Chloro-5'-deoxy-(±)-ENBA, a Potent and Selective Adenosine A₁ Receptor Agonist, Alleviates Neuropathic Pain in Mice Through Functional Glial and Microglial Changes without Affecting Motor or Cardiovascular Functions. *Molecules*. 2012; 17:13712–13726. [PubMed: 23174891]
- Mamedova L, Joshi BV, Gao ZG, von Kügelgen I, Jacobson KA. Diisothiocyanate derivatives as potent, insurmountable antagonists of P2Y₆ nucleotide receptors. *Biochem Pharmacol*. 2004; 67:1763–1770. [PubMed: 15081875]
- Magni G, Ceruti S. P2Y purinergic receptors: New targets for analgesic and antimigraine drugs. *Biochem Pharmacol*. 2013; 85:466–477. [PubMed: 23146663]
- Malmsjö M, Hou M, Pendergast W, Erlinge D, Edvinsson L. The stable pyrimidines UDPβS and UTPγS discriminate between contractile cerebrovascular P2 receptors. *Eur J Pharmacol*. 2003; 458:305–311.
- Mehta N, Kaur M, Singh M, Chand S, Vyas B, Silakari P, Bahia MS, Silakari O. Purinergic receptor P2X₇: a novel target for anti-inflammatory therapy. *Bioorg Med Chem Lett*. 2014; 22:54–88.

- Meis S, Hamacher A, Hongwiset D, Marzian C, Wiese M, Eckstein N, et al. NF546 [4,4'-(carbonylbis(imino-3,1-phenylene-carbonylimino-3,1-(4-methyl-phenylene)-carbonylimino))-bis(1,3-xylene- γ,γ' -diphosphonic acid) tetrasodium salt] is a non-nucleotide P2Y₁₁ agonist and stimulates release of interleukin-8 from human monocyte-derived dendritic cells. *J Pharmacol Exp Ther.* 2011; 332:238–247. [PubMed: 19815812]
- Mo G, Bernier LP, Zhao Q, Chabot-Doré AJ, Ase AR, Logothetis D, Cao CQ, Séguéla P. Subtype-specific regulation of P2X3 and P2X2/3 receptors by phosphoinositides in peripheral nociceptors. *Mol Pain.* 2009; 5:47. [PubMed: 19671169]
- Miller KJ, Michel AD, Chessell IP, Humphrey PP. Cibacron blue allosterically modulates the rat P2X4 receptor. *Neuropharmacology.* 1998; 37:1579–1586. [PubMed: 9886680]
- Müller CE. Medicinal chemistry of P2X receptors: allosteric modulators. *Curr Med Chem.* 2015; 22:929–941. [PubMed: 25524251]
- Müller CE. Emerging structures and ligands for P2X3 and P2X4 receptors-towards novel treatments of neuropathic pain. *Purinergic Signal.* 2010; 6:145–148. [PubMed: 20806006]
- Müller CE, Schiedel AC, Baqi Y. Allosteric modulators of rhodopsin-like G protein-coupled receptors: opportunities in drug development. *Pharmacol Ther.* 2012; 135:292–315. [PubMed: 22728155]
- Müller CE, Jacobson KA. Recent developments in adenosine receptor ligands and their potential as novel drugs. *Biochem Biophys Acta – Biomembranes.* 2011; 1808:1290–1308.
- Mulryan K, Gitterman DP, Lewis CJ, Vial C, Leckie BJ, Cobb AL, Brown JE, Conley EC, Buell G, Pritchard CA, Evans RJ. Reduced vas deferens contraction and male infertility in mice lacking P2X1 receptors. *Nature.* 2000; 403:86–89. [PubMed: 10638758]
- Nagata K, Imai T, Yamashita T, Tsuda M, Tozaki-Saitoh H, Inoue K. Antidepressants inhibit P2X4 receptor function: a possible involvement in neuropathic pain relief. *Mol Pain.* 2009; 5:20.10.1186/1744-8069-5-20 [PubMed: 19389225]
- Navarro G, Borroto-Escuela DO, Fuxe K, Franco R. Purinergic signaling in Parkinson's disease. Relevance for treatment. *Neuropharmacology.* 2015;10.1016/j.neuropharm.2015.07.024
- Nicke A, Baumert HG, Rettinger J, Eichele A, Lambrecht G, Mutschler E, Schmalzing G. P2X1 and P2X3 receptors form stable trimers: a novel structural motif of ligand-gated ion channels. *EMBO J.* 1998; 17:3016–3028. [PubMed: 9606184]
- Nörenberg W, Sobottka H, Hempel C, Plötz T, Fischer W, Schmalzing G, Schaefer M. Positive allosteric modulation by ivermectin of human but not murine P2X7 receptors. *Br J Pharmacol.* 2012; 167:48–66. [PubMed: 22506590]
- Ochoa-Cortes F, Liñán-Rico A, Jacobson KA, Christofi FL. Potential for developing purinergic drugs for gastrointestinal diseases. *Inflamm Bowel Dis.* 2014; 20:1259–1287. [PubMed: 24859298]
- Ohlmann P, Lecchi A, El-Tayeb A, Müller CE, Cattaneo M, Gachet C. The platelet P2Y₁₂ receptor under normal and pathological conditions. Assessment with the radiolabeled selective antagonist [³H]PSB-0413. *Purinergic Signal.* 2013; 9:59–66. [PubMed: 22892887]
- Okuro M, Fujiki N, Kotorii N, Ishimaru Y, Sokoloff P, Nishino S. Effects of Paraxanthine and Caffeine on Sleep, Locomotor Activity, and Body Temperature in Orexin/Ataxin-3 Transgenic Narcoleptic Mice. *Sleep.* 2010; 33(7):930–942. [PubMed: 20614853]
- Omura S, Crump A. The life and times of ivermectin - a success story. *Nat Rev Microbiol.* 2004; 2:984–989. [PubMed: 15550944]
- Ostrovskaya O, Asatryan L, Wyatt L, Popova M, Li K, Peoples RW, Alkana RL, Davies DL. Ethanol is a fast channel inhibitor of P2X4 receptors. *J Pharmacol Exp Ther.* 2011; 337:171–179. [PubMed: 21212160]
- Paoletta S, Tosh DK, Finley A, Gizewski E, Moss SM, Gao ZG, Auchampach JA, Salvemini D, Jacobson KA. Rational design of sulfonated A₃ adenosine receptor-selective nucleosides as pharmacological tools to study chronic neuropathic pain. *J Med Chem.* 2013; 56:5949–5963. [PubMed: 23789857]
- Paoletta S, Sabbadin D, von Kügelgen I, Hinz S, Katritch V, Hoffmann K, Abdelrahman A, Straßburger J, Baqi Y, Zhao Q, Stevens RC, Moro S, Müller CE, Jacobson KA. Modeling ligand recognition at the P2Y₁₂ receptor in light of X-ray structural information. *J Computer-Aided Mol Des.* 2015; 29:737–756.

- Puchałicz K, Tarnowski M, Baranowska-Bosiacka I, Chlubek D, Dziedziczko V. P2X and P2Y Receptors—Role in the Pathophysiology of the Nervous System. *Int J Mol Sci.* 2014; 15:23672–23704. [PubMed: 25530618]
- Rivera-Oliver M, Díaz-Ríos M. Using caffeine and other adenosine receptor antagonists and agonists as therapeutic tools against neurodegenerative diseases: A review. *Life Sci.* 2014; 101:1–9. [PubMed: 24530739]
- Rettinger J, Schmalzing G, Damer S, Müller G, Nickel P, Lambrecht G. The suramin analogue NF279 is a novel and potent antagonist selective for the P2X(1) receptor. *Neuropharmacology.* 2000; 39:2044–2053. [PubMed: 10963748]
- Robichaud J, Fournier JF, Gagné S, Gauthier JY, Hamel M, Han Y, Hénault M, Kargman S, Levesque JF, Mamane Y, Mancini J, Morin N, Mulrooney E, Wu J, Black WC. Applying the pro-drug approach to afford highly bioavailable antagonists of P2Y₁₄. *Bioorg Med Chem Lett.* 2011; 21:4366–4368. [PubMed: 21689930]
- Rudolph DA, Alcazar J, Ameriks MK, Anton AB, Ao H, Bonaventure P, Carruther NI, Chrovian CC, De Angelis M, Lord B, Rech JC, Wang Q, Bhattacharya A, Andres JI, Letavic MA. Novel methyl substituted 1-(5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazine-7(8*H*))methanones are P2X7 antagonists. *Bioorg Med Chem Lett.* 2015; 25:3157–3163. [PubMed: 26099534]
- Schaddelee MP, Collins SD, DeJongh J, de Boer AG, IJzerman AP, Danhof M. Pharmacokinetic/pharmacodynamics modelling of the anti-hyperalgesic and anti-nociceptive effect of adenosine A₁ receptor partial agonists in neuropathic pain. *Eur J Pharmacol.* 2005; 514:131–140. [PubMed: 15910799]
- Serchov T, Clement HW, Schwartz MK, Iasevoli F, Tosh DK, Idzko M, Jacobson KA, de Bartolomeis A, Normann C, Biber K, van Calker D. Increased Signaling via Adenosine A₁ Receptors, Sleep Deprivation, Imipramine, and Ketamine Inhibit Depressive-like Behavior via Induction of Homer1a. *Neuron.* 2015; 87:549–562. [PubMed: 26247862]
- Shaver SR, Rideout JL, Pendergast W, Douglass JG, Brown EG, Boyer JL, Patel RI, Redick CC, Jones AC, Picher M, et al. Structure-activity relationships of dinucleotides: potent and selective agonists of P2Y receptors. *Purinergic Signal.* 2005; 1:183–191. [PubMed: 18404503]
- Shryock JC, Snowdy S, Baraldi PG, Cacciari B, Spalluto G, Monopoli A, et al. A_{2A}-Adenosine receptor reserve for coronary vasodilation. *Circulation.* 1998; 98:711–718. [PubMed: 9715864]
- Sim JA, North RA. Amitriptyline does not block the action of ATP at human P2X₄ receptor. *Br J Pharmacol.* 2010; 160:88–92. [PubMed: 20331601]
- Soto F, Lambrecht G, Nickel P, Stühmer W, Busch AE. Antagonistic properties of the suramin analogue NF023 at heterologously expressed P2X receptors. *Neuropharmacology.* 1999; 38:141–149. [PubMed: 10193905]
- Stock TC, Bloom BJ, Wei N, Ishaq S, Park W, Wang X, Gupta P, Mebus CA. Efficacy and safety of CE-224,535, an antagonist of P2X₇ receptor, in treatment of patients with rheumatoid arthritis inadequately controlled by methotrexate. *J Rheumatol.* 2012; 39:720–727. [PubMed: 22382341]
- Springthorpe B, Bailey A, Barton P, Birkinshaw TN, Bonnert RV, Brown RC, et al. From ATP to AZD6140: the discovery of an orally active reversible P2Y₁₂ receptor antagonist for the prevention of thrombosis. *Bioorg Med Chem Lett.* 2007; 17:6013–6018. [PubMed: 17827008]
- Stoddart, LA.; Kilpatrick, LE.; Briddon, SJ.; Hill, SJ. Probing the pharmacology of G protein-coupled receptors with fluorescent ligands. *Neuropharmacology.* 2015. in press. <http://dx.doi.org/10.1016/j.neuropharm.2015.04.033>
- Tendera M, Gaszewska- urek E, Parma Z, Ponikowski P, Jankowska E, Kawecka-Jaszcz K, Czarnecka D, Krzemi ska-Pakuła M, Bednarkiewicz Z, Sosnowski M, Ochan Kilama M, Agrawal R. The new oral adenosine A₁ receptor agonist capadenoson in male patients with stable angina. *Clin Res/ Cardiol.* 2012; 101(7):585–591.
- Tian M, Abdelrahman A, Weinhausen S, Hinz S, Weyer S, Dosa S, El-Tayeb A, Müller CE. Carbamazepine derivatives with P2X₄ receptor-blocking activity. *Bioorg Med Chem.* 2014; 22:1077–1088. [PubMed: 24411477]
- Tosh DK, Deflorian F, Phan K, Gao ZG, Wan TC, Gizewski E, Auchampach JA, Jacobson KA. Structure-guided design of A₃ adenosine receptor-selective nucleosides: combination of 2-

arylethynyl and bicyclo[3.1.0]hexane substitutions. *J Med Chem.* 2012a; 55:4847–4860. [PubMed: 22559880]

Tosh DK, Paoletta S, Deflorian F, Phan K, Moss SM, Gao ZG, Jiang X, Jacobson KA. Structural sweet spot for A₁ adenosine receptor activation by truncated (N)-methanocarba nucleosides: Receptor docking and potent anticonvulsant activity. *J Med Chem.* 2012b; 55:8075–8090. [PubMed: 22921089]

Tosh DK, Finley A, Paoletta S, Moss SM, Gao ZG, Gizewski E, Auchampach J, Salvemini D, Jacobson KA. In vivo phenotypic screening for treating chronic neuropathic pain: Modification of C2-arylethynyl group of conformationally constrained A₃ adenosine receptor agonists. *J Med Chem.* 2014; 57:9901–9914. [PubMed: 25422861]

Tosh DK, Padia J, Salvemini D, Jacobson KA. Efficient, large-scale synthesis and preclinical studies of MRS5698, a highly selective a₃ adenosine receptor agonist that protects against chronic neuropathic pain. *Purinerg Signal.* 2015; 11:371–387.

Trujillo CA, Nery AA, Martins AH, Majumder P, Gonzalez FA, Ulrich H. Inhibition mechanism of the recombinant rat P2X(2) receptor in glial cells by suramin and TNP-ATP. *Biochemistry.* 2006; 45:224–233. [PubMed: 16388598]

Trujillo K, Paoletta S, Kiselev E, Jacobson KA. Molecular modeling of the human P2Y₁₄ receptor: A template for structure-based design of selective agonist ligands. *Bioorg Med Chem.* 2015; 23:4056–4064. [PubMed: 25868749]

Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K. P2X₄ receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature.* 2003; 424:778–783. [PubMed: 12917686]

Ulmann L, Levavasseur F, Avignone E, Peyroutou R, Hirbec H, Audinat E, Rassendren F. Involvement of P2X₄ receptors in hippocampal microglial activation after status epilepticus. *Glia.* 61:1306–1319. [PubMed: 23828736]

van Galen PJM, van Bergen AH, Gallo-Rodriguez C, Melman N, Olah ME, IJzerman AP, Stiles GL, Jacobson KA. A binding site model and structure-activity relationships for the rat A₃ adenosine receptor. *Mol Pharmacol.* 1994; 45:1101–1111. [PubMed: 8022403]

Varma R, Chai Y, Troncoso J, Gu J, Xing H, Stojilkovic SS, Mattson MP, Haughey NJ. Amyloid-beta induces a caspase-mediated cleavage of P2X₄ to promote purinotoxicity. *Neuromolecular Med.* 2009; 11:63–75. [PubMed: 19562525]

Vincenzi F, Martina Targa M, Romagnoli R, Merighi S, Gessi S, Baraldi PG. TRR469, a potent A₁ adenosine receptor allosteric modulator, exhibits anti-nociceptive properties in acute and neuropathic pain models in mice. *Neuropharmacology.* 2014; 81:6–14. [PubMed: 24486382]

Waldo GL, Harden TK. Agonist binding and Gq-stimulating activities of the purified human P2Y₁ receptor. *Mol Pharmacol.* 2004; 65:426–436. Erratum in: *Mol Pharmacol.* 2004 65, 811. [PubMed: 14742685]

Weisman GA, Woods LT, Erb L, Seye CI. P2Y receptors in the mammalian nervous system: pharmacology, ligands and therapeutic potential. *CNS Neurol Disord Drug Targets.* 2012; 11(6): 722–738. [PubMed: 22963441]

Wiedon A, Tölle M, Bastine J, Schuchardt M, Huang T, Jankowski V, Jankowski J, Zidek W, van der Giet M. Uridine adenosine tetraphosphate (Up4A) is a strong inductor of smooth muscle cell migration via activation of the P2Y₂ receptor and cross-communication to the PDGF receptor. *Biochem Biophys Res Commun.* 2012; 417:1035–1040. [PubMed: 22214933]

Wilkinson SM, Gunosewoyo H, Barron ML, Boucher A, McDonnell M, Turner P, Morrison DE, Bennett MR, McGregor IS, Rendina LM, Kassiou M. The first CNS-active carborane: a novel P2X₇ receptor antagonist with antidepressant activity. *ACS Chem Neurosci.* 2014; 21:335–339. [PubMed: 24689484]

Wolf C, Rosefort C, Fallah G, Kassack MU, Hamacher A, Bodnar M, Wang H, Illes P, Kless A, Bahrenberg G, Schmalzing G, Hausmann R. Molecular determinants of potent P2X₂ antagonism identified by functional analysis, mutagenesis, and homology docking. *Mol Pharmacol.* 2011; 79:649–661. [PubMed: 21191044]

- Xu F, Wu H, Katritch V, Han GW, Jacobson KA, Gao ZG, Cherezov V, Stevens RC. Structure of an agonist-bound human A_{2A} adenosine receptor. *Science*. 2011; 332:322–327. [PubMed: 21393508]
- Yelovitch S, Camden J, Weisman GA, Fischer B. Boranophosphate isoster controls P2Y-receptor subtype selectivity and metabolic stability of dinucleoside polyphosphate analogues. *J Med Chem*. 2012; 55:437–448. [PubMed: 22107038]
- Yi CL, Liu YW, Xiong KM, Stewart RR, Peoples RW, Tian X, Zhou L, Ai YX, Li ZW, Wang QW, Li CY. Conserved extracellular cysteines differentially regulate the inhibitory effect of ethanol in rat P2X₄ receptors. *Biochem Biophys Res Commun*. 2009; 381:102–106. [PubMed: 19351603]
- Zemkova H, Tvrdonova V, Bhattacharya A, Jindrichova M. Allosteric modulation of ligand gated ion channels by ivermectin. *Physiol Res*. 2014; 63:S215–224. [PubMed: 24564661]
- Zhang K, Zhang J, Gao ZG, Zhang D, Zhu L, Han GW, Moss SM, Paoletta S, Kiselev E, Lu W, Fenalti G, Zhang W, Müller CE, Yang H, Cherezov V, Katritch V, Han GW, Jacobson KA, Stevens RC, Wu B, Zhao Q. Structure of the human P2Y₁₂ receptor in complex with an antithrombotic drug. *Nature*. 2014a; 509:115–118. [PubMed: 24670650]
- Zhang K, Zhang J, Gao ZG, Paoletta S, Zhang D, Han GW, Li T, Ma L, Zhang W, Müller CE, Yang H, Jiang H, Cherezov V, Katritch V, Jacobson KA, Stevens RC, Wu B, Zhao Q. Agonist-bound structure of the human P2Y₁₂R receptor. *Nature*. 2014b; 509:119–122. [PubMed: 24784220]
- Zhang D, Gao ZG, Zhang K, Kiselev E, Crane S, Wang J, Paoletta S, Yi C, Ma L, Zhang W, Han GW, Liu H, Cherezov V, Katritch V, Jiang H, Stevens RC, Jacobson KA, Zhao Q, Wu B. Two disparate ligand-binding sites in the human P2Y₁ receptor. *Nature*. 2015; 520:317–321. [PubMed: 25822790]
- Zimmermann H, Zebisch M, Sträter N. Cellular function and molecular structure of ecto-nucleotidases. *Purinergic Signal*. 2012; 8:437–502. [PubMed: 22555564]
- Zylka MJ. Pain-relieving prospects for adenosine receptors and ectonucleotidases. *Trends Mol Med*. 2011; 17:188–196. [PubMed: 21236731]

Highlights

Selective agonists and antagonists define action at adenosine receptors.

Some selective and stable P2Y receptor ligands are known.

Ligand pharmacology of P2X receptors is still in nascent stages.

Nonselective agonists and precursors

A₁ agonists A_{2A} agonists A_{2B} agonist A₃ agonists

AR modulators

Figure 1.
AR agonists and modulators

Figure 2.
AR antagonists.

Figure 3.
Representative P2YR agonists that are useful as pharmacological tool compounds.

Figure 4.
Representative P2YR antagonists that are useful as pharmacological tool compounds.

Figure 5.
Selected agonists and allosteric modulators at P2XR.

Figure 6.
Positive allosteric modulators and antagonists of P2XR.

Figure 7.
P2X4- and P2X7-selective antagonists

Table 1

AR subtypes and their ligands. Only representative useful ligands are shown.

	A ₁	A _{2A}	A _{2B}	A ₃
Expression (selection)	brain > heart, kidney, lung	thymus gland > heart, lung > spleen, leukocytes	bronchial smooth muscle, jejunum, ileum, colon	liver, lung, placenta, adrenal glands > spleen, small intestine
Knockout phenotypes (selection)	hyperalgesia, blood pressure and plasma renin activity ↑ anxiety ↑ tubuloglomerular feedback	acute pain response anxiety and aggression ↑ damage by focal brain ischemia neonatal brain ischemic damage ↑	leukocyte adhesion to vascular endothelium ↑ inflammation ↑ vascular leakiness ↑	airway responsiveness mast cell degranulation intraocular pressure resistance to cardiac ischemia-reperfusion injury
Adenosine Potency (approx, EC ₅₀)	1–10 nM	30 nM	1000 nM	100 nM
Useful Agonists	CCPA 6b , 5'-Cl-5-d-ENBA 10	CGS21680 15 , UK-432097 17	BAY 60-6583 18	Cl-IB-MECA 20 , MRS5698 24
Useful Antagonists	WRC-0571 36 , PSB-36 40	SCH442416 51	PSB-603 15 , MRS1754 17	MRS1523 62 , MRS1191 59a
Other tool compounds	PD-81,723 (PAM)			LUF6000 30 (PAM)

AR subtype selective agonists are generally synthetic adenosine derivatives that are long lasting metabolically.

Table 2

P2YR subtypes and their ligands. Only representative useful ligands are shown.

	P2Y ₁	P2Y ₂	P2Y ₄	P2Y ₆	P2Y ₁₁	P2Y ₁₂	P2Y ₁₃	P2Y ₁₄
Expression (selection)	placenta > prostate > brain (nucleus accumbens) > intestine > skeletal muscle, heart	epithelial cells, putamen, striatum	intestine > pituitary ≧ brain > adipose > lung, skeletal muscle, spleen, lymphocytes, prostate	placenta, skeletal muscle > brain, heart, liver, pancreas	brain > pituitary > lymphocytes > spleen > intestine, macrophages > lung, stomach, adipose, pancreas (human)	vascular smooth muscle, platelets, brain	brain, spleen, bone marrow, lymph nodes	placenta, adipose tissue, intestine > stomach, skeletal muscle > spleen, lung, heart > peripheral blood mononuclear cells, pituitary, brain
Knockout phenotypes (selection)	platelet aggregation ↓ atherosclerosis in ApoE KO ↓ renal disease ↓	Cl [−] secretion in trachea and jejunum ↓ salt-resistant arterial hypertension ↑ Na ⁺ and water renal reabsorption ↑ monocyte and macrophage recruitment, clearance of apoptotic thymocytes ↓	intestinal K ⁺ and Cl [−] secretion ↓ cardiac angiogenic defect, exercise capacity ↓ effort-induced adaptive cardiac hypertrophy ↓	altered IP ₃ and Ca ²⁺ levels in rat proximal tubule, macrophages and glial cells	not found in mouse	allodynia after nerve injury ↓ platelet activation/adhesion ↓ microglial extension toward cortical damage ↓	hepatic HDL and cholesterol/cholesterol uptake/biliary cholesterol output ↓ bone volume ↓	neuropathic pain ↓ high-fat diet induced insulin resistance ↓ neurophil/macrophage chemotaxis ↓
Native agonists Potency (approx. EC ₅₀)	10 μM (ADP), ~1 μM (Ap ₄ U)	0.1 μM (ATP), 0.01 μM (UTP),	~1 μM (UTP)	~0.3 μM (UDP)	~10 μM (ATP)	0.1 μM (ADP)	0.01 μM (ADP)	0.1 μM (UDP), 0.3 μM (UDP-glucose),
Useful Agonists	2-MeS-ADP 65 , MRS2365 68	PSB-11114 75	MRS4062 77	MRS2957 82 , PSB-0474 81	γ-thio-ATP, NF546 83	2-MeS-ADP 65	2-MeS-ADP 65	MRS2690 85
Useful Antagonists	MRS2500 88 , BPTU 89	AR-C1189251XX 91		MRS2578 92	NF340 98	PSB-0739 99 , ticagrelor 95 , AZD1283 108	MRS2211 107	PPTN 109
Other tool compounds		MRS4162 (fluorescent)	MRS4162 (fluorescent)	MRS4129 (fluorescent), MRS4162 (fluorescent)				MRS4183, MRS4174 (fluorescent)

Table 3

P2XR subtypes and their ligands.

	P2X1	P2X2	P2X3	P2X4	P2X5	P2X6	P2X7
Expression (selection)	smooth muscle, platelets, CNS	smooth muscle, CNS, pancreas, autonomic and sensory ganglia	nociceptive sensory neurons, sympathetic neurons, CNS	microglia, testis, colon, endothelial cells, CNS	skeletal muscle, skin, epithelia	CNS, peripheral nerves	macrophages, mast cells, microglia, pancreas, skin, immune cells, oligodendrocytes
Knockout phenotypes (selection)	male infertility kidney function ↓ arterial thrombosis ↓	inflammatory pain ↓ urinary bladder reflex ↓ taste sensing ↓ intestinal motility ↓	hippocampal LTD ↓ pain ↓ bladder reflex ↓ taste sens. ↓ temperature sens. ↓ intestinal motility ↓	hippocampal LTD ↓ Inflammatory & neuropathic pain ↓ heart function and blood pressure ↑			inflammation ↓ pain ↓ fluid secretion (pancreas, salivary gland) ↓ skeletal abnormalities
ATP Potency (EC ₅₀)	0.1–0.7 μM (fast desensitization)	2–8 μM	~1 μM (fast desensitization)	1–10 μM	0.5 μM non-functional in most humans (splice variant)		2–4 mM
Useful Agonists	ATP 2-MeS-ATP	ATP	ATP 2-MeS-ATP	ATP γ-thio-ATP Benzoyl-ATP			ATP Benzoyl-ATP
Useful Antagonists	NF449	PSB-1011 NF770	A-317491 RO-4/AF353 RO-51/AF906	5-BDBD PSB-12054 BX-430			A-740003
Other tool compounds			Spinorphin (PAM)	Ivermectin (PAM)			Ivermectin (PAM), only hP2X7R