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ORIGINAL ARTICLE

P2X receptor antagonists for pain management: examination of binding and physicochemical properties

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Abstract Enhanced sensitivity to noxious stimuli and the perception of non-noxious stimuli as painful are hallmark sensory perturbations associated with chronic pain. It is now appreciated that ATP, through its actions as an excitatory neurotransmitter, plays a prominent role in the initiation and maintenance of chronic pain states. Mechanistically, the ability of ATP to drive nociceptive sensitivity is mediated through direct interactions at neuronal P2X3 and P2X2/3 receptors. Extracellular ATP also activates P2X4, P2X7, and several P2Y receptors on glial cells within the spinal cord, which leads to a heightened state of neural-glial cell interaction in ongoing pain states. Following the molecular identification of the P2 receptor superfamilies, selective small molecule antagonists for several P2 receptor subtypes were identified, which have been useful for investigating the role of specific P2X receptors in preclinical chronic pain models. More recently, several P2X receptor antagonists have advanced into clinical trials for inflammation and pain. The development of orally bioavailable blockers for ion channels, including the P2X receptors, has been traditionally difficult due to the necessity of combining requirements for target potency and selectivity with suitable absorption distribution, metabolism, and elimination properties. Recent studies on the physicochemical properties of marketed orally bioavailable drugs, have identified several parameters that appear critical for increasing the probability of achieving suitable bioavailability, central nervous system exposure, and acceptable safety necessary for clinical efficacy. This review provides an overview of the antinociceptive pharmacology of P2X receptor antagonists and the chemical diversity and drug-like properties for emerging antagonists of P2X3, P2X2/3, P2X4, and P2X7 receptors.

Keywords Physicochemical parameters · P2X4 · P2X7 · P2X3 · Pain

Abbreviations

clogP	Calculated partition coefficient (octanol/water) of a unionized organic compound—ionization not considered and pH independent. A measure of lipophilicity
ClogD	Calculated distribution coefficient (octanol/buffer) of a compound in all forms (ionized and unionized)—pH dependent. A pH dependent measure of lipophilicity
PSA	Polar surface area—surface sum over all polar atoms in a molecule (generally N and O) units in Angstrom squared. A measure of polarity
HBD	Hydrogen bond donors
HBA	Hydrogen bond acceptors
MW	Molecular weight
BEI	Binding efficiency index
MPO	Multi-parameter optimization
ADME	Absorption, distribution, metabolism, and elimination
CFA	Complete Freund's adjuvant

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Introduction

The ability to detect and react to noxious environmental stimuli is a highly adaptive physiological response that is mediated through the coordinated processing of sensory information by the peripheral and central nervous systems [1–4]. Chronic pain in the presence or absence of tissue trauma is characterized by increased sensitivity to painful stimuli (i.e., hyperalgesia) and the perception of pain in response to normally innocuous stimuli (i.e., allodynia) [4]. Based on many studies of the neurobiology of pain over the last 20 years, it is now appreciated that both peripheral and central neural pathways become "sensitized" in response to ongoing nociceptor stimulation [3]. This sensitized state of sensory function is mediated, at least in part, by neurochemical and phenotypic changes within sensory neurons as well as changes in neural-glial cell interactions at multiple sites including peripheral nerves, spinal cord and brain [3, 4].

A wide variety of pronociceptive neurotransmitters and neuromodulators including glutamate, substance P, CGRP, pro-inflammatory cytokines (e.g., interleukin-1β and tumor necrosis factor-α), and neurotrophic factors (e.g., nerve growth factor) contribute to initiation and maintenance of heightened states of sensory neuron excitability associated with chronic pain [4]. Emerging data also support a key role for ATP, acting as an excitatory neurotransmitter, in both acute and persistent nociception. It had been appreciated for many years that extracellular ATP can excite nerves [5, 6]. ATP administered intradermally elicits pain in humans under normal conditions and enhances inflammatory-mediated pain [6, 7]. Similar effects have been produced in experimental pain models [8–11] using ATP and structurally related agonists such as α,β-methylene ATP [1]. A mechanistically specific role for ATP as a pain neurotransmitter was provided by demonstrations that distinct ATP-gated channels are expressed on sensory neurons and that genetic and/or pharmacological inhibition of channel function led to decreased pain sensitivity in experimental models [5–7, 11].

The ability of ATP to modulate neuronal excitability associated with nociception is mediated by at least two mechanistically distinct pathways. ATP can directly activate homomeric and heteromeric P2X3 receptors on central and distal processes of peripheral nerves which results in the sensation of acute pain. ATP can also indirectly modulate chronic nociception by activating both P2X and P2Y receptors on non-neural-glial cells (astrocytes and microglia) in the periphery and spinal cord [11].

Based on the emerging appreciation for the role of P2X receptors in mediating nociceptive neurotransmission, the development of P2X receptor-selective antagonists as research tools and, in the context of pain management, as potential therapeutics has received much interest. This effort has incorporated a wide variety of medicinal chemistry research strategies including iterative structure activity relationship studies based on the prototypical purinergic antagonists suramin and pyridoxalphosphate-6-azophenyl-2′,5′-disulfonic acid (PPADS), as well as the application of high throughput screening of large chemical libraries [10, 11]. To date, structurally diverse receptor-selective antagonists for P2X3 and P2X7 receptors have been identified. Additionally, three dimensional crystal structures of P2X2 [12] and P2X4 [13] receptors have been identified which may aid the discovery of potent antagonists for these P2X receptor subunits.

Physicochemical properties of orally bioavailable drugs Over the last decade, the number of drugs reaching the market has continued to decrease with the rate of attrition rising to as much as 98% [14–20]. One noticeable trend during this timeframe is that the molecular weight and lipophilicity of molecules entering clinical studies has continued to increase [15]. In this context, the physicochemical properties of successful drugs and drug candidates can be used as key parameters for in silico predictions of in vitro absorption, distribution, metabolism, and elimination (ADME), oral bioavailability, and off-target toxicity, in attempts to improve the probabilities of success throughout the drug discovery process (Table 1). Lipinski et al. [14] first articulated a series of physicochemical parameters for drug solubility and permeability that may improve the probability of identifying compounds with acceptable (>30%) oral bioavailability. These parameters have subsequently become known as the rule of (RO)-5 because they are based on 5 s or multiples of 5 s and include the following parameter thresholds: MW<500, ClogP<5, HBA<10, and HBD<5. Lipinski found that 90% of the drugs from the World Drug Index violated not more than one of these rules [14]. Since then, a number of investigators have generated more quantitatively correlated subsets of molecular physicochemical properties related to the absorption, solubility, permeability, oral bioavailability, target specificity, and toxicity of drugs and

Table 1 Physicochemical properties of orally bioavailable drugs

Property	Marketed oral drugs	Marketed CNS drugs	References
MW	≤473	≤427	[18, 23]
CLogP	≤5.5	≤5.1	[18, 23]
PSA	≤120	≤86	[16, 23]
HBD	≤4	≤3	[18, 23]
CLogD	≤4.3	≤3.8	[18, 23]
BEI	≥19		
MPO		≥4	[24]

Additional information on the properties of drug-like molecules can be found in [74–76]

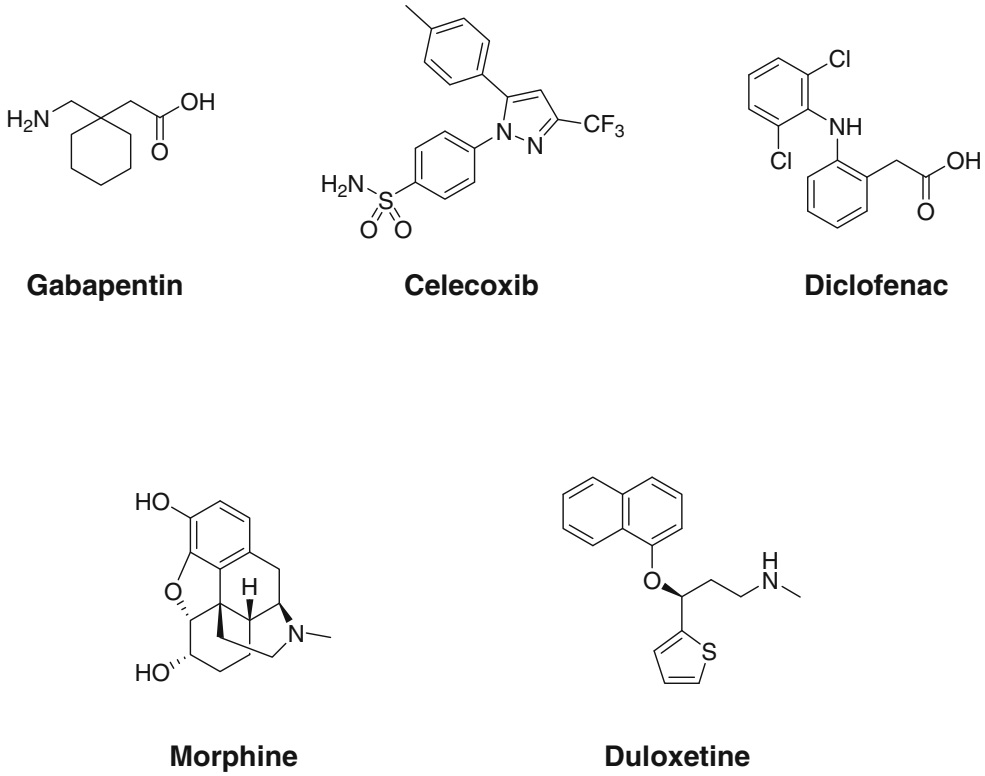

Fig. 1 Chemical structures of representative orally bioavailable analgesic drugs

clinically evaluated molecules [15–23]. These parameters have been used to assess the relative probability of success of clinical lead optimization research for specific drug-like compounds that have demonstrated proof of concept in subsequent clinical testing [15–23].

Recently, Wager et al. [24] analyzed currently marketed drugs for a variety of central nervous system (CNS) disorders and developed a multi-parameter optimization (MPO) score, which is calculated from six in silico physicochemical properties (ClogP, ClogD, TPSA, MW, HBD, and pK_a). This score is expressed as a single number ranging from 0 to 6. The reader is referred to this review and the references therein for full details [24]. In this study, the authors found that 74% of currently marketed CNS drugs have an MPO score greater than 4, which defines the desirable drug-like space. They also demonstrate that CNS drugs and clinical candidates with higher MPO scores also have a higher probability of achieving ADME and safety criteria typically necessary for successful advancement into clinical trials. Based on this analysis, novel lead molecules for CNS disorders with MPO scores greater than 4 should also have a higher probability of having acceptable oral bioavailability.

The intrinsic molecular efficiency for which novel molecules bind their targets is also another critical parameter for achieving an acceptable efficacy and side-effect profile for drug candidates. Abad-Zapatero and Metz [25] illustrated the importance of binding efficiency (BEI) for a target as defined by (pIC_{50})/(molecular weight (MW)/1,000)), which is a measure of target potency relative to MW. This parameter is based on the hypothesis that increasing the potency of compounds within a chemical series solely by increasing the MW is less likely to improve the drug-like properties of the series relative to increasing the intrinsic binding efficiency [23]. For many CNS drugs (e.g., G-protein-coupled receptor antagonists), it can be assumed that a lead-like molecules would have an IC_{50}

Table 2 In vitro potency and physicochemical summary of selected analgesic drugs

Name	MOA	IC_{50} (nM)	BEI	MPO score	MW	CLogP	PSA	HBA	HBD	LOGD	References
Gabapentin	Voltage-gated calcium channel 2 subunit ligand	140	40.03	4.2	171.3	−0.7	63.32	3	2	−1.6	[77]
Celecoxib	COX2 inhibitor	40	19.4	3.7	381.4	4.4	77.98	3	1	3.4	[78]
Diclofenac	NSAID	3	28.78	4.7	296.2	4.7	49.33	3	2	1.4	[79]
Morphine	μ opioid receptor agonist	4	29.43	4.9	285.4	0.6	52.93	4	2	0.1	[80]
Duloxetine	SNRI	2.6	28.87	3.7	297.4	4.3	21.26	2	1	2.2	[81]

COX-2 cyclooxygenase-2, *NSAID* non-steroidal anti-inflammatory drug, *SNRI* serotonin norepinepherine reuptake inhibitor

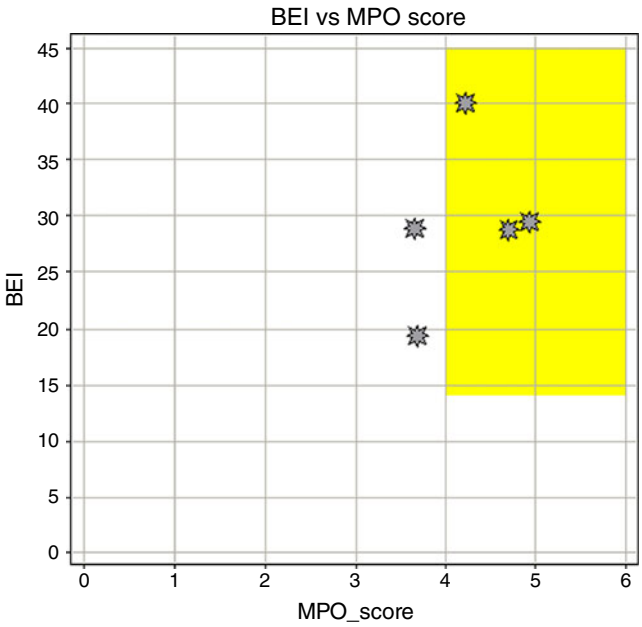

Fig. 2 Comparison of binding efficiency and multi-parameter analysis (*MPO*) for the orally bioavailable drugs shown in Fig. 1

value less than or equal to 100 nM and a MW threshold of 500, thus producing a BEI greater than or equal to 14. For successful marketed oral drugs, the majority have a BEI greater than 19 (Cox, unpublished analysis). For the purposes of this review, P2X antagonists with BEI greater than 14 are characterized in lead-like chemical space and those with BEI greater than 19 are considered in drug-like space. Table 1 summarizes physicochemical properties, MPO score and BEI parameters that define the characteristics of the majority of marketed orally bioavailable drugs and a subset of orally bioavailable CNS drugs.

Drug-like properties of current analgesics Figure 1 and Table 2 show five marketed, orally bioavailable analgesic drugs from different chemical classes along with their putative mechanism(s) of action. To test how these oral analgesic drugs (Fig. 1) compare with respect to these properties, we have plotted the BEI against the MPO score for the five drugs (Fig. 2). This plot demonstrates how the five analgesic drugs fit into or close to BEI/MPO drug space (i.e., the yellow box represents the optimal lead-like chemical space based on the analysis described above). Three out of five of the analgesics meet the MPO criteria for the more desirable drug space while they all meet the proposed drug space in terms of binding efficiency index. Celecoxib and duloxetine are the two drugs just under the desirable CNS MPO score of >4. Although this is a relatively small sample size, this MPO analysis is fairly consistent with the observed data from the original publication where 26% of CNS drugs have MPO scores of <4 [24]. This analysis can be readily applied to the evaluation of novel ligands with the expectation that

Table 3 In vitro potency and physicochemical summary of antagonists for P2X3 receptors

Compound no.	Name	P2X3 IC_{50} (nM)	P2X2/3 IC_{50} (nM)	BEI P2X3	MPO score	MW	CLogP	PSA	HBA	HBD	LOGD	References
1	TNP-ATP	1	7	12.6	3.5	714	−6.4	398	23	5	−1.7	[82]
2	PPADS	1,000		11.8	3.8	507	−9.5	262	15	5	−2.6	[82]
3	Suramin	3,000		4.3	2.0	1,291	−27.4	501	23	12	−2.5	[82]
4	Spinorphin	0.008	>10,000	12.6	2.9	877	1.0	285	11	10	0.4	[82]
5	NF-110	36		7.4	2.0	1,005	−17.9	386	17	10	−2.1	[83]
6	IP5I	3	2,800	9.4	3.0	913	−8.1	483	28	11	−8.6	[82]
7	A-317491	100	100	12.4	3.8	564	−0.9	147	8	3	0.7	[82]
8		10		17.9	3.4	447	6.0	93	5	1	2.3	[84]
9	RO-3	100	1,000	23.2	4.5	302	2.7	96	6	2	2.3	[36]
10	RO-4	13	25	19.7	3.4	400	3.9	96	6	2	3.3	[36]
11	RO-51	2	5	18.4	3.0	474	3.6	123	8	4	2.5	[85]
12	RO-85	398	>5,000	14.6	4.8	440	3.3	70	4	1	2.7	[86]
13		2.8	10	21.5	5.5	399	2.4	86	5	1	2.0	[84]
14		2	10	22.4	3.7	394	4.0	93	5	2	2.3	[84]
15		11	11	16.8	4.1	475	2.9	86	5	1	3.6	[84]
16		8		18.8	3.9	430	4.0	68	4	1	4.0	[87]
17		9	27	20.8	5.0	387	3.1	87	7	1	3.6	[84]
18		7	9	19.4	4.7	420	3.1	92	5	1	2.9	[88]
19	AZ-2	13	>3,900	16.3	3.8	485	3.8	82	6	1	3.5	[38]
20	MK-3901	24		15.8	3.8	482	3.4	89	6	1	4.4	[37]

compounds falling within the yellow box will generally have more drug-like characteristics compared to compounds falling outside of the yellow box. This prediction may be even more apparent for compounds that fail to meet both criteria for lead-like chemical space (i.e., BEI, <14 and MPO, <4) relative to compounds that fulfill both criteria. In this review, we have used this analysis to evaluate the drug-like properties of antagonists for the P2X receptors involved in persistent nociception.

Analgesic pharmacology and drug-like properties of P2X receptor antagonists

P2X3 receptors

Table 3 and Fig. 3 show summary data and chemical structures, respectively, for known P2X3/P2X2/3 receptor antagonists. PPADS (compound 2) and Suramin (compound 3) are two nonselective P2X receptor antag-

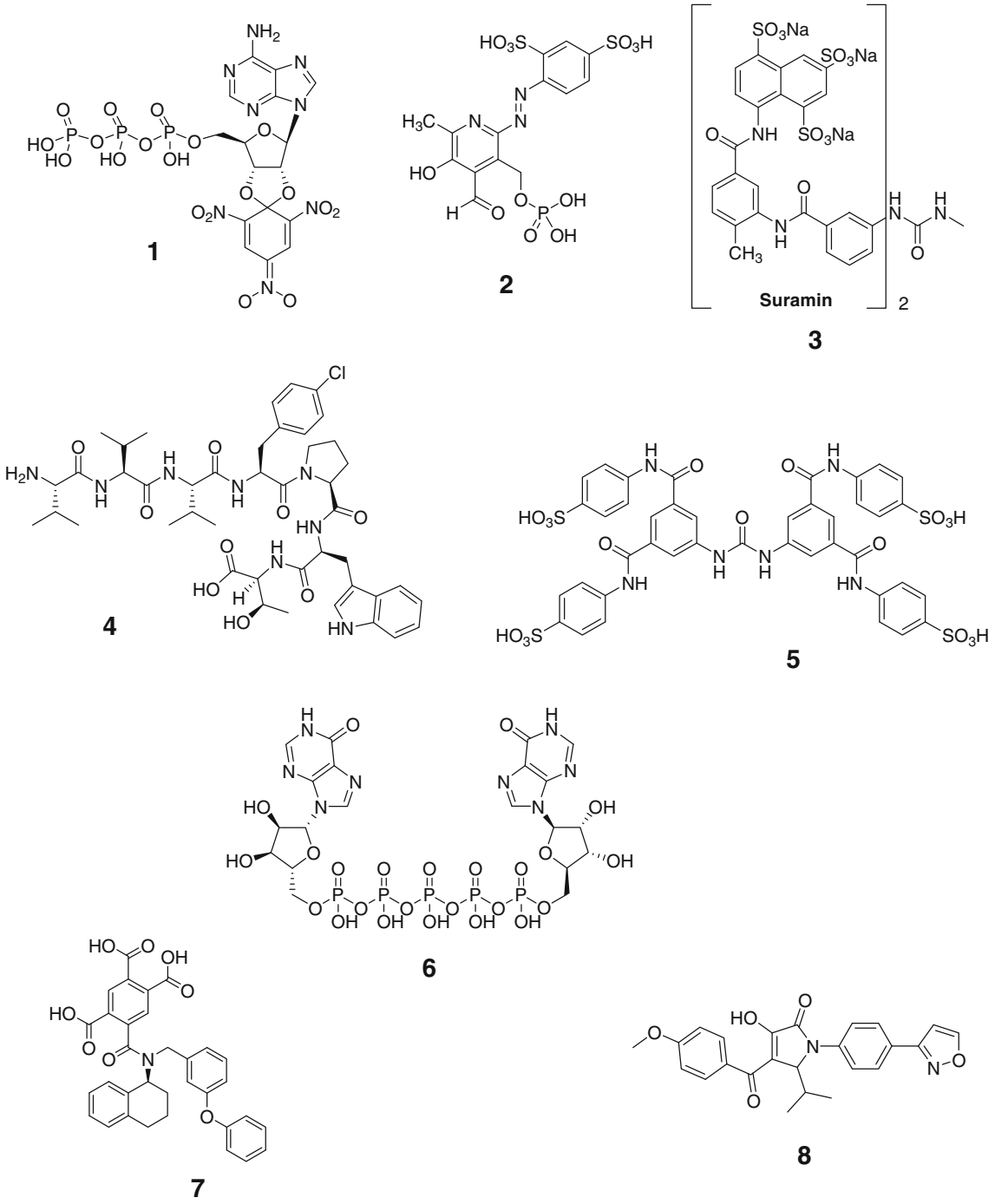

Fig. 3 Chemical structures of antagonists for P2X3 receptors

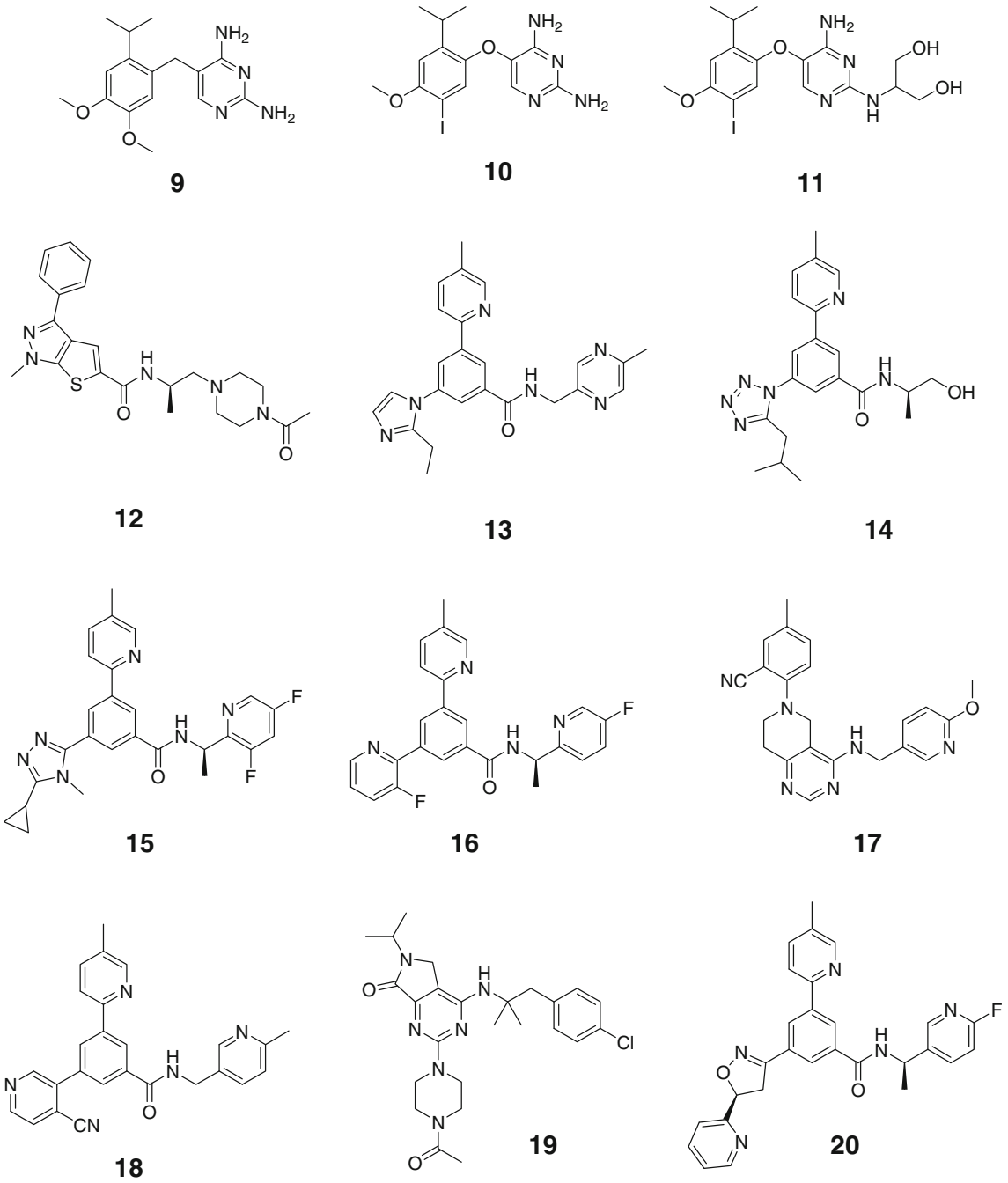

Fig. 3 (continued)

onists that have been studied in a wide variety of animal pain models [8, 26–31]. The utility of these antagonists for delineating mechanistically specific contributions of individual P2X receptors to pain is limited by their nonselective pharmacology and generally weak potency [10]. The poly-pharmacological activities of early P2X receptor antagonists have also generated conflicting reports of both pronociceptive and antinociceptive effects following P2X receptor blockade [26].

2′(3′)-*O*-(2,4,6-Trinitrophenyl) ATP (TNP-ATP; compound 1) is a nonselective but highly potent antagonist of P2X1 receptors and P2X3 receptors [9, 29]. The ability to use this antagonist for preclinical pain studies in rodents is limited by its poor metabolic stability in plasma [30]. However, direct administration of TNP-ATP into relevant sites has been shown to block the pronociceptive effects of P2 receptor agonists [9, 31].

A-317491 (compound 7) has nanomolar affinity for blocking both P2X3 and P2X2/3 receptors and is a

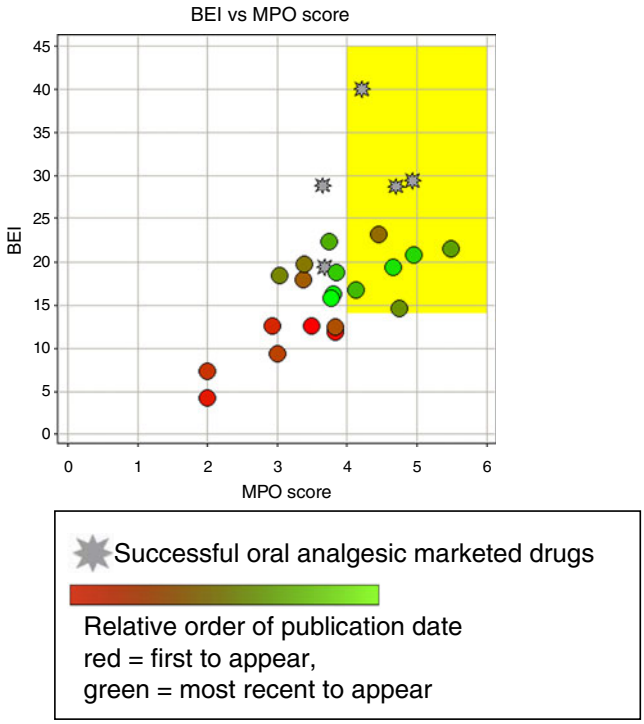

Fig. 4 Analysis of P2X3 antagonist efficiency and physical chemical properties. Early identified antagonists lacked acceptable lead-like characteristics and are associated with low oral bioavailability and generally poor drug-like properties. In contrast, recently disclosed P2X3 antagonists have improved physicochemical properties that are consistent with orally bioavailable leads and drugs. These compounds demonstrate the significant improvement in the quality of P2X3 antagonist chemical matter over time. The newer compounds have helped to solidify the preclinical validation of the role of P2X3 in pain (see text), as well as demonstrate the feasibility of successfully advancing lead molecules into clinical trials

competitive antagonist [32]. Peripheral and spinal administration of A-317491 attenuates complete Freund's adjuvant (CFA)-induced inflammatory hyperalgesia [33]. A-317491 has limited CNS penetration following systemic administration. However, systemic administration of high doses or intrathecal administration of this antagonist effectively attenuates tactile allodynia caused by peripheral nerve injury [32, 33]. Consistent with these data, ATP-evoked activation of capsaicin-insensitive spinal P2X2/3 receptors underlies an *N*-methyl-D-aspartate (NMDA)-dependent long lasting allodynic sensitivity in rodents [34]. Another structurally different and potent P2X2/3 and P2X3 antagonist, RO-4 (compound 4), has been reported to reverse both inflammatory and bone cancer pain in experimental models [35, 36]. Following peripheral administration, RO-4 is effective in nerve injury induced pain models, presumably resulting from its ability to readily cross the blood–brain barrier [36]. Scientists at Merck have also recently disclosed a novel P2X3 antagonist, MK-3901 (compound 20), that effectively attenuates chronic inflammatory and neuropathic pain in experimental models [37]. Interestingly, AZ-2 (compound 19) represents another novel antagonist that has been reported to have greater than 300-fold selectivity for homomeric P2X3 receptors over heteromeric P2X2/3 receptors [38]. AZ-2 effectively reversed CFA-induced mechanical allodynia following systemic and intraplantar dosing but was ineffective when dosed intrathecally [38]. These data indicate that peripheral homomeric P2X3 receptors may play a key role in inflammatory pain. Taking all the available data into account, it appears that the heteromeric P2X2/3 receptor at key synapses in the spinal cord are essential for the modulation of nociceptive input from the periphery.

Figure 4 shows the BEI/MPO analysis for existing P2X3 receptor antagonists. Early P2X3 antagonists including compounds 1–6 in Table 3 (colored red in Fig. 4) are high molecular weight antagonists with multiple phosphonate and sulfonate groups, and as expected, do not fit well into lead-like chemical space. A-317491(compound 7) was the first selective small molecule compound for P2X3 and P2X2/3. Subsequently, other potent P2X3 antagonists were reported including RO-3 (compound 9), and RO-4 (compound 10) that are at least 100-fold less active across a wide range of kinases, receptors, and ion channels [35, 36, 39]. These compounds have improved drug-like properties relative to earlier P2X3 antagonists (e.g., compounds 1–6) (Fig. 4). Comparing potent and selective P2X3 receptor antagonists, RO-3 and RO-4 have significantly improved BEI and MPO scores that correlate with enhanced oral bioavailability, lower plasma protein binding, and good CNS penetration relative to A-317491 [35, 36].

Table 4 In vitro potency and physicochemical summary of antagonists for P2X4 receptors

Compound no.	Name	P2X4 IC_{50} (nM)	BEI	MPO score	MW	CLogP	PSA	HBA	HBD	LogD	References
1	TNP-ATP	15,000	6.8	3.5	714.3	−6.4	398	23	5	−1.7	[82]
21	Brilliant Blue G	3,160	6.6	2.8	831	−3.7	142	9	3	1.0	[63]
22		500	17.7	5.1	355.2	4.2	41	2	1	3.2	[89]
23		~8,000	13.7	4.7	371.3	4.8	25	2	1	3.9	[84]
24		6,100	16.0	3.6	326.5	4.3	34	3	1	3.2	[84]
25		5,300	15.9	4.6	332.9	4.3	34	4	1	2.8	[84]
26	Paroxetine	1,870	17.4	5.7	329	3.3	40	4	1	0.9	[52]

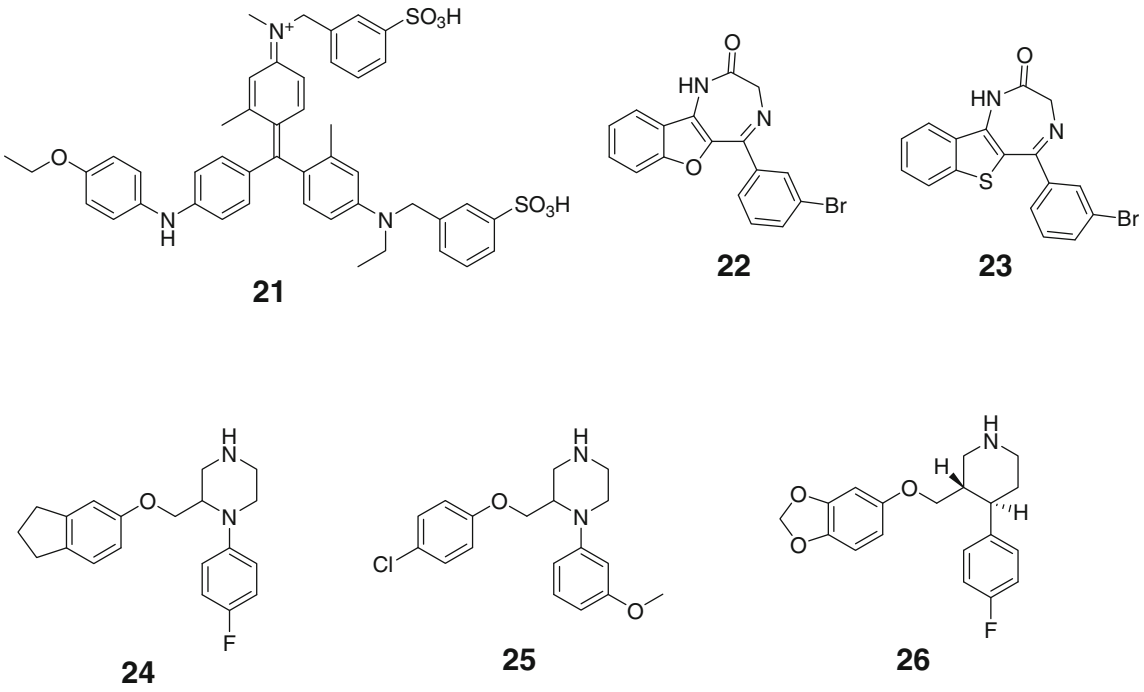

Fig. 5 Chemical structures of antagonists for P2X4 receptors

More recently, other potent P2X3 antagonists that are much more drug like have been disclosed (colored green in Fig. 4). Many of these newer molecules fit into lead-like space. RO-85 (compound 12), a potent and selective P2X3 antagonist with a high MPO score of 4.75, is reported to have high oral bioavailability (%*F*=89) in rats [36]. MK-3901 has an MPO score of 3.8 and has good (%*F*=60) oral bioavailabilty [37]. Another potent P2X3 antagonist, AF-219 (structure undisclosed), is reported to have advanced into clinical studies [39].

P2X4 receptors

A role for P2X4 receptors in the pathophysiology of chronic pain has largely been delineated based on receptor expression and genetic disruption studies. Within the CNS, cell surface expression of P2X4 receptors on microglial cells follows trauma or exposure to pro-inflammatory agents (e.g., bacterial lipopolysaccharides) [40]. This increased expression appears to be specific since P2X4 receptor protein expression is not increased in neurons or astrocytes following trauma or injury [27, 41]. Several intracellular signaling pathways have been implicated in the upregulation of P2X4 receptor expression including the PI3K/AKT pathway [42], and lyn kinase via an associated fibronectin/integrin mechanism [43–49].

P2X4 (−/−) mice show normal sensory function but diminished nociceptive sensitivity following nerve injury [45]. Additionally, intrathecal administration of a specific P2X4 receptor antisense oligodeoxynucleotide decreases

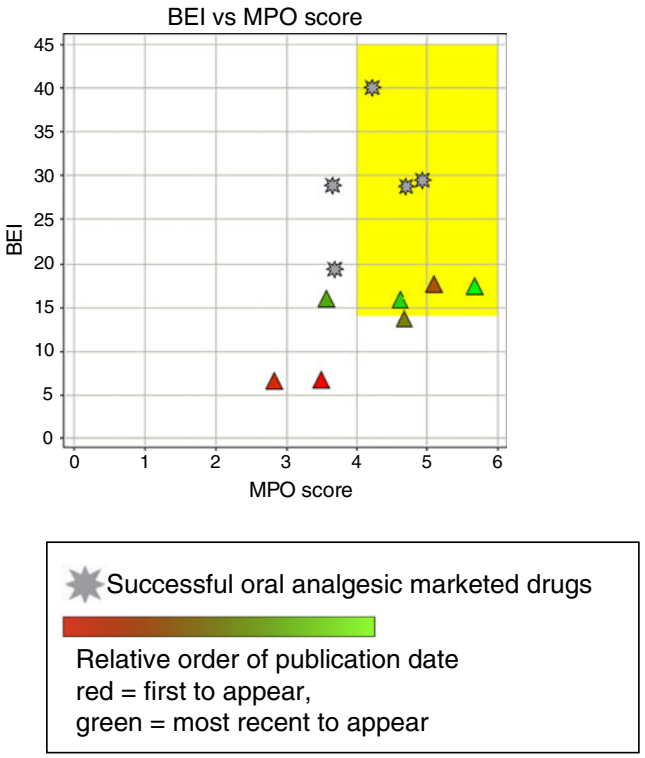

Fig. 6 Analysis of P2X4 antagonist efficiency and physical chemical properties. Early identified P2X4 antagonists suffer from low affinity and selectivity for blocking P2X4 receptors. Additionally, these antagonists also lacked lead-like characteristics. While some recently disclosed P2X4 antagonists have improved drug-like properties, potency and selectivity for specifically blocking only P2X4 receptors remains a challenge. These factors have hampered the pharmacological validation of P2X4 for pain indications

P2X4 receptor expression and suppressed tactile allodynia following peripheral nerve injury [27]. Intrathecal infusion of ATP-stimulated microglia cells that express P2X4 receptors also produces allodynia in normal rats [27]. This action appears to be mediated by increased intracellular Cl^- concentrations in lamina I neurons that is dependent on brain-derived neurotrophic factor (BDNF) and neurotropin (TrkB) receptor signaling pathways [45, 47]. P2X4 receptor stimulation results in down-stream signaling involving PIP2, PIP3, and p38 MAPK and subsequent release of BDNF [48, 49]. Microglial P2X4 receptor stimulation also leads to phosphorylation of the NMDA receptor NR1 on subunit spinal neurons [45] that is likely mediated by P2X7-dependent release of interleukin-1β (IL-1β). These events lead to increased synthesis of microglial BDNF that contributes to a slow and prolonged release of this trophic factor [49].

Pharmacological validation of the role of P2X4 receptors in chronic pain states is limited due primarily to the lack of receptor-selective antagonists. TNP-ATP has also been used as a putative antagonist of P2X4 receptors [41]). While TNP-ATP (compound 1) does block P2X4 receptor activation (IC_{50}=15 μM), it is more than 1,000-fold more potent in blocking P2X1 and P2X3 receptors [9, 50]. Since presynaptic P2X3 and P2X2/3 receptors are also expressed on the superficial lamina of the dorsal horn of the spinal cord, the relative contributions of blocking P2X4, in

Table 5 In vitro potency and physicochemical summary of antagonists for P2X7 receptors

Compound #	Name	P2X7 IC_{50} (nM)	P2X7 IC_{50} (rat, nM)	BEI	MPO score	MW	CLogP	PSA	HBA	HBD	LOGD	References
2	PPADS	3,500		10.8	3.8	507	−9.5	262	15	5	−2.6	[63, 64]
21	Brilliant Blue G	1,950	603	6.9	2.8	831	−3.7	142	9	3	1.0	[63, 64, 90]
27	NF-279	20,000		7.0	3.0	676	−13.9	271	12	7	−1.7	[82]
28	KN-62	14		10.9	2.7	722	5.4	130	9	0	5.1	[89]
29		1	19	21.0	3.6	423	5.6	86	5	4	2.7	[10]
30		398		16.5	4.3	389	4.4	57	4	2	2.5	[89]
31		16		18.6	4.1	420	4.3	65	4	2	2.6	[89]
32		40		23.9	4.0	309	5.5	58	2	2	3.7	[62]
33		14	>10,000	17.0	3.5	464	3.6	103	7	0	3.7	[63, 64]
34		10		22.6	2.9	354	6.8	24	2	1	4.0	[89]
35		<200		15.2	3.1	442	4.2	76	4	2	3.9	[89]
36	A-438079	126	316	22.5	5.2	306	3.2	56	4	0	2.7	[63, 64]
37	A-740003	44	18	15.5	2.4	475	3.2	121	8	3	3.4	[63, 64]
38	A-804598	11	10	25.2	4.5	315	3.2	73	5	2	3.0	[84]
39		100		12.4	3.7	564	1.8	104	8	3	1.9	[91, 92]
40		12		18.5	5.4	428	3.4	83	5	1	1.8	[62]
41		0.079		22.9	3.9	442	4.3	94	3	2	4.2	[62]
42		0.398		21.5	4.1	436	2.9	71	3	2	3.6	[62]
43		10		23.9	5.8	335	2.3	49	2	1	2.4	[62]
44		10	45	24.9	4.3	321	3.5	54	3	2	3.2	[62]
45		15	12	20.1	4.6	389	4.0	59	5	1	3.6	[62]
46		11	40	19.8	3.8	402	4.6	56	4	1	3.9	[62]
47	GSK314181A, AACBA	18	29	17.4	3.9	402	4.6	58	3	2	2.9	[70]
48		<100		20.0	5.8	351	2.7	59	3	1	1.8	[62, 84]
49		<2,000		12.5	3.2	455	6.1	69	4	1	5.4	[84]
50		<2,000		11.9	3.7	464	5.0	79	4	2	3.6	[84]
51		1		19.9	4.9	444	2.9	89	5	2	3.3	[84]
52		4		18.8	4.1	447	2.1	98	6	4	1.7	[84]
53		<1,000		16.1	4.2	373	3.7	68	4	1	3.5	[93]
54		<2,000		11.8	4.3	483	0.5	101	6	2	1.0	[84]
55		3		17.3	3.9	490	3.2	99	5	3	2.3	[62]
56		27		18.9	4.9	100	1.7	75	4	2	2.6	[94]
57		13	13	16.4	3.7	480	3.5	56	4	0	3.5	[95]

addition to P2X3, receptors in chronic pain states has not been delineated. P2X4 receptor-mediated currents are insensitive to block by the nonselective antagonists suramin or PPADS and can be potentiated by ivermectin (IC_{50}> 500 μM) which has been used as a pharmacological indicator of P2X4 activity [51].

While the genetic and neurochemistry data described above provides significant insights into the potential role of P2X4 receptors in the maintenance of chronic pain states, very few P2X4 antagonists have been reported in the literature, which may reflect the difficulty of identifying potent leads or lead-like chemical matter for this P2X receptor. A summary of the available P2X4 antagonists described to date is provided in Table 4 with corresponding chemical structures shown in Fig. 5. It should be noted that compounds 22 and 23 represent a single chemical class and compounds 24–26 represent another distinct chemical series, but this is the only other small molecule chemical class known to interact with the P2X4 receptor. Importantly, compounds 24–26 belong to a class of potent serotonin reuptake inhibitors [52]. For

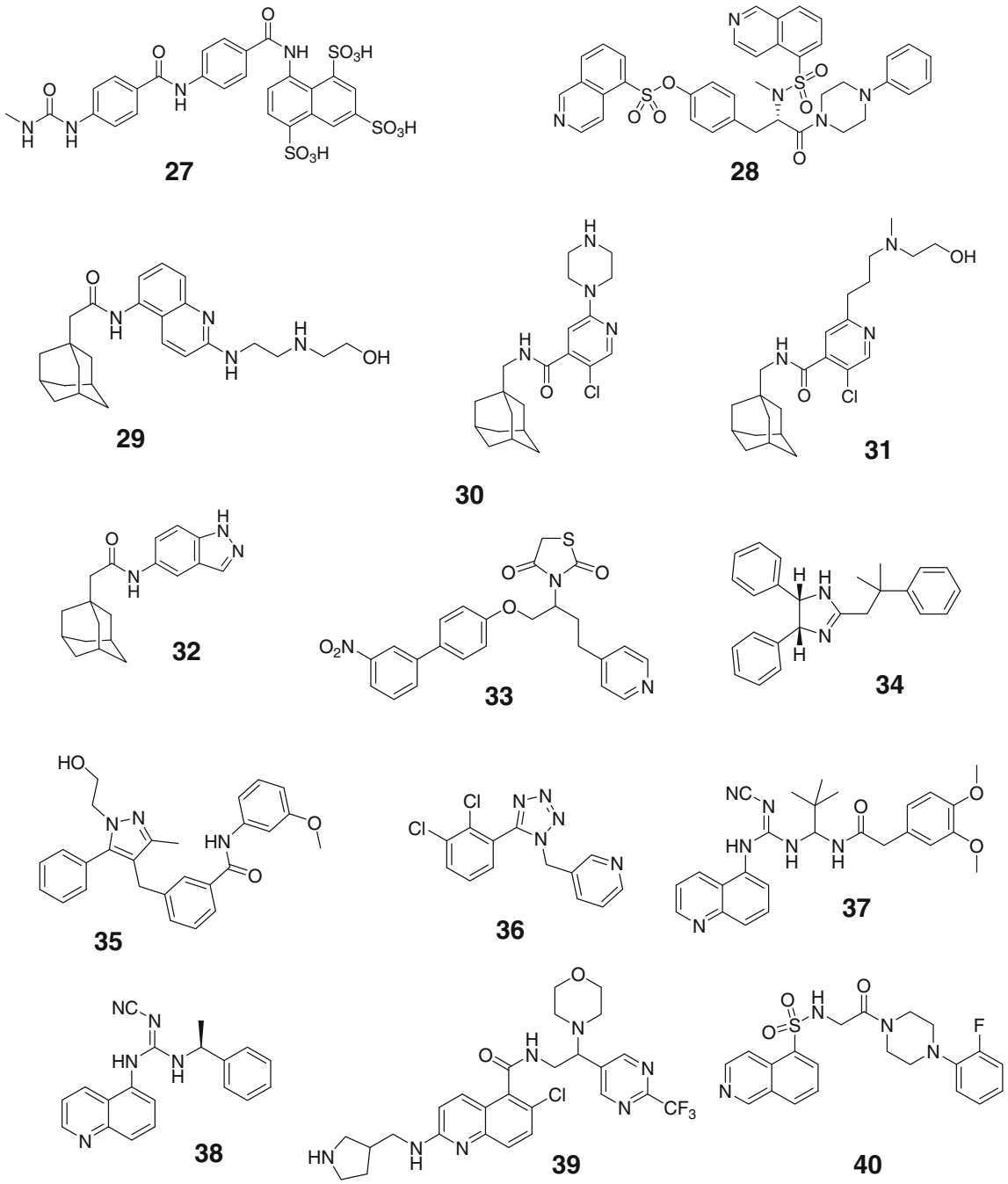

Fig. 7 Chemical structures of antagonists for P2X7 receptors

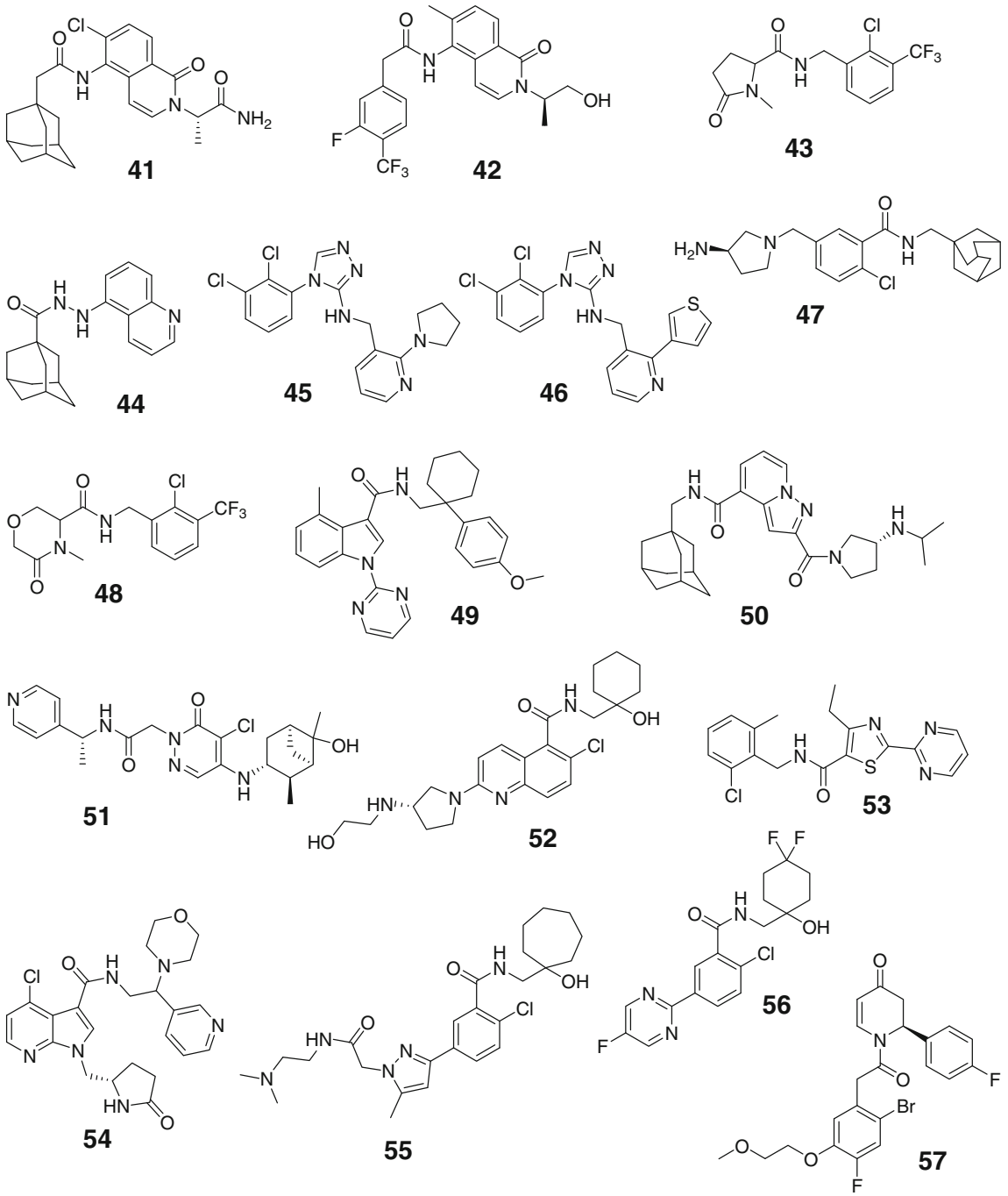

Fig. 7 (continued)

example, compound 26, paroxetine, is a clinically useful antidepressant. Compound 22 is reported to have an IC_{50} value of 500 nM and has appropriate physicochemical properties to place it within lead-like chemical space (Fig. 6). However, the relative selectivity of this compound for other targets including other P2X receptors has not been reported. Although compounds 25 and 26 also fall within lead-like space (Fig. 6), they are weak antagonists and meet the BEI and MPO lead-like criteria only as a result of their low molecular weight and superior physicochemical properties. The recent elucidation of the closed state crystal structure of the P2X4 receptor [13] offers hope that potent antagonists can now be rationally designed.

P2X7 receptors

P2X7 receptors are expressed on quiescent and activated microglia in the CNS and on macrophages in the periphery

[5, 30, 40, 53]. Agonist stimulation of P2X7 receptors opens a nonselective cation channel and also leads to the rapid maturation and release of IL-1β [53–55]. Prolonged (>60 s) receptor activation leads to the formation of large cytolytic pores in the cell membrane that are produced, at least in part, by receptor-mediated down-stream signaling events linked to the recruitment of hemichannels to the cell surface [5, 48–57].

P2X7(−/−) mice exhibit disrupted cytokine signaling cascades and attenuated ATP-induced processing of pro-IL-1β [58]. In a collagen monoclonal antibody model of arthritis, P2X7(−/−) mice also show a decreased incidence and severity of arthritis as compared to wild-type control mice [58]. P2X7(−/−) mice also show reduced nociceptive sensitivity in inflammatory and neuropathic pain states compared to wild-type controls and this phenotype is consistent with the antinociceptive phenotype of mice lacking both isoforms of IL-1 (IL-1α and IL-1β) [59–61].

Given the demonstrated role of P2X7 in both inflammatory and pronociceptive signaling, the development of receptor-selective antagonists has received much attention. Multiple chemically distinct selective P2X7 receptor antagonists have been recently described (Table 5; Fig. 7) [62]. Many of the new antagonists are systemically bioavailable. One limitation for some P2X7 antagonists is their differential activity at rodent and human receptor isoforms [63, 64]. Examples include compounds like KN-62 (compound 28) and Brilliant Blue G (BBG; compound 21) that show preferential activity at human or rat P2X7 receptors, respectively [63]. Additionally, many of the adamantane-based antagonists (e.g., compounds 30–32) also show less activity at rat versus human P2X7 receptors [64]. Early putative P2X7 receptor antagonists also have significant ancillary pharmacological actions. Examples include oxidized-ATP which is a weak (IC_{50}>1 mM) antagonist that has anti-inflammatory actions independent of its affinity for P2X7 receptors [5, 10, 65]. BBG (compound 21) has recently been shown to potently block sodium channels and P2X4 receptors [66]. NF-279 (compound 27) also is active against P2X1 [64]). KN-62 (compound 28) was the first potent P2X7 antagonist identified [10]. None of these four early compounds (shaded red) fall into lead-like space as exemplified by the yellow box in Fig. 8. However, nearly half of the more recently identified P2X7 antagonists are within lead or drug-like space, potentially indicating P2X7 is a highly druggable target (shaded green in Fig. 8).

The discovery of selective P2X7 receptor antagonists has helped clarify the role of this receptor in states of ongoing pain and inflammation [64, 67–69]. For example, AACBA (compound 47) has been reported to attenuate collagen-induced arthritis following prophylactic dosing in rats [70]. An analog from a series of adamantine P2X7 receptor-selective antagonists from AstraZeneca has provided

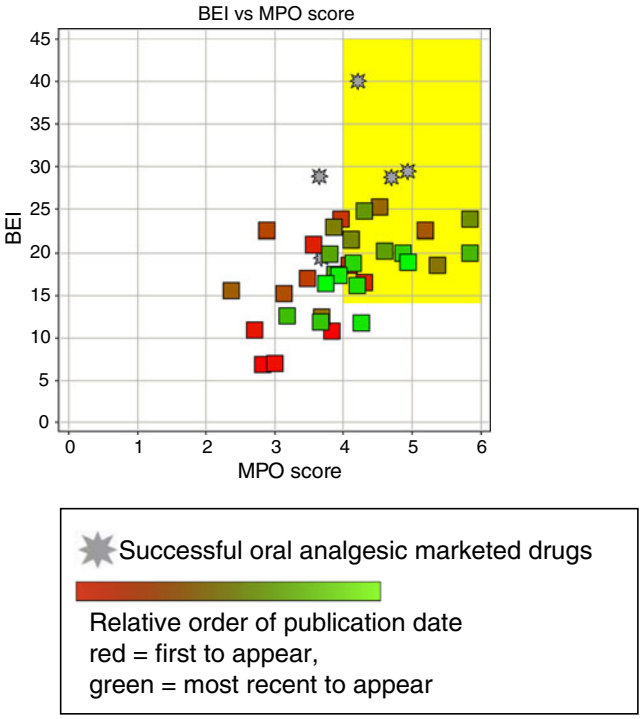

Fig. 8 Analysis of P2X7 antagonist efficiency and physical chemical properties Early identified antagonists lacked acceptable lead-like characteristics and are associated with low oral bioavailability and generally poor drug-like properties. Further, many of these antagonists also had generally low potency for blocking P2X7 receptors and showed significant species differences. In contrast, recently disclosed P2X7 antagonists have improved potency, selectivity, and physico-chemical properties that are consistent with orally bioavailable leads and drugs. These compounds demonstrate the significant improvement in the quality of P2X7 antagonist chemical matter over time. The newer compounds have helped to solidify the preclinical validation of the role of P2X7 in inflammatory and chronic pain states (see text). Compared with the available chemical matter for antagonists of P2X3 and P2X4 receptors, the diversity of chemical matter for P2X7 antagonists suggests it may be a highly druggable target of pain and inflammation

signals of clinical efficacy in reducing the symptoms of rheumatoid arthritis [71].

In experimental models of chronic nociception, A-740003 (compound 37) and A-438079 (compound 36), represent selective and competitive P2X7 antagonists that reduce inflammatory hyperalgesia in rodents [72, 73]. These effects do not appear to be secondary to P2X7-mediated anti-inflammatory effects in models of acute inflammation that are largely driven to prostaglandin E2 [72, 73]. P2X7 receptor-selective antagonists also reduce nociception in some, but not all, experimental models of neuropathic pain [67–69]). These effects have been reported to be mediated by spinal and/or supra-spinal sites of action based on the of ability A-438079 (compound 36) to reduce the firing of nociceptive specific and wide dynamic range spinal neurons in neuropathic rats [69]. Taken together, these data support a role for P2X7 receptors

on microglial cells in mediating pronociceptive cytokine production (e.g., IL-β) in states of persistent pain. Thus, the contribution of the P2X7 receptor to spinal nociceptive processing is enhanced following a neuropathic injury and appears to modulate a diverse spectrum of inputs affecting spinal neuronal excitability. However, this action may not be mechanistically relevant in all neuropathic pain conditions. Recent data from Heegaard and colleagues have demonstrated the lack of microglial cell involvement and P2X7 antagonist mediated antinociception in two murine models of cancer pain [73].

Conclusions

The molecular cloning and characterization of the P2 receptor superfamilies has greatly contributed to the development of P2 receptor-selective antagonists. In the context of pain research, these advances have enabled initial investigations into the individual contributions of specific P2 receptors that are involved in the initiation and maintenance of chronic pain. Data based on the use of receptor-selective antagonists has clearly demonstrated mechanistically specific roles for P2X3 and P2X7 receptors in mediating nociceptive sensitivity. In contrast, definitive pharmacological validation for P2X4 receptors in modulating microglial cells during ongoing pain remains to be demonstrated. From a ligand recognition perspective, it is surprising that there is a diversity of pharmacophores that have potent and selective antagonist actions at P2X7 receptors. Such diversity is much more constrained for antagonists of P2X3 receptors and essentially absent for P2X4 receptor antagonists. The present analysis also shows that progress has been made in the generation of useful pharmacological tools for P2X3 and P2X7 receptors that have drug-like characteristics. Further, P2X3 and P2X7 receptor leads have advanced into early clinical evaluation.

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