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Mendeleev Commun., 2024, **34**, 8–10
Mendeleev Communications

Novel AMPA receptor allosteric modulators of bis(pyrimidine) series: synthesis and SAR evaluation

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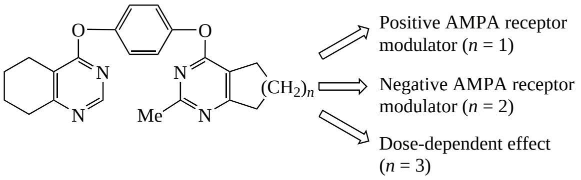
DOI: 10.1016/j.mencom.2024.01.002

A series of new bis(pyrimidines) with *p*-hydroquinone linker was prepared and evaluated as AMPA receptor modulators. Both positive and negative modulators were found among the investigated compounds.



Keywords: pyrimidines, aromatic nucleophilic substitution, AMPA receptor, allosteric modulators, bivalent ligands.

α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA receptors, AMPARs), which belong to ionotropic glutamate receptors family, represent an attractive target for the development of drugs for the treatment of the central nervous system diseases, as they play a key role in fast excitatory synaptic transmission and synaptic plasticity.[1–5] Positive allosteric modulators (PAMs)[6] of AMPAR are reported to act as neuroprotectors and may be applied to treat neurodegenerative and psychoneurological disorders,[7–13] while negative allosteric modulators (NAMs) possess anticonvulsant activity and can be used as antiepileptic drugs.[14–17]

Molecules with a hydrophobic linker and polar substituents that bind to two receptor subunits at the allosteric binding site of AMPAR represent a promising structural type for the development of potent AMPARs modulators.[18–21] Previously, we have reported the synthesis and studies of bivalent AMPAR PAMs and NAMs of bis(benzo[*d*][1,3]dioxole),[22] bis(isoxazole),[23] bis(pyrimidine)[24–26] and other[22] series acting in nanomolar concentration range. Compound **1** (Figure 1, Table 1) was found to be one of the most potent PAMs in bis(pyrimidine) series.[26] In order to investigate the options for fine tuning of modulating activity of bis(pyrimidines), in the present work we obtained several analogues of compound **1** retaining one heterocyclic part and varying the other. Thus, we planned to introduce a substituent into position 2 of the pyrimidine ring and to vary the size of the alicycle or to replace it with *tert*-butyl as an acyclic hydrophobic substituent.

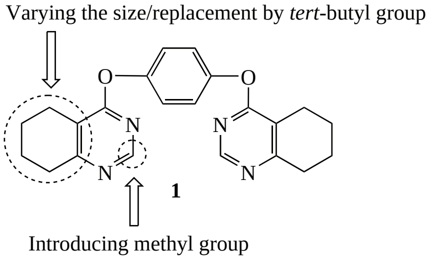


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Figure 1 Compound **1** and the directions of its modification.

Novel bis(pyrimidines) **2a–d** containing two different pyrimidine moieties were synthesized using the approach including two subsequent aromatic nucleophilic substitution reactions that was previously elaborated in our group[26] (Scheme 1).† The S$_N$Ar reaction of 4-chloropyrimidines **3a–c** or **5** with 4-(benzyloxy)phenol in the presence of Cs$_2$CO$_3$ followed by hydrogenative debenzylation yielded phenols **4a–c** or **6** bearing the first pyrimidine moiety. Then, compounds **4a–c** or **6** were involved in S$_N$Ar reaction with 4-chloropyrimidines **5** or **7**, respectively, to afford the target bis(pyrimidines) **2a–d** in moderate to good yields.

The influence of bis(pyrimidines) **2a–d** on kainate-induced currents in Purkinje cells was studied by electrophysiological patch-clamp method as described earlier[22] (see Table 1). A well-known AMPAR potentiator cyclothiazide[27] was used as a positive control. It was found that modulating action of the described compounds drastically depended on the molecular structure. The introduction of a methyl group to position 2 of one pyrimidine moiety led to the change of modulating action from positive towards negative (**1** *vs*. **2b**). However, the contraction of the alicycle down to five-membered ring restored the potentiating action (**2a** *vs*. **2b**), though, it became lower than in the case of

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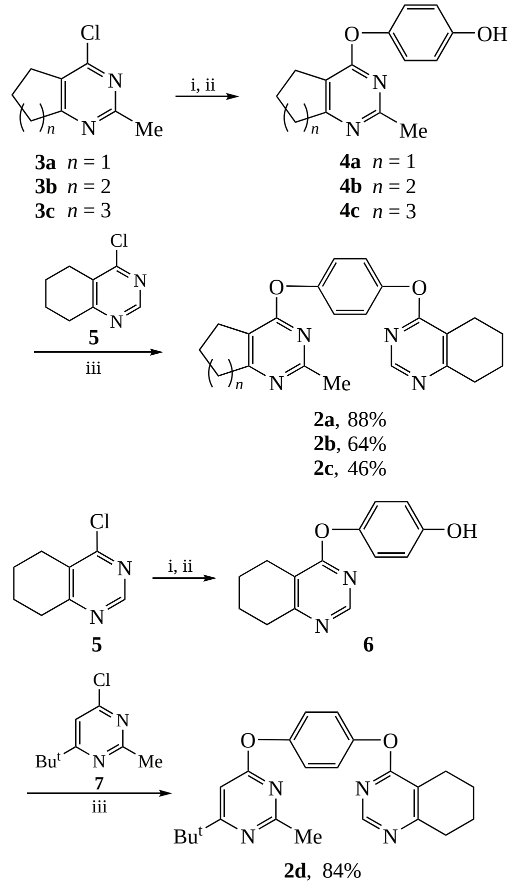
† *Synthesis of bis(pyrimidines)* **2a–d** *(general procedure)*. A mixture of the corresponding 4-(pyrimidin-4-yloxy)phenol **4a–c** or **6** (1.0 mmol) and Cs$_2$CO$_3$ (652 mg, 2.0 mmol) in absolute DMF (10 ml) was stirred for 10 min at room temperature, under argon. 4-Halogenopyrimidine **5** or **7** (2.0 mmol) was added. The reaction mixture was stirred at 85 °C for 6 h, allowed to cool down to room temperature, quenched with an equal volume of water and extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with brine (3 × 10 ml) and dried over MgSO$_4$; the solvent was evaporated under reduced pressure. The products were isolated by preparative column chromatography (SiO$_2$).

Table 1 The changes in the kainate-induced Purkinje-cell currents upon treatment with compounds **1** and **2a**–**d**.

Compound	Number of neurons	Currents (%) for various concentrations of compounds (M) (control = 100%)						
		10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}
1[26]	4	101 ± 9	118 ± 10	147 ± 12	166 ± 12	157 ± 11	144 ± 12	122 ± 8
2a	3	100 ± 3	119 ± 5	130 ± 5	141 ± 6	128 ± 5	102 ± 6	100 ± 5
2b	5	100 ± 2	89 ± 9	88 ± 9	72 ± 8	93 ± 2	96 ± 5	100 ± 4
2c	4	125 ± 4	135 ± 5	147 ± 6	152 ± 7	128 ± 6	61 ± 6	65 ± 6
2d	4	100 ± 3	122 ± 5	131 ± 6	142 ± 5	136 ± 6	131 ± 7	105 ± 5
Cyclothiazide	8	–	–	–	–	–	100 ± 3	145 ± 11



Scheme 1 *Reagents and conditions*: i, 4-BnOC$_6$H$_4$OH, Cs$_2$CO$_3$, DMF, 85 °C, 4 h; ii, H$_2$, 10%-Pd/C, EtOAc–MeOH, 4 h (**4a**–**c**) or 96 h (**6**); iii, Cs$_2$CO$_3$, DMF, 85 °C, 6 h.

compound **1**. The enlargement of the alicycle up to seven-membered one also restored the potentiating action (**2b** *vs.* **2c**) in low concentration range (10^{-12}–10^{-8} M), whereas in high concentration range (10^{-7}–10^{-6} M) compound **2c** was found to block the currents. The replacement of the five-membered annulated ring by the acyclic *tert*-butyl substituent had only negligible effect on potentiating properties (**2a** *vs.* **2d**), therefore, demonstrating that bicyclic moiety may be simplified to a substituted pyrimidine without the loss of positive modulation.

In order to elucidate the probable mechanism of action of the compounds, their interactions with the GluA2 AMPA receptor were modelled by means of molecular docking using AutoDock Vina 1.1.2 software[28] and molecular dynamics simulations using the CHARMM36/CGenFF 4.6 force field[29,30] in the GROMACS 2023.0 software[31] following the previously published[23,26,32,33] computational workflow. The binding modes of compound **2a**–**d** in the PAM binding site at the interface between the dimeric ligand-binding domains remained stable over the entire course of the simulation [100 ns, see root mean squared deviation (RMSD) plots in Figure S1, Online Supplementary Materials]. Similar to other medium-sized modulators,[3,6] the modulator molecules occupied a symmetrical position in the central subpocket of the symmetrical PAM binding site. The binding was primarily stabilized by steric fit and hydrophobic interactions. Interestingly, the preferred conformations for the ligands are different: in bound **2a** molecule, the cyclohexane ring is oriented inside the pocket and cyclopentane ring towards the solution, while in **2b**–**d** the cyclohexane, cycloheptane, and *tert*-butyl moieties are all oriented towards the pocket (see Figure S2). As an example, the binding mode of compound **2c** is shown in Figure 2. However, as is often the case in series of closely related ligands acting on targets with complex structures and mechanisms of operation,[34] no simple explanation of the differences in activity profiles for compounds **2a**–**d** could be derived from the inspection of their

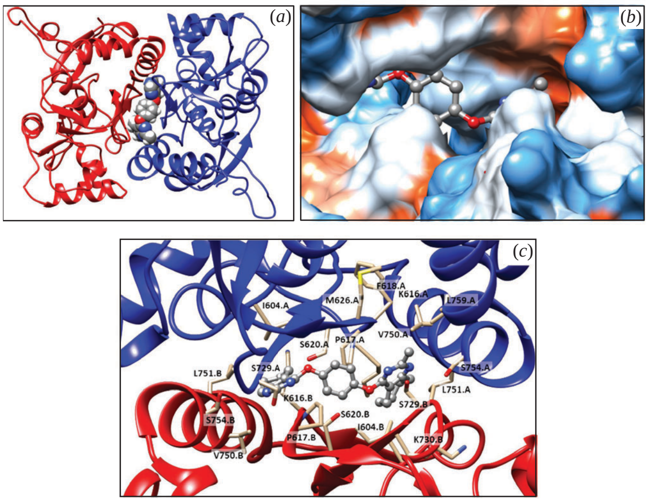


Figure 2 Binding mode of the PAM **2c**, refined using molecular dynamics simulation (100 ns). (*a*) General view of the dimeric ligand binding domain of AMPA receptor (GluA2) and location of the binding site. (*b*) Binding pockets in the protein molecular surface colored by local hydrophobicity (brown for hydrophobic and blue for hydrophilic). (*c*) Detailed view of the binding site. The ligand is represented by a grey ball-and-stick model, the amino acid residues located within 3 Å of it are represented by beige stick models.

Table 2 Predicted MM/GBSA binding free energies and physicochemical and ADMET profiles of compounds **2a**–**d**.[a]

Compound	ΔG_b	LogP_{ow}	pS_{aq}	LogBB	HIA	hERG pK_i	hERG pIC$_{50}$	QED
2a	–54.0 ± 0.2	4.46	5.82	–1.01	84	5.25	4.58	0.67
2b	–56.5 ± 0.3	4.86	6.28	–0.98	84	5.37	4.46	0.63
2c	–54.2 ± 0.3	5.16	6.65	–0.95	84	5.38	4.61	0.55
2d	–53.9 ± 0.3	4.86	6.46	–0.69	97	6.39	4.64	0.60

[a] ΔG_b is MM/GBSA binding free energy (kcal mol^{-1}), LogP_{ow} is octanol–water partition coefficient, pS_{aq} is aqueous solubility [–log(M)], LogBB is blood–brain barrier permeability, HIA is human intestinal absorption (%), hERG pK_i is hERG potassium-channel affinity [–log(M)], hERG pIC$_{50}$ is hERG potassium channel inhibitory activity [–log(M)], QED is quantitative estimate of drug-likeness.

binding positions, RMSD plots, or binding free energies calculated using the MM/GBSA (molecular mechanics, generalized Born, surface area) method (Table 2). We believe[33] that the differences in the PAM activity profiles could be attributable to finer differences in the receptor subunit dynamics that could, in turn, affect the receptor gating and desensitization.[1,3]

Computational evaluation of the physicochemical, ADMET, and PAINS profiles for the compounds included the predictions of their lipophilicity and aqueous solubility,[35] human intestinal absorption,[36] blood–brain barrier permeability,[37] and hERG-mediated cardiac toxicity risk,[38] as well as calculation of quantitative estimate of drug-likeness[39] and pan-assay interference compounds (PAINS) alert check using RDKit version 2020.03.4 software.[40] The results (see Table 2) were quite acceptable for potential lead compounds at the early drug development stages, although additional checks and structure optimization would likely be required.

To conclude, novel potent bivalent AMPARs modulators of bis(pyrimidine) series were obtained, and the possibility of fine tuning of modulating action of the bis(pyrimidines) *via* small changes of molecular structure was demonstrated.

We thank the Russian Science Foundation (grant no. 22-15-00041) for financial support of this work. This study was fulfilled using an Agilent 400-MR NMR spectrometer purchased by the MSU Development Program. The study of cyclothiazide was supported by the State Assignment of IPAC RAS (topic no. FFSN-2021-0005).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.01.002.

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Received: 25th September 2023; Com. 23/7257