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REVIEW ARTICLE

Recent Advances in the Development of Antidepressants Targeting the Purinergic P2X7 Receptor

Shinyoung Lee[1,†], Hyunsoo Ha[1,†], Jaebong Jang[1] and Youngjoo Byun[1,2,*]

[1]*College of Pharmacy, Korea University, 2511 Sejong-ro, Sejong 30019, Korea;* [2]*Institute of Pharmaceutical Science and Translational Research, Korea University, 2511 Sejong-ro, Sejong 30019, Korea*

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Abstract: The purinergic P2X7 receptor (P2X7R) is an adenosine triphosphate (ATP)-gated cation channel protein. Although extracellular ATP (eATP) is maintained at the nanomolar concentration range under normal conditions, it is elevated to micromolar levels in response to cell stress or damage, resulting in activation of P2X7R in the brain. The binding of eATP to P2X7R in glial cells in the brain activates the NLRP3 inflammasome and releases pro-inflammatory cytokines, such as IL-1β, IL-6, IL-18, and TNFα. Depression has been demonstrated to be strongly associated with neuroinflammation activated by P2X7R. Therefore, P2X7R is an attractive therapeutic target for depression. Multinational pharmaceutical companies, including AstraZeneca, GlaxoSmithKline, Janssen, Lundbeck, and Pfizer, have developed CNS-penetrating P2RX7 antagonists. Several of these have been evaluated in clinical trials. This review summarizes the recent development of P2X7R antagonists as novel antidepressant agents in terms of structural optimization, as well as *in vitro/in vivo* evaluation and physicochemical properties of representative compounds.

Keywords: Purinergic P2X7 receptor, depression, neuroinflammation, ATP, structure-activity relationship, BBB penetration.

1. INTRODUCTION

Depression is a mood disorder that causes a persistent feeling of sadness and leads to a loss of interest in affected individuals. It is one of the major challenges to overcome in public health worldwide. According to the World Health Organization (WHO), more than 260 million people suffer from depression [1]. In particular, 8-12% of the population in industrialized countries have been affected by depression at least once in their lifetime [2]. Patients with depression are treated clinically using selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), or atypical antidepressants [3, 4]. However, treatment resistance occurs in up to 30% of the treated patients diagnosed with major depressive disorder (MDD), the most common psychiatric disorder [5], suggesting that new therapeutics are urgently needed. Esketamine, an antagonist of the *N*-methyl-*D*-aspartate receptor, has been clinically tested as an alternative in adults with MDD and suicidal ideation [6, 7]. However, concerns about the duration of esketamine long-term prescription were reported [8].

Recent studies have demonstrated that depression is strongly associated with neuroinflammation and microglial activation. The purinergic signaling pathway plays an important role in the communication between neurons and microglia cells in the brain [9]. In particular, the purinergic P2X7 receptor (P2X7R), an ATP-gated cation channel, is overexpressed in immune cells, including microglia. This receptor can be a potential target for controlling neuroinflammation because P2X7R is ranked at the top of the neuroinflammation hierarchy. P2X7R is activated by high concentrations of extracellular adenosine triphosphate (eATP), which mainly occurs during cellular damage or stress [10]. The activation of P2X7R allows the inward passage of Na^+ and Ca^{2+} ions and the outward passage of K^+ ions through the cell membrane of the glial cells. In addition, as repeated and prolonged activation of P2X7R results in the opening of membrane pores, large organic cations, such as the fluorescent dye YO-PRO-1, could pass through the cell membrane [11].

*Address correspondence to this author at the College of Pharmacy, Faculty of Pharmacy, Korea University, P.O. Box: 30019, Sejong City, South Korea; Tel: +82-44-860-1619;
Fax: +82-44-860-1606; E-mail: yjbyun1@korea.ac.kr
†*These authors contributed equally to this work.*

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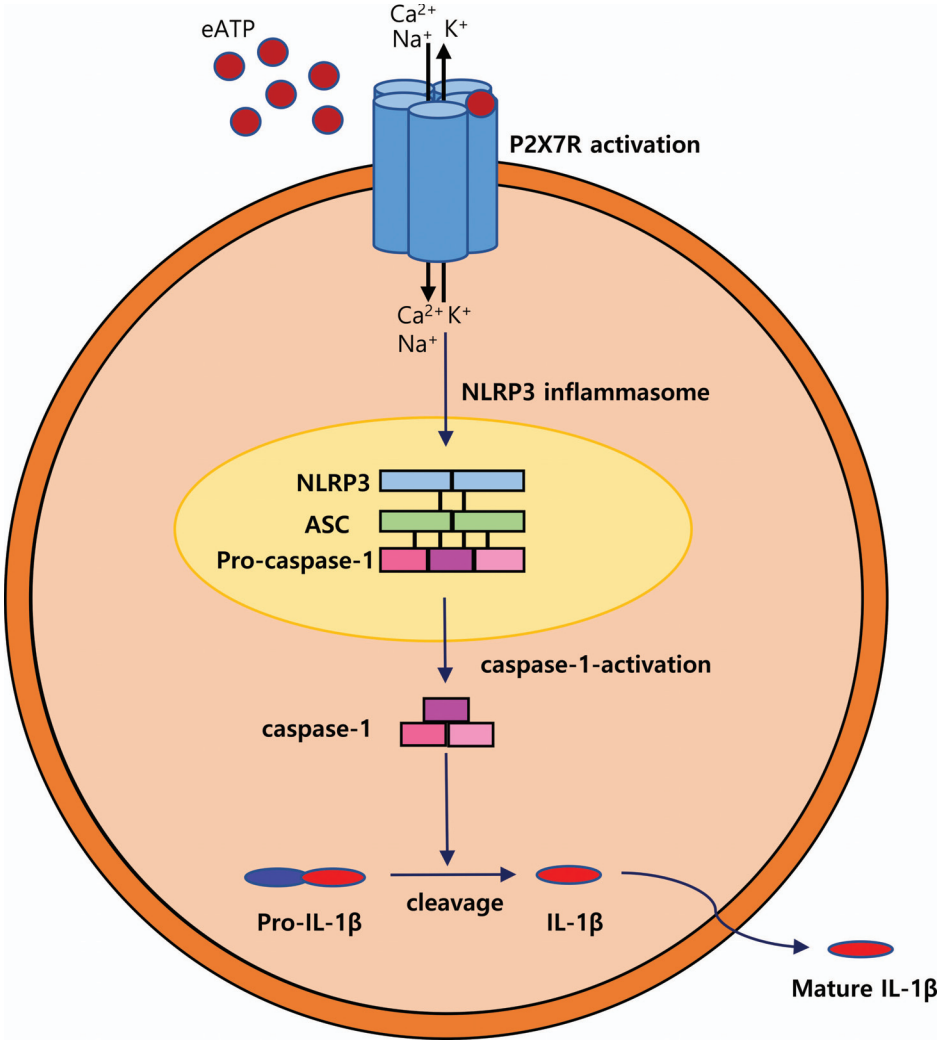

Fig. (1). P2X7R/NLRP3 inflammasome/IL-1β pathway for neuroinflammation. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The mechanism of P2X7R in the pathogenesis of depression has recently been reported. Upon external and internal stimulation, ATP is synthesized in terminal neurons, glial cells, or astrocytes and is released into the extracellular space. Extracellular ATP binds to P2X7R in microglial cells and activates the innate immune response by interacting with the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome [12]. The NLRP3 inflammasome contains NLRP3 as a sensor molecule, apoptosis-associated speck like protein containing a caspase recruitment domain (ASC) as an adaptor protein, and pro-caspase-1. The formation of this complex generates caspase-1-activation, which leads to the cleavage of pro-interleukin 1 beta (pro-IL-1β) to produce mature IL-1β (Fig. **1**). IL-1β and downstream cytokines (*e.g.*, IL-6, IL-18, and TNFα) alter neuron-glial network functions, affecting mood disorders [13].

P2X7R is different from other P2X receptors in terms of protein structure and sensitivity to P2X receptor ligands. P2X1−P2X6 receptors have a structure of 379−472 amino acids, while the P2X7R subunit contains 595 amino acids with a long COOH-terminal chain. P2X7R is generally 100-1000 times less sensitive to eATP than other P2X receptors [14]. Therefore, millimolar concentrations of eATP are necessary to activate P2X7R. P2X7R has two allosteric ligand-binding sites located in the extracellular domain [15]. Most of the reported P2X7R antagonists bind to the allosteric ligand-binding pocket placed in the upper vestibule of the receptor, hindering the conformational changes of the residues that are involved in the pore opening. Therefore, P2X7R is considered an important gatekeeper of neuroinflammation and can be a potential therapeutic target for depression.

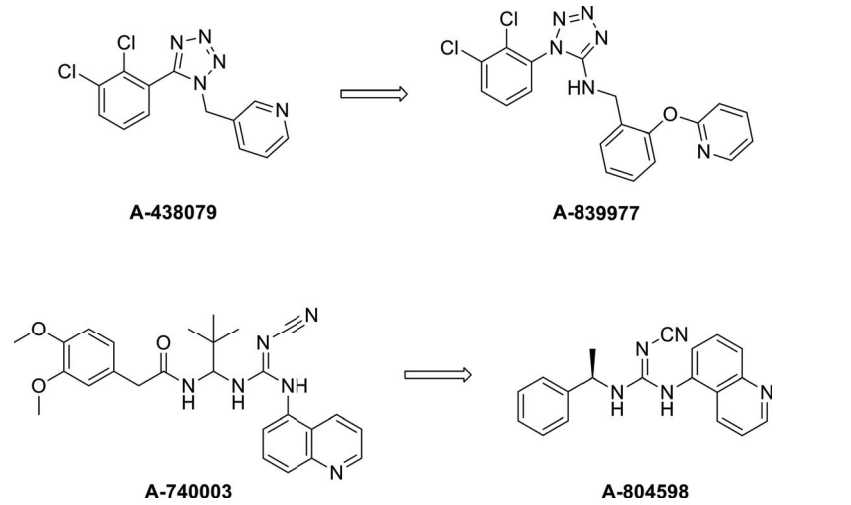

Fig. (2). Representative P2X7R antagonists developed by Abbott Laboratories.

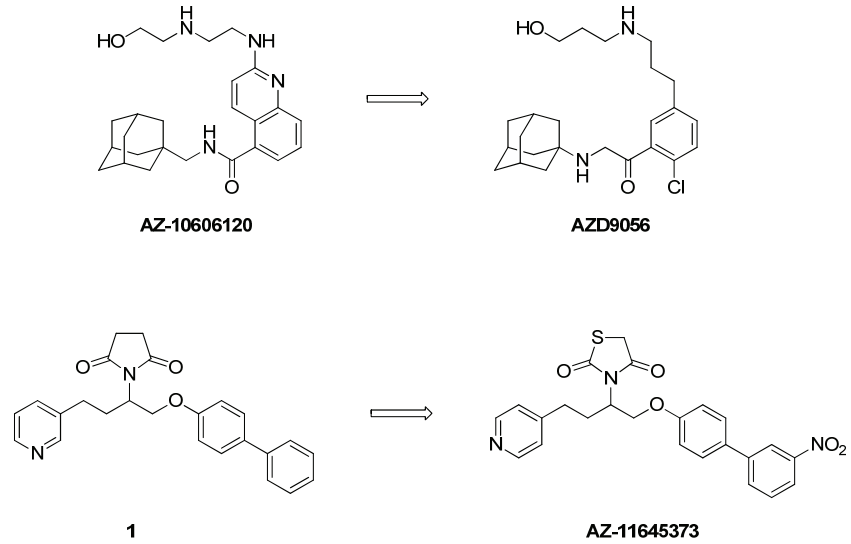

Fig. (3). Representative P2X7R antagonists developed by AstraZeneca.

In this review, we describe the recent development of P2X7R antagonists as a new option for antidepressant agents. We discuss the structural characteristics of the current P2X7R antagonists, their mechanism of action, *in vitro* activities, *in vivo* efficacy in animal models, and clinical studies on representative drugs.

2. P2X7R ANTAGONISTS

2.1. Abbott Laboratories

2.1.1. A-438079

Abbott Laboratories performed a high-throughput screening (HTS) of the compound libraries and identified that the disubstituted phenyl tetrazole analog is a novel P2X7R antagonist (Fig. **2**). A-438079 (3-[[5-(2,3- dichlorophenyl)-1*H*-tetrazol-1-yl]methyl] pyridine) displayed moderate antagonism with IC_{50} values of 126 nM and 316 nM against human P2X7R and rat P2X7R cell lines by determining Ca^{2+} flux, respectively [16]. The brain-to-plasma ratio of A-438079 was approximately 2, suggesting that it could penetrate the blood-brain barrier (BBB) [17]. However, structural optimization of A-438079 was needed because of its weak P2X7R-inhibitory activity [16].

2.1.2. A-839977

When the pyridin-3-ylmethyl moiety of A-438079 was replaced with a benzyl group, human and rat P2X7R inhibition significantly increased [18]. However, this replacement lowered the solubility of the benzyl-containing compounds. To improve the physicochemical properties, the pyridine-2-oxybenzyl group was introduced to maintain the 2,3-dichlorophenyl tetrazole ring, which led to the discovery of A-839977.

A-839977 potently blocked Ca^{2+} flux in 1321N1 cells stably expressing human P2X7R (IC_{50} = 20 nM) and rat P2X7R (IC_{50} = 42 nM), demonstrating that A-839977 is a more potent P2X7R antagonist than A-438079. A-839977 displayed antinociceptive effects in rat and mouse models of inflammatory pain. However, A-839977 showed a relatively low brain-to-plasma ratio of 0.15-0.25 [18].

2.1.3. A-740003

The cyanoguanidine analog A-740003 (*N*-(1-{[(-cyanoimino)(5-quinolinylamino) methyl] amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide) blocked BzATP-evoked changes in intracellular calcium concentrations in 1321N1 cells with IC_{50} values of 44 and 18 nM for rat and human P2X7R, respectively [19]. The analgesic activity of A-740003 suggested the pharmacological role of P2X7R in controlling pain, which is related to neural-glial cell interactions [16, 20]. A-740003 labeled with [^{11}C] was assessed for P2X7R affinity using positron emission tomography (PET) scanning in healthy male Wistar rats. A-740003 showed low brain uptake and moderate metabolic rate [21].

2.1.4. A-804598

A-804598 (*N*-cyano-*N*'-[(1*S*)-1-phenylethyl]-*N*''-5-quinolinyl-guanidine) has quinoline and cyanoguanidine moieties as key pharmacophore groups found in A-740003. A-804598 exhibited strong inhibition for human P2X7R (IC_{50} = 20 nM) and even higher activity in mouse and rat P2X7Rs by Ca^{2+} flux assay (mouse = 8.9 nM and rat = 9.9 nM) [22]. As cross-species differences of P2X7Rs were not observed, A-804598 could be swiftly translated into preclinical studies [23]. Pharmacokinetic studies of A-804598 showed that it penetrated the blood-brain barrier and maintained a high concentration in the brain tissue to block P2X7R [24]. Furthermore, the pharmacological profile using A-804598 radiolabeled with [^{3}H] demonstrated high affinity and selectivity for P2X7R *in vivo* [25].

2.2. AstraZeneca

2.2.1. AZ-10606120

AZ-10606120 (*N*-[2-[[2-[(2-hydroxyethyl)amino] ethyl]amino]-5-quinolinyl]-2-tricyclo[3.3.1.13.7]dec-1-ylacetamide) selectively attenuated Bz-ATP induced calcium flux in human P2X7R with an IC_{50} value of 1.3 nM. Under the same conditions, it showed weak inhibition of rat P2X7R (IC_{50} = 2 μM) [22]. Intraperitoneal administration of AZ-10606120 inhibited LPS-induced depression-like symptoms in mice [26].

2.2.2. AZD-9056

AZD-9056, an amantadine analog, is a potent and orally active P2X7R antagonist. AZD-9056 displayed strong inhibition of human and rat P2X7R with IC_{50} values of 3.5 and 4.4 nM by Ca^{2+} flux assay, respectively [27]. However, AZD-9056 did not have significant efficacy in phase II clinical studies for the treatment of rheumatoid arthritis [28, 29]. However, there are no reports on the antidepressant activities of AZD-9056.

2.2.3. AZ-11645373

AZ-11645373, a thiazolidinedione analog developed from compound **1** (Fig. **3**), is a highly selective and potent antagonist of human P2X7R. The IC_{50} values of human and rat P2X7R were 10 and >10,000 nM by Ca^{2+} flux assay, respectively [30]. It also inhibited IL-1β release mediated by eATP *in vitro* [30]. AZ-11645373 was evaluated for its effect on hyperinflammation during severe influenza A virus infection in C57BL/6 mice. Intranasal treatment in mice significantly reduced the clinical signs of the disease, including weight loss and prolonged survival [31].

2.3. GlaxoSmithKline

2.3.1. GSK-1482160

Prior to the discovery of GSK-1482160, a series of pyrazol-4-yl acetamide analogs were evaluated using HTS to identify compound **2** (Fig. **4**) [32]. Structural modification of compound **2** led to the discovery of compound **3**, which was selected for the *in vivo* pain model study [33]. However, it inhibited CYP3A4 and metabolic pathways because of the oxidation of the methyl substituents of the pyrazole ring [34]. The next step was to introduce a pyroglutamic amide ring instead of a pyrazole ring, resulting in the identification of compound **4** (Fig. **4**). While removal of the isopropyl group of **4** resulted in the complete loss of activity, its replacement with methyl, ethyl, or propyl groups produced similar or slightly better P2X7R inhibition [34]. GSK-1482160, a pyroglutamic amide analog with a methyl substituent, is a potent and selective P2X7R antagonist that emerged as a front-runner among those developed by GlaxoSmithKline [35]. GSK-1482160 possessed high potency for human P2X7R (IC_{50} = 3 nM) but the lower affinity for rat P2X7R (IC_{50} = 316 nM) in a dye uptake assay. In addition, no noticeable CYP inhibition was observed at concentrations up to 100 μM [34]. It displayed a moderate

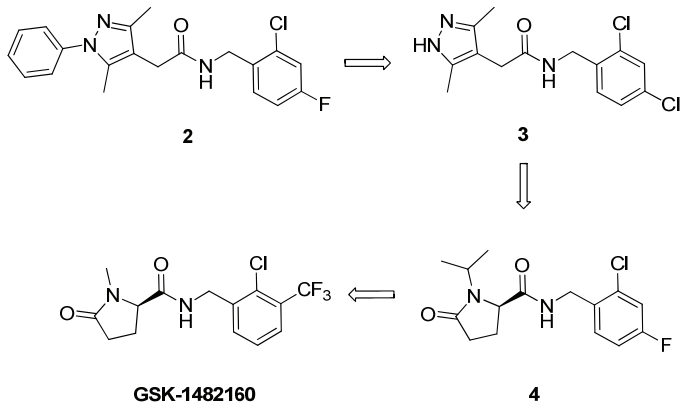

Fig. (4). Representative P2X7R antagonists developed by GlaxoSmithKline.

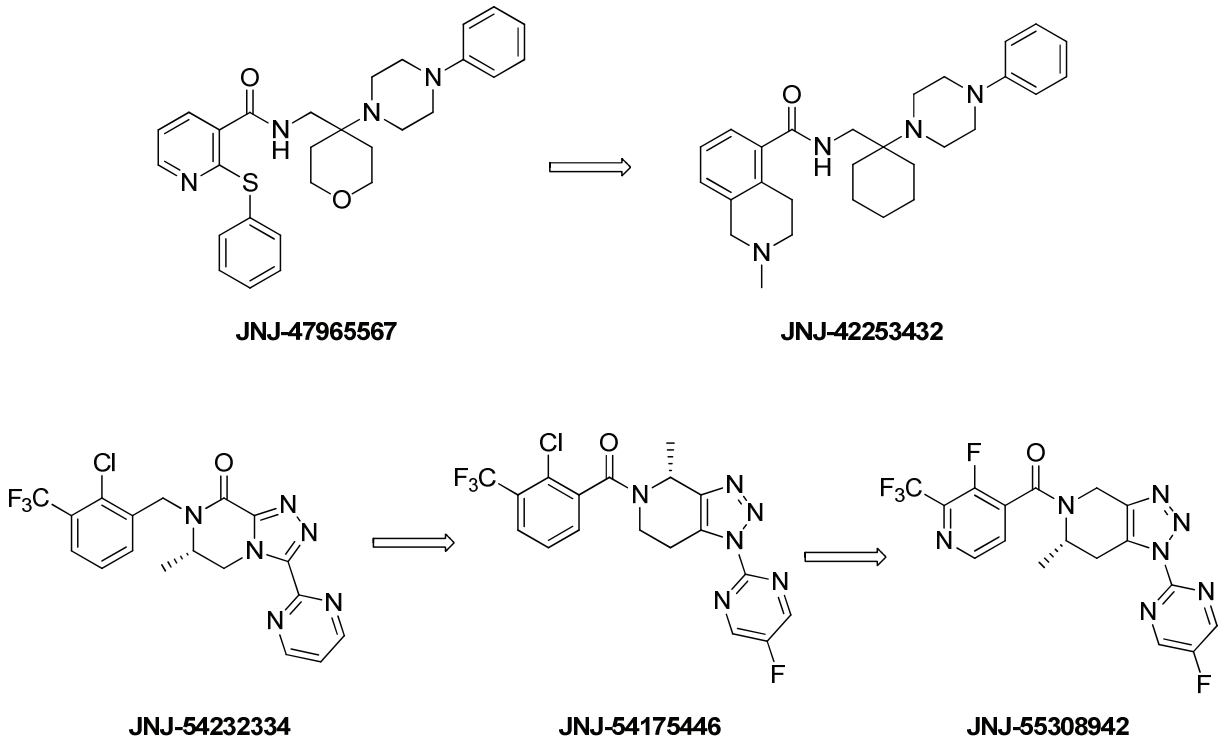

Fig. (5). Representative P2X7R antagonists developed by Janssen Pharmaceuticals.

brain-to-plasma ratio of approximately 0.5. It was evaluated as a therapeutic agent in a clinical phase I human study of depression. Although the PK profile of GSK-1482160 was excellent, the development was discontinued owing to a lack of efficacy [36].

2.4. Janssen

2.4.1. JNJ-47965567

The first CNS-penetrant compound reported by Janssen was JNJ-47965567 (Fig. **5**). The IC_{50} values of JNJ-47965567 using a Ca^{2+} flux assay were 5 and 63 nM for human and rat P2X7R, respectively [22, 37]. The efficacy of JNJ-47965567 was tested in standard models of depression, mania, and neuropathic pain. However, analysis of both plasma and brain tissue after oral dosing revealed that JNJ-47965567 lacked occupancy owing to poor oral bioavailability [22].

2.4.2. JNJ-42253432

JNJ-42253432 (2-methyl-*N*-([1-(4-phenylpiperazin-1-yl)cyclohexyl]methyl)-1,2,3,4-tetrahydroisoquino-

line-5-carboxamide) is also a potent P2X7R antagonist. The IC_{50} values of JNJ-42253432 in the calcium flux assay were 20 and 16 nM against human and rat P2X7R, respectively [38]. JNJ-42253432 exhibited five-fold higher potency against P2X7R than that of JNJ-47965567 [37]. It penetrated the BBB with a brain-to-plasma ratio of approximately 1. This compound was orally active and exhibited improved drug--like properties compared to that of JNJ-47965567. Administration of JNJ-42253432 in rats decreased the release of the proinflammatory cytokine IL-1β in the brain [38].

2.4.3. JNJ-54232334

New P2X7R antagonists with a triazolopyridine scaffold were reported by Janssen Pharmaceuticals, starting in 2015. Based on structure-activity relationship (SAR) studies, the 2-chloro-3-trifluoromethylbenzyl moiety was found to be important for high human P2X7R affinity [39]. The IC_{50} value of JNJ-54232334 using Ca^{2+} flux assay for human P2X7R was 0.3 nM, which was more potent than for rat P2X7R (32 nM) [40]. JNJ-54232334 was labeled with tritium for *ex vivo* autoradiography. The ED_{50} value of JNJ-54140515 was 0.8 mg/kg using [^{3}H]JNJ-54232334 as the radiotracer [40].

2.4.4. JNJ-54175446

JNJ-54166060 is an (*R*)-enantiomer separated from a racemic imidazopyridine analogue. It showed higher P2X7R affinity for humans (IC_{50} = 4 nM) than for rats (IC_{50} = 115 nM) by Ca^{2+} flux assay [39]. Based on the significant effect of the methyl group on the P2X7R affinity of JNJ-54166060, JNJ-54175446 was synthesized based on the 4-(*R*)-methyl-6,7-dihydro-4*H*-triazolo[4,5-*c*] pyridine scaffold. JNJ-54175446 exhibited similar inhibition for human and rat P2X7Rs with IC_{50} values of 1.5 nM and 3.5 nM in a Ca^{2+} flux assay, respectively. It also displayed good BBB penetration, with a brain-to-plasma ratio of 1 [41]. The introduction of a fluoro group at the 5-position of the pyrimidine ring increased metabolic stability, which was found to be less sensitive to oxidation and more stable in liver microsomes [41]. This compound showed high selectivity for P2X7R over other P2XRs, including P2X1R, P2X2R, P2X3R, and P2X4R [41]. The first-in-human phase I clinical trial (NCT02475148) with JNJ-54175446 was performed [42]. In this clinical trial, it was administered orally to 77 participants, with a maximum study duration of 6 weeks. Relatively low doses (30-50 mg/day) were sufficient to elicit a biological effect, and no serious adverse effects were reported [43]. Brain penetration of JNJ-54175446 was also confirmed. Phase II studies of JNJ-54175446 in 142 participants with MDD are underway (NCT04116606).

2.4.5. JNJ-55308942

JNJ-55308942 has a 2-fluoro-3-(trifluoromethyl) phenyl moiety instead of the 2-chloro-3-trifluoromethylphenyl moiety of JNJ-54175446. JNJ-55308942 exhibited strong inhibition of both human P2X7R (IC_{50} = 10 nM) and rat P2X7R (IC_{50} = 15 nM) by Ca^{2+} flux assay, respectively. The affinity (K_i) values of JNJ-55308942 for human and rat P2X7R using radioligand binding assay were 7.59 and 3.16 nM, respectively [44]. It displayed good BBB permeability with a brain-to-plasma ratio of approximately 1. It also exhibited a dose-dependent occupancy of rat brain P2X7R with an ED_{50} of 0.07 mg/kg. Oral administration of JNJ-55308942 (30 mg/kg) attenuated LPS-induced microglial activation in mice on day 2 after a single LPS injection (0.8 mg/kg, *i.p*). In addition, JNJ-55308942 was stable in both human and murine liver microsomes and demonstrated insignificant inhibition of nine CYP isoforms (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) [45].

2.5. Lundbeck

Compound **5** (Fig. **6**) inhibited human P2X7R with an IC_{50} of 0.33 nM [46]. It was the most active compound in a series of benzamide derivatives. To improve CNS permeability and PK properties, Lundbeck focused on synthesizing compounds with structural characteristics suitable for preclinical studies. Structural modification of compound **5** focused on the 2,3-dichlorophenyl moiety. Replacement of the 2,3-dichlorophenyl ring with the 2-pyrimidyl thiazole ring increased P2X7R activity [27]. Lu AF27139 ((*S*)-*N*-(2-(4-chlorophenyl)-2-morpholinoethyl)-2-(pyrimidin-2-yl)-4-(trifluoromethyl)thiazole-5-carboxamide showed similar P2X7R potency against rat (IC_{50} = 2.4 nM, K_i = 13 nM), mouse (IC_{50} = 22 nM, K_i = 180 nM), and human P2X7R (IC_{50} = 12 nM, K_i = 55 nM) by Ca^{2+} flux assay and a radioligand assay using [^{3}H]-A-804598. Lu AF27139 displayed high permeability in the MDR1-MDCK assay without efflux action. Lu AF27139 was 7.5- and 40-fold more potent than the corresponding (*R*)-enantiomer (Lu AF27138) against human and rat P2X7R, respectively. The measured log D of Lu AF27139 was 3.3. Intrinsic clearances in rat and human liver microsomes were 5.1 and 2.6 L/(h·kg), and in rat and human hepatocytes were 4.8 and 0.85 L/(h·kg), respectively. The rat pharmacokinetic profile of LuAF27139 is favorable with high oral

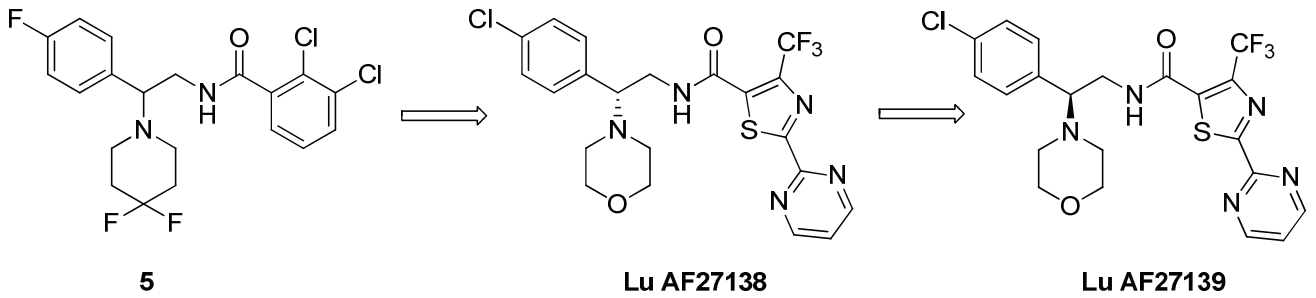

Fig. (6). Representative P2X7R antagonists developed by Lundbeck.

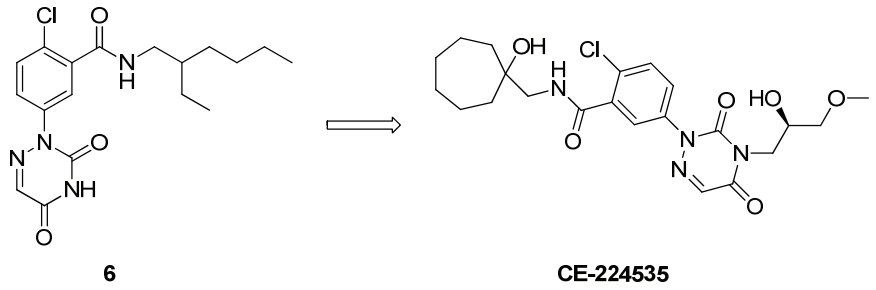

Fig (7). Representative P2X7R antagonists developed by Pfizer.

bioavailability, modest clearance [0.79 L/(h·kg)], and good CNS permeability, suggesting that it can be developed as a clinical candidate compound [27].

2.6. Pfizer

2.6.1. CE-224535

CE-224535 (2-chloro-5-{4-[(*2R*)-2-hydroxy-3-methoxypropyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazin-2-yl}-*N*-[(1-hydroxycycloheptyl)methyl]benzamidea) is a potent and highly

a selective human P2X7R antagonist with IC_{50} values of 0.76 nM in Ca^{2+} flux assay [27]. CE-224535 was developed from compound **6** (Fig. **7**), which was identified in the HTS study [47]. Pfizer advanced a P2X7R antagonist into a phase IIa clinical trial to treat rheumatoid arthritis [48]. Although CE-224535 was safe and well tolerated, it did not demonstrate efficacy outcomes compared to the placebo group in a clinical study. As the topological polar surface area of CE-224535 is 135.68, it is expected that it cannot cross the BBB.

2.7. ITH15004

ITH15004 (2-(6-chloro-9*H*-purin-9-yl)-1-(2,4-dichlorophenyl)ethan-1-one, Fig. **8**) is the first non-nucleotide purine analog that penetrates the BBB and displays selectivity for P2X7R over the other P2X receptors. The IC_{50} value of ITH15004 was high (9 μM) in hP2X7-HEK293 cells stimulated by BzATP 30 μM [45]. However, in a parallel artificial membrane permeability assay (PAMPA), ITH15004 showed higher permeability through lipid membranes than the other synthesized compounds. Although the potency of ITH15004 is in the micromolar range, its pharmacodynamic profile and BBB permeability data suggest that this scaffold could serve as a starting point for the design of new P2X7R antagonists [49].

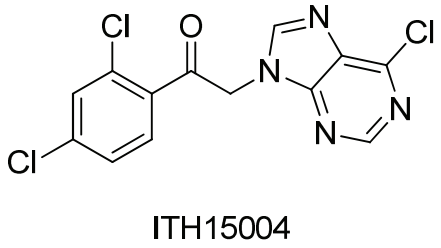

Fig. (8). Non-nucleotide P2X7R antagonists.

Table 1. Chemical structure, *in vitro* activities, and physicochemical properties of representative P2X7R antagonists.

Compound	P2X7R IC_{50} (nM)	P2X7R K_i (nM)	Brain/Plasma Ratio	M.W	# of HBA/HBD	# of RB	CLogP	tPSA	Ref.
A-438079	(h) 126 [a] (r) 316 [b]	(h) 7.1 [e] (r) 6.7 [e]	2 [g]	306.15	5/0	3	3.24	52.68	[16] [17]
A-839977	(h) 20 [a] (r) 42 [b]	-	0.15~0.25 [h]	413.26	7/1	6	4.33	73.94	[18]
A-740003	(h) 44 [a] (r) 18 [b]	-	0.11 [h]	474.56	9/3	10	3.15	120.13	[19]
A-804598	(h) 20 [a] (r) 158 [b]	(h) 8.0 [e] (r) 8.8 [e]	-	315.38	5/2	5	3.21	72.57	[22]
AZ-10606120	(h) 1.3 [a] (r) 2 μM [b]	(h) 8.5 [e] (r) 8.0 [e]	-	422.57	6/4	9	5.28	85.75	[22]
AZD9056	(h) 3.5 [a] (r) 4.4 [b]	(h) 15 [e] (r) 130 [e]	very low [h]	419.00	4/3	10	4.91	61.36	[27]
AZ-11645373	(h) 10 [a] (r) >10,000 [b]	-	-	463.50	8/10	9	3.57	110.78	[30]
GSK-1482160	(h) 3 [c] (r) 316 [d]	-	2.1 [h]	344.72	4/1	4	2.26	49.41	[34]
JNJ-47965567	(h) 5 [a] (r) 63 [b]	(h) 11 (r) 2	0.5~0.58 [h]	488.65	6/1	7	4.25	57.17	[22] [37]
JNJ-42253432	(h) 20 [a] (r) 16 [b]	(h) 11 [e] (r) 0.8 [e]	1.23 [h]	446.63	5/1	5	5.08	38.82	[38]
JNJ-54232334	(h) 0.3 [a] (r) 32 [b]	(r) 0.5 [e]	-	422.79	7/0	4	2.00	72.99	[40]
JNJ-54175446	(h) 1.5 [a] (r) 3.5 [b]	(h) 3.98 [e] (r) 4.47 [e]	~1 [h]	440.78	7/0	3	2.98	72.99	[41]
JNJ-55308942	(h) 10 [a] (r) 15 [b]	(h) 7.59 [f] (r) 3.16 [f]	1 [h]	425.32	8/0	3	1.42	85.35	[44] [45]
Lundbeck Compound **5**	(h) 0.33[a]	-	-	431.28	3/1	5	4.26	32.34	[46]
Lu AF27138	(h) 90 [a] (r) 95 [b]	-	-	497.92	7/1	7	2.16	78.65	[27]
Lu AF27139	(h) 12 [a] (r) 2.4 [b]	(h) 55 [e] (r) 13 [e]	-	497.92	7/1	7	2.16	78.65	[27]
CE-224535	(h) 0.76 [a] (r) 470 [b]	-	-	464.94	9/2	8	2.34	111.54	[27]
ITH15004	(h) 9 μM[a]	-	-	341.57	5/0	3	2.58	57.39	[49]

Abrreviations: h: Human, r: Rat,
[a] Measured in a Ca^{2+} flux assay, [b] Measured in a Ca^{2+} flux assay, [c] Measured in a dye uptake assay,
[d] measured in a dye uptake assay, [e] using [^{3}H]-A-804598 as the radioligand, [f] Radioligand binding assay, [g] Obtained from intraperitoneal administration, [h] Obtained from oral administration, HBA: number of hydrogen-bonding acceptors, HBD: number of hydrogen-bonding donors, # of RB: number of rotational bonds, tPSA: topological polar surface area.

3. DISCUSSION

Research papers were searched using Google Scholar and Web of Sciences for this review. Low-molecular-weight P2X7R antagonists were selected, and their physicochemical properties were calculated by ChemDraw (ver. 20.0.0.41) and Molinspiration site (http://www.molinspiration.com). Representative physicochemical values are summarized in Table **1**. It was reported that successful CNS drugs have the following properties: Molecular weight < 450 amu, number of hy-

drogen-bonding acceptors < 7, number of hydrogen-bonding donors < 3, number of rotational bonds < 8 [50, 51]. Some of the representative P2X7R antagonists, including A-740003, AZ-10606120, AZD-9056, AZ-11645373, and CE-224535, do not meet these criteria. Indeed, their brain-to-plasma ratio was significantly low or not observed.

The *in vitro* IC_{50} and K_i values of the reported P2X7R antagonists were determined using a calcium flux assay and a YO-PRO-1 dye uptake assay using P2X7R-expressing cell lines. The *in vitro* assay system should be considered when comparing the *in vitro* IC_{50} or K_i activities of the reported P2X7R antagonists. For *in vitro* and *in vivo* studies, 2(3)-*O*-(4-benzoylbenzoyl)adenosine-5-triphosphate (Bz-ATP), a synthetic ATP analog, is commonly used because it is a more potent P2X7R activator than natural ATP. With respect to the BBB penetration of P2X7R antagonists, PAMPA and P-glycoprotein ATPase assays were used.

P2X7R antagonists were initially developed as therapeutic agents for rheumatoid arthritis in the early 2000s by Pfizer and AstraZeneca. Although CE-224535 and AZD-9056 strongly inhibited human P2X7R and blocked IL-1β release in the patient plasma samples, neither compound met the clinical endpoints of rheumatoid arthritis. More recently, P2X7R antagonists have been evaluated as antidepressant agents. The first clinical compound was found to be GSK1482160. However, it was reported to fail in phase I clinical study conducted on 29 volunteers. Although the administered dose of GSK1482160 was proportional to C_{max} and AUC, twenty volunteers experienced headache as an adverse event, and one volunteer withdrew from the clinical trial because of accelerated idioventricular rhythm. GSK1482160 did not achieve a pharmacological level to prove the mechanism of P2X7R in a safe state. The second compound was JNJ-54175446, which was completed in phase I clinical trials. Phase I clinical trials were conducted on 77 volunteers. The administered dose was confirmed to be proportional to AUC and C_{max}. Furthermore, the BBB penetration of JNJ-54175446 was confirmed. As no serious side effects have been reported, a phase II clinical trial is currently being conducted.

When eATP binds to P2X7R, the cation channel opens, and the allosteric site of P2X7R becomes narrow. However, upon P2X7R antagonist binding to the allosteric site, the opening of the ion channel is inhibited. P2X7R antagonists in preclinical and clinical trials have used this allosteric site. The allosteric site of P2X7R contains a hydrophobic subpocket consisting of Phe88, Met105, Phe108, and Ile310 and a hydrophilic entrance site consisting of Asp89, Asp92, Lys110, and Lys297. The docking results of the P2X7R monomer with JNJ-54175446 showed that the trifluoromethyl benzyl moiety was stacked deep in the hydrophobic subpocket, while the rest extended toward the entrance site. The P2X7R 3-D complex structures of *Ailuropoda melanoleuca* with the reported P2X7R antagonists (*e.g.*, A-740003, A-804598, AZ-10606120, GW-791343, and JNJ-4796567) also reported the importance of the hydrophobic subpocket and the hydrophilic entrance site [52], which should be considered for designing and optimizing the current P2X7R antagonists.

An *in silico* docking study of P2X7R antagonists, including the clinical compounds (GSK1482160, JNJ-54175446, and JNJ-55308942), was performed using the Surflex Dock GeomX module of SYBYL-X (*ver* 2.1.1., Tripos Inc., St. Louis, MO, USA). The Surflex--Dock Protomol site was generated using the residue method by selecting amino acids, including Phe88, Phe95, Phe103, Met105, Phe108, Trp167, Phe293, Tyr295, and Val312. The 3-D structure of *Homo sapiens* P2X7R was downloaded from the Alphafold protein structure database (https://alphafold.ebi.ac.uk). As shown in Fig. (**9**), the hydrophobic regions of the three compounds were located in the hydrophobic region, and the heteroatom-containing cyclic ring was projected onto the hydrophilic entrance site.

Significant cross-species differences among P2X7R and insufficient CNS permeability hinder the development of new antidepressants based on the P2X7R/NLRP3/interleukin axis mechanism. Most P2X7R antagonists lack one of the following criteria: sufficient potency for rodent and human P2X7R, BBB penetration, metabolic stability, or target selectivity. Despite numerous hurdles in developing P2X7R antagonists in the treatment of depression, P2X7R has several advantages as a therapeutic target protein. From the viewpoint of drug discovery, P2X7R in neuroinflammation tends to induce fewer side effects because it is activated only by a millimolar concentration of eATP, which is much higher in the normal state. The second advantage is that IL-1β released by blood cells can be used as a biomarker of P2X7R activity in both preclinical and clinical assessments. Third, the hydrophobic moiety that interacts with the hydrophobic subpocket is essential for P2X7R affinity and is favorable for optimizing BBB penetration.

In addition to neuroinflammation, other factors, such as dysregulation of neural circuits within the limbic-cortical system and bidirectional immune-to-brain communications, are also associated with depression

[53, 54]. Recent results of the Depression Genome-Wide Association Study (GWAS) with human brain proteomes have identified 19 genes, including calcium signaling genes (P2X7R, PPP3CC, ADCY3), that contribute to the pathogenesis of depression, suggesting that P2RX7 is a potential target for novel therapeutics of depression [55].

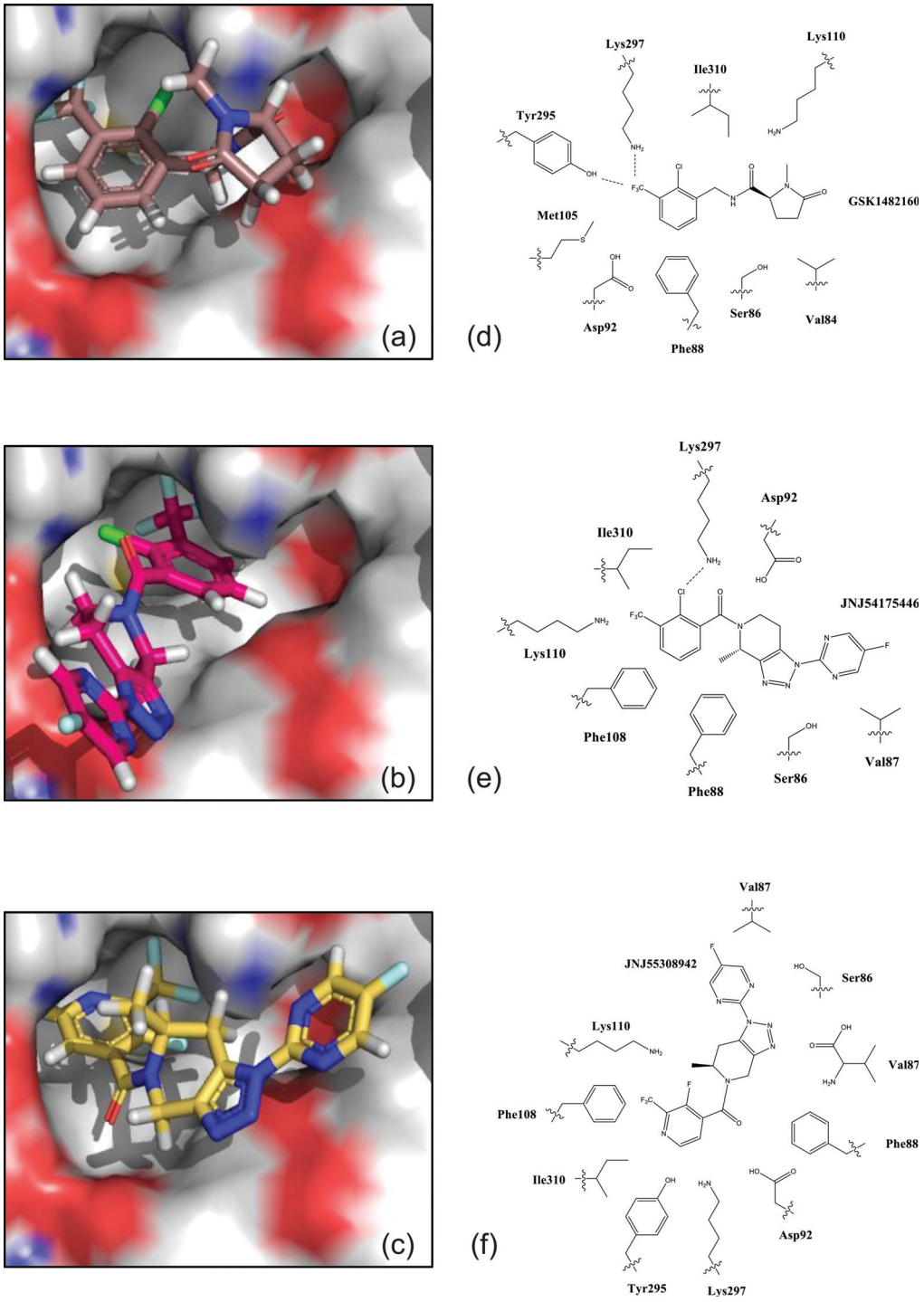

Fig. (9). The 3D docked pose of (**a**) GSK1482160, (**b**) JNJ-54175446, and (**c**) JNJ-55308942 in the allosteric site of P2X7R. Amino acid residues within 3 Å of (**d**) GSK1482160, (**e**) JNJ-54175446, and (**f**) JNJ-55308942. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

CONCLUSION

P2X7R has emerged as a novel, innovative therapeutic target to control neuroinflammation closely associated with a variety of CNS diseases. In particular, P2X7R has been proposed as an option to treat depression, which has an unmet medical need in patients who do not respond to current treatments, such as SSRIs and TCAs. The binding of eATP to P2X7R activates the NLRP3 inflammasome, producing and releasing proinflammatory cytokines. BBB-penetrating P2X7R antagonists have shown promising efficacy in preclinical and clinical studies. Two compounds are currently being evaluated in clinical trials to assess the use of P2X7R antagonists as novel antidepressants.

LIST OF ABBREVIATIONS

ATP	=	Adenosine Triphosphate
BBB	=	Blood-Brain Barrier
BZ-ATP	=	2(3)-O-(4-Benzoylbenzoyl) Adenosine-5-Triphosphate
cLogP	=	Calculated Partition Coefficient
CNS	=	Central Nervous System
CYP	=	Cytochrome P450
MDD	=	Major Depressive Disorder
eATP	=	Extracellular ATP
HBA	=	Hydrogen-Bonding Acceptor
HBD	=	Hydrogen-Bonding Donor
IL	=	Interleukin
LPS	=	Lipopolysaccharide
NLRP3	=	Nod-Like Receptor Family Pyrin Domain Containing 3
MDCK	=	Madin-Darby Canine Kidney Cells
MDR1	=	Multidrug Resistance Protein 1
P2X7R	=	Purinergic P2X7 Receptor
PAMPA	=	Parallel Artificial Membrane Permeability Assay
PET	=	Positron Emission Tomography
PK	=	Pharmacokinetics
RB	=	Rotational Bonds
SAR	=	Structure-Activity Relationship
SSRI	=	Selective Serotonin Reuptake Inhibitor
TCA	=	Tricyclic Antidepressant
tPSA	=	Topological Polar Surface Area
YO-PRO-1	=	4-((3-Methyl-2(3*H*)-Benzoxazolylidene)Methyl)-1-(3-(Trimethyl Ammonio)Propyl)-Quinolinium Diiodide

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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