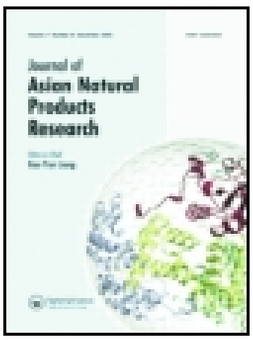

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Synthesis and *in vitro* anti-epileptic activities of novel [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one derivatives

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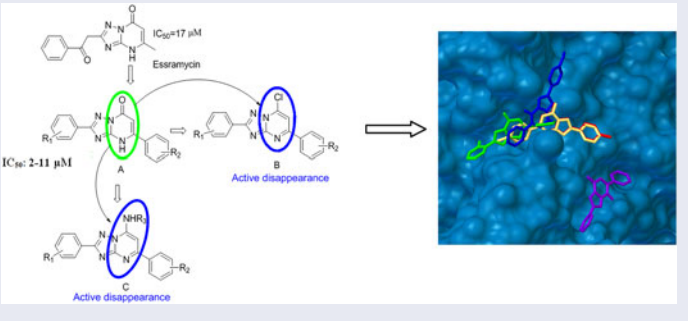
ABSTRACT

In this investigation, eight novel 2,5-disubstituted [1,2,4]-triazolo [1,5-a]pyrimidin-7(4H)-one and eight novel 2,5-disubstituted [1,2,4]-triazolo[1,5-a]pyrimidine amine derivatives were synthesized based on the novel marine natural product Essramycin. Their anti-epileptic activities were evaluated by 4-aminopyridine (4-AP)-induced hyper excitability model in primary cultured neocortical neurons. Five compounds with [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one skeleton showed remarkable anti-epileptic activities. The preliminary structure–activity relationship (SAR) showed that the pyrimidine-7(4H)-one motif is the necessary "active core" of anti-epileptic activity. To understand the action mechanism of anti-epileptic activity of [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one compounds, docking studies using the model of $GABA_A$ as docking scaffolds were performed and the docking results were in concordance with the experiment observations.



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1,2,4-triazolo[1,5-a] pyrimidine-7(4H)-one; synthesis; anti-epileptic; molecular docking

1. Introduction

Epilepsy, a common neurological disorder characterized by recurrent spontaneous seizures, is considered to be a major health problem that affects approximately 1–2% of the population worldwide [1]. Despite the considerable progress in our

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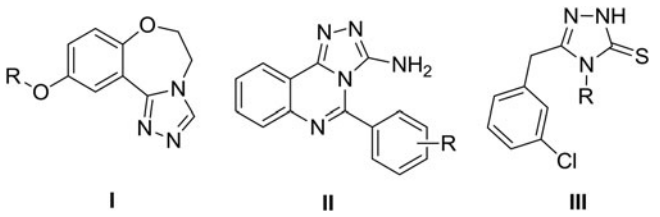


Figure 1. Examples of triazole derivatives with anti-epileptic activity.

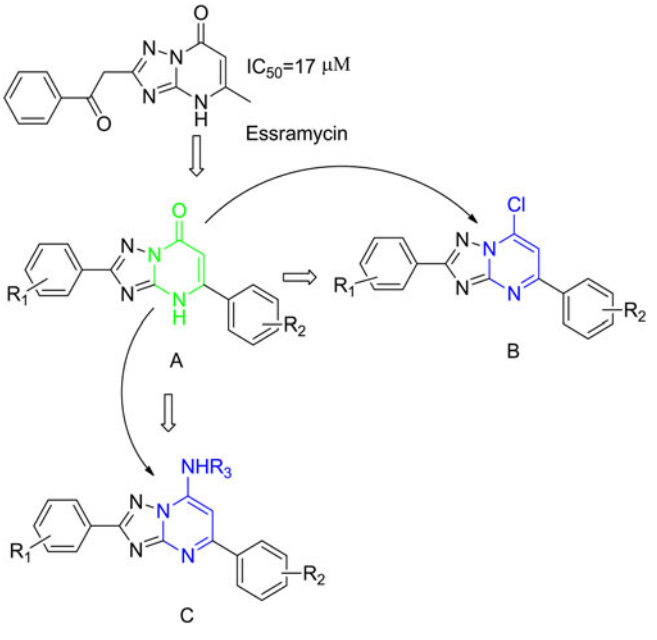


Figure 2. The modification strategy based on Essramycin.

understanding of pathophysiology and pharmacotherapy of seizures and epilepsy, the cellular basis of human epilepsy remains an enigma [2]. Over years, success has been made in the development of anti-epileptic drugs (AEDs). However, current AEDs either exhibit unfavorable side effect profiles or fail to adequately control seizures [3]. It is necessary and urgent to develop new generation of effective AEDs [4].

1,2,4-triazole is a key pharmacophore in pesticide and pharmaceutical synthesis, and several AEDs with 1,2,4-triazole moiety have successfully entered the pharmaceutical market such as Alprazolam, Estazolam, and Etizolam [5–9]. Due to their importance in medicinal chemistry, especially in the field of anti-epilepsy, the heterocyclic compounds bearing the 1,2,4-triazole moiety have received considerable attentions [10–13]. For example, 1,2,4-triazole derivatives **I**, **II,** and **III** (Figure 1) showed remarkable anti-epileptic activities in cell or animal models [10,11]. Interestingly, most of these compounds showed anti-epileptic activity through activating $GABA_A$ receptors or GABA-mediated mechanism (direct or indirect) [10,11,14–17].

Encouraged by anti-epileptic activities of 1,2,4-triazole compounds, we hypothesized that the novel marine natural product essramycin bearing [1,2,4]-triazolo[1,5-a] pyrimdin-7(4H)-one motif (Figure 2) possesses anti-epileptic activity. Just as we

expected, in our preliminary experiments, essramycin showed better anti-epileptic activity (IC_{50} 17 μM) than positive control carbamazepine (CBZ, IC_{50} 35 μM) in 4-AP-induced hyperexcitability model in primary cultured neocortical neurons. The result prompted us to synthesize a series of compounds bearing [1,2,4]-triazolo[1,5-a] pyrimidin-7(4H)-one motif as potential anti-epileptic agents.

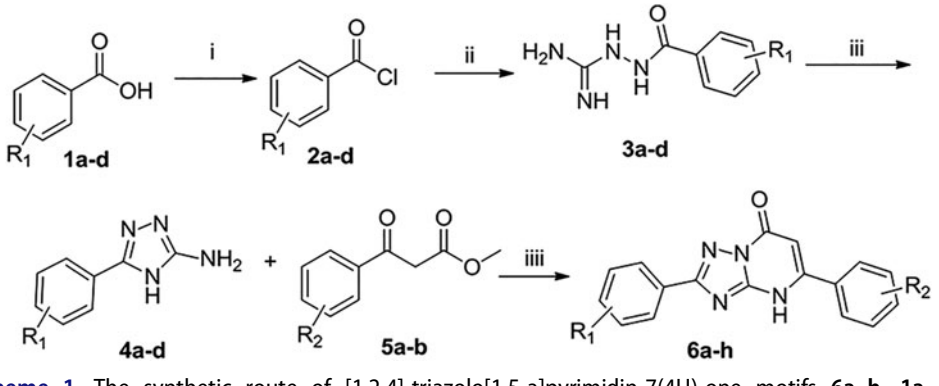
In this work, a series of [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one derivatives (Figure 2 structure **A**) based on essramycin scaffold were synthesized and their anti-epileptic activities were evaluated. The preliminary SAR was studied by modifying active structure **A** to structures **B** and **C** (Figure 2). In order to test whether the anti-epileptic activity of our [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one derivatives act through A-type GABA receptors ($GABA_A$) like many other AEDs with the 1,2,4-triazolo moiety, we performed docking studies on a native $GABA_A$ model.

2. Results and discussion

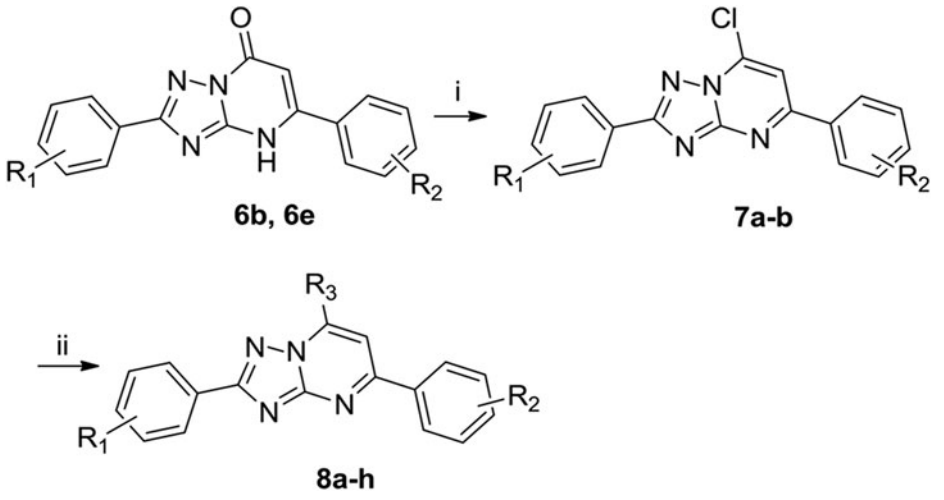
2.1. Chemistry

Essramycin was prepared according to the procedure reported by Huang L. H. [18]. The synthetic routes of [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one motif **6a-h** were showed in Scheme 1. Acids **1a-d** were reacted with $(COCl)_2$/DMF to afford acyl chlorides **2a-d**. The acyl chlorides **2a-d** reacted with aminoguanidine bicarbonate under base condition to afford the products **3a-d**. Compounds **3a-d** were cyclized under acid condition to afford 2-amino-1,2,4-triazole derivatives **4a-d**. 2-amino-1,2,4-triazole derivatives **4a-d** reacted with β-ketone esters **5a-b** to afford **6a-h** with [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one motif.

The synthetic route of [1,2,4]-triazolo[1,5-a]pyrimidin-7-amine derivatives **8a-h** was showed in Scheme 2. The [1,2,4]-triazolo[1,5-a]pyrimidin-7-one derivatives **6b** and **6e** were reacted with $POCl_3$ in reflux under catalytic amount of DMF to afford compounds **7a-b**. Compounds **7a-b** reacted with different amines to afford the targets **8a-h**.



Scheme 1. The synthetic route of [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one motifs **6a–h**. **1a–4a**: R_1=H; **1b–4b**:R_1=4-CH_3; **1c–4c**:R_1=4-OCH_3; **1d–4d**: R_1=2-F; **5a**:R_2=H; **5b**:R_2=4-Cl; **6a**:R_1=H, R_2=H; **6b**:R_1=4-CH_3, R_2=H; **6c**:R_1=4-OCH_3, R_2=H; **6d**:R_1=2-F, R_2=H; **6e**:R_1=H, R_2=4-Cl; **6f**:R_1=4-CH_3, R_2=4-Cl; **6g**:R_1=2-F, R_2=4-Cl; **6h**: R_1=4-OCH_3, R_2=4-Cl. Reagents and conditions: i: oxaloyl chloride, DCM, rt, 2.5 h, yield 75%–85%; ii: aminoguanidine carbonate, dry DCM, rt, 12 h, yield 50–90%; iii: water, 100 °C, 12 h, yield 80–90%; iiii: n-BuOH, TsOH, 125 °C, 24 h, yield of 70–98%.



Scheme 2. The synthetic route of [1,2,4]-triazolo[1,5-a]pyrimidin-7-amine derivatives **8a–h**. **7a**:R_1=4-CH_3, R_2=H; **7b**: R_1= H, R_2=4-Cl; **8a**:R_1=4-CH_3, R_2=H, R_3=-$NHCH_2CH_3$; **8b**:R_1=4-CH_3, R_2=H, R_3=-NC_5H_{10}; **8c**:R_1=4-CH_3, R_2=H, R_3=-NC_6H_5; **8d**:R_1=4-CH_3, R_2=H, R_3=-$NHCH_2C_6H_5$; **8e**:R_1=H, R_2=4-Cl, R_3=-$NHCH_2CH_3$; **8f**: R_1=H, R_2=4-Cl, R_3=-NC_5H_{10}; **8g**:R_1=H, R_2=4-Cl, R_3=-NHC_6H_5; **8h**: R_1=H, R_2=4-Cl, R_3=-$NHCH_2C_6H_5$. Reagents and conditions: i: $POCl_3$, DMF, 90 °C, 5 h, yield 80–90%; ii: Et_3N, amine, EtOH, rt, yield 50–80%.

2.2. Anti-epileptic activity

Primary cultured neocortex neurons can form neuronal networks and display spontaneous Ca^{2+} oscillations (SCOs). These SCOs occur simultaneously with action potential generation. SCO activity is dependent on the balanced excitatory/inhibitory neuronal inputs. 4-AP can induces hyper-excitability in neurons, therefore has been considered to be an *in vitro* seizurogenic model widely used for *in vitro* anti-epileptic activity test [19]. The activities toward 4-AP-induced SCO changes of 17 compounds were tested in primary cultured neocortical neurons. Compounds **6a-h** containing [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one motif inhibited the intracellular calcium ion concussion of mouse neuronal cells. Compounds **6a**, **6b**, **6c**, **6d**, and **6e** showed inhibitory effect in 4-AP induced hyper excitability model in primary cultured neocortical neurons (Figure 3). In order to determine the "active core" of these compounds, we selected the best active compound **6b** as the model and modified the structure of compound **6b** to **7a** and **8a-d** (Scheme 2). Interestingly, all of the compounds **7a** and **8a-d** showed no any inhibitory effects on 4-AP induced SCO changes (Figure 4). The result indicated that the pyrimidine-7(4H)-one motif in the structure of **6a-e** is the probably "active core". In order to confirm this, the active compound **6e** was modified to compounds **7b** and **8e-h** by using the same strategy with compound **6b** (Scheme 2). Compounds **7b** and **8e-h** had no effect on 4-AP-induced SCO changes (Figure 4). All of the results indicated that the pyrimidin-7(4H)-one motif in the structure of **6a-e** is necessary to their activity.

Through a continuous assay procedure, different concentrations of compounds **6a-e** were tested in 4-AP-induced hyper excitability model in primary cultured

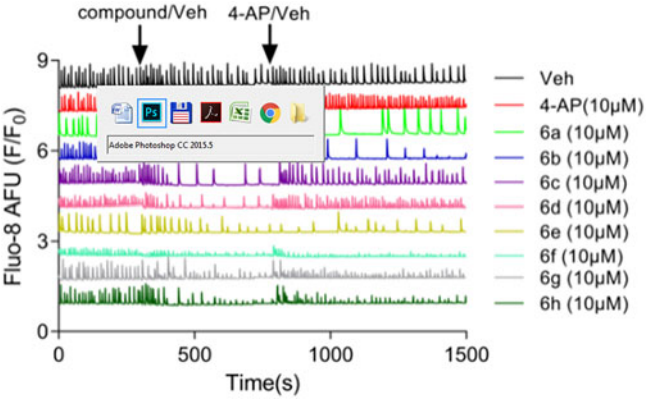


Figure 3. Effects of compounds **6a–h** on 4-AP-induced SCO changes in neocortical neurons.

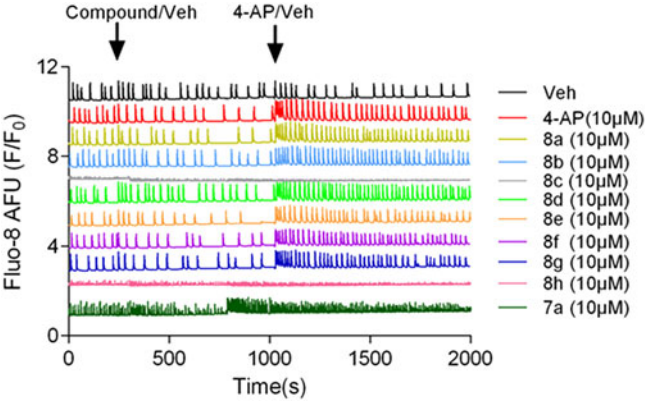


Figure 4. Effects of compounds **8a–h** and **7a** on 4-AP-induced SCO changes in neocortical neurons.

neocortical neurons. As shown in Figure 5, it was found that with the increase of the drug concentration, the compounds (**6a**, **6b**, **6c**, **6d**, **6e**) can inhibit the spontaneous calcium oscillation of primary cortical neurons (Figure 5(A)) and 4-AP induced hyper excitability model in primary cultured neocortical neurons (Figure 5(B)) in a dose-dependent manner. Compounds **6a**, **6b**, **6c**, **6d,** and **6e** showed inhibitory effect on the intracellular calcium ion concussion of mouse neuronal cells with IC_{50} values between 1.68 μM and 7.24 μM (Table 1). In the 4-AP induced hyper excitability model in primary cultured neocortical neurons, IC_{50} values of compounds **6a**, **6b**, **6c**, **6d,** and **6e** were ranged between 2.35 μM and 15.40 μM (Table 1). Compounds **6b** and **6e** (IC_{50} 2.35 μM and 3.21 μM, respectively) significantly outperformed essramycin (IC_{50} 17 μM) and postive control-CBZ (IC_{50} 35 μM).

2.3. Molecular docking studies

CBZ can inhibit seizures by enhancing the transmission or activity of GABAergic neurons and activating $GABA_A$-mediated mechanisms [20], most of 1,2,4-triazole

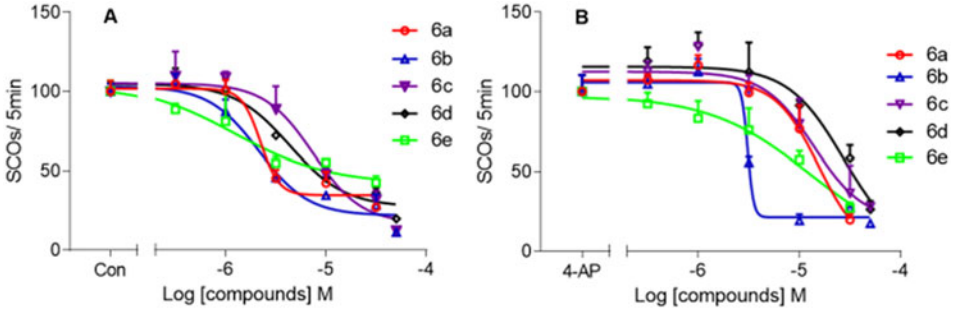


Figure 5. A: Concentration response curve for compounds **6a–6e** inhibiting the frequency of SCO in primary cortical neuron; B: Concentration response curve for compounds **6a–6e** inhibiting 4-AP-induced SCOs in neocortical neurons.

Table 1. The IC_{50} values of the active compounds against 4-AP-induced neuron excitability.

	Primary cortical cells		4-AP primary cerebral cortex hyperstimulation model	
Compd	IC_{50} (μM)	95%CI (μM)	IC_{50} (μM)	95%CI (μM)
6a	2.04	1.44–2.88	11.03	6.39–19.04
6b	2.27	1.67–3.08	2.35	1.77–3.12
6c	7.24	4.87–10.77	12.35	8.46–18.03
6d	3.23	2.49–4.20	15.40	8.79–26.98
6e	1.68	0.58–4.87	3.21	2.70–3.83
CBZ	–	–	35.0	32.21–37.79

95% CI: 95% confidence intervals.

derivatives with anti-epileptic activity also have a similar mechanism [21–23]. In order to study the action mechanism of compounds **6a-e**, the docking mode of $GABA_A$ protein with compounds **6a-e** was studied. Compounds **6a-e** were docked to subunits of extracellular domain of $GABA_A$ interacting with CBZ. [20].

As shown in Figure 6, from top view of $GABA_A$ protein model, all of compounds **6a-e** can bind with active region of $GABA_A$ and the binding sites of compounds **6a**, **6c**, **6d** with $GABA_A$ were different. Compounds **6b** and **6e** shared same binding sites with $GABA_A$. The detailed binding sites of compounds **6b** and **6e** with the amino acid residues of $GABA_A$ were shown in Figures 7 and 8.

In 2017, Meeta Sahu et al. proposed binding interactions of standard drug CBZ with $GABA_A$ receptor (PDB ID: 4COF) and the result showed that CBZ made crucial H-bonds with Arg216 and Ile218 of $GABA_A$ active site [20]. In our experiment, the most effective molecules **6b** (as showed in Figure 7) and **6e** (as showed in Figure 8) made the same crucial H-bonds with Arg216 and Ile218 of $GABA_A$ active site. In addition, the methyl group on the benzene ring attached on the trizole ring of compound **6b** had a hydrophobic effect with residues Pro-276, the pyrimidone ring and two benzene rings have pi-Cation, pi-Sigma, and pi-Alkyl interactions with the amino acid residues Lys-215, Pro-276 and Leu-145, respectively. Same with the compound **6b**, the pyrimidone ring and two benzene rings of compound **6e** had pi-Cation, pi-Sigma, and pi-Alkyl interactions with the amino acid residues Lys-215, Pro-276 and Leu-145, respectively.

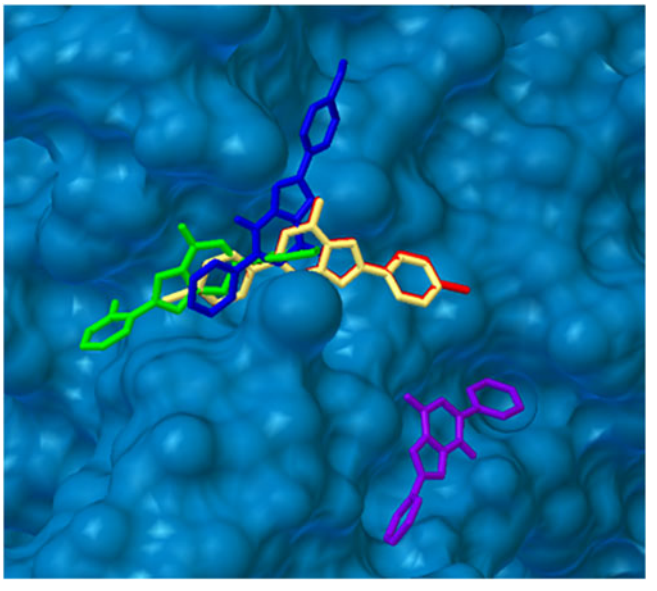


Figure 6. Top view of $GABA_A$ protein model (PDB Code: 4COF): active region of $GABA_A$ and **6a-e** docking conformation with $GABA_A$.

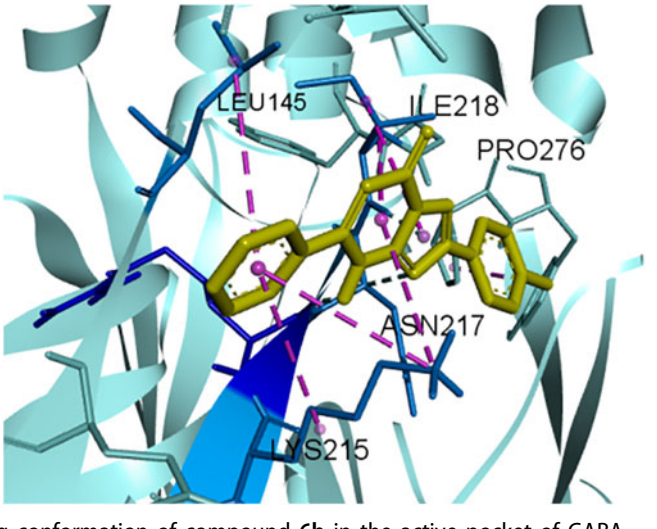


Figure 7. Docking conformation of compound **6b** in the active pocket of $GABA_A$.

The docking results indicated that compounds **6b** and **6e** had same action mechanism with the receptor protein $GABA_A$. Compounds **6a**, **6c,** and **6d** bound the different active sites of $GABA_A$ receptor as compared with **6b** and **6e**. The H-bonds binding energy of compounds **6a-e** to the amino acid residues of $GABA_A$ receptor was calculated and shown in Table 2. These docking results were in concordance with the observations on the 4-AP induced hyper excitability model in primary cultured neocortical neurons.

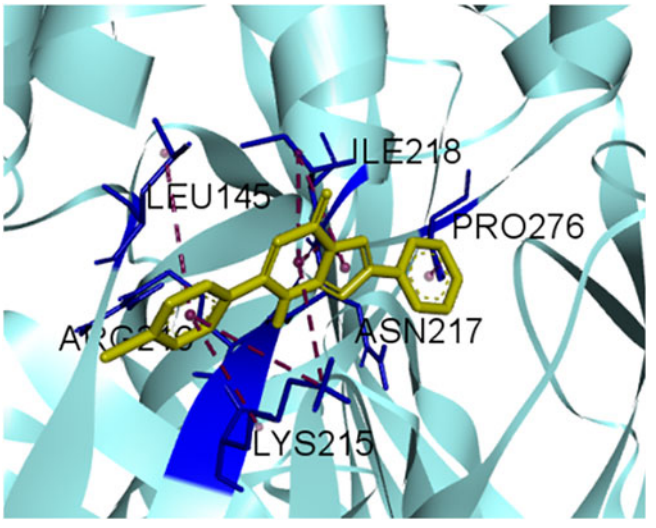


Figure 8. Docking conformation of compound **6e** in the active pocket of $GABA_A$.

Table 2. The H-bonds binding energy of compounds **6a–e** to the amino acid residues of $GABA_A$ receptor.

Compound	Binding energy	Amino acid residues
6a	8.69	ASN-217, GLU-182
6b	7.85	ARG-216, ILE-218
6c	7.38	ILE-218
6d	7.38	ARG-142, ASP-146
6e	7.39	ARG-216, ILE-218

3. Experimental

3.1. Chemistry

All of the solvents and materials were analytical grade and used without further purification. General ^{1}H NMR and ^{13}C NMR spectra were recorded on a 500 MHz, 125 MHz (Bruker Avance III) instrument, respectively, and chemical shifts were given in ppm relative to tetramethylsilane. Mass spectra were measured on an UPLC-Q/TOF Xevo G2-XS (Waters, MA, USA). All chemical shifts are reported as d (ppm) values. Splitting patterns are designated as follows: s: singlet; d: doublet; t: triplet; q: quartet; and m: multiplet.

3.2. Synthesis of the compounds

3.2.1. 3-Amino-1,2,4-triazole derivatives 4a-d

To a solution of benzoic acid derivatives (**1a-d**) (132 mmol) in dry dichloromethane (200 ml), was added oxalyl chloride (158.4 mmol) and a catalytic amount of DMF (two drops) at 0 °C, and the mixture was allowed to warm to room temperature and stirred at room temperature for 2.5 h. The resulting mixture was concentrated under vacuum to afford benzoyl chloride derivatives (**2a-d**) as light yellow oil, which was used directly without further purification.

To a solution of aminoguanidine carbonate (20.0 mmol) in pyridine (50 ml) was added benzoyl chloride derivatives **2a-d** (10.0 mmol), and the reaction mixture was stirred for 12 h at room temperature. The resulting mixture was concentrated under vacuum to give a residue, which was dissolved in water (30 ml) and adjusted to pH 12 with sodium hydroxide. The product was precipitated and collected by filtration and dried in an oven at 40 °C under reduced pressure to afford **3a-d**.

A solution of compounds **3a-d** (10.75 mmol) in water (100 ml) was stirred overnight at 100 °C. The reaction was cooled down to room temperature; the product was precipitated and collected by filtration and dried in an oven at 40 °C under reduced pressure to afford **4a-d**.

3.2.2. Methyl benzoylacetate derivatives 5a–b

To a solution of NaH (166.7 mmol) in toluene (40 ml) was added dimethyl carbonate (166.7 mmol). After the mixture was heated to 100 °C, a solution of acetophenone derivatives (83.3 mmol) in toluene (20 ml) was added dropwise over 0.5 h, and the reaction mixture was stirred for 2 h at 100 °C. The reaction was cooled down to room temperature. Glacial acetic acid (30 ml) was added dropwise and a heavy pasty solid separated. Ice-cold water was slowly added until the solid was dissolved completely. Then, the reaction system was diluted with 200 ml of EtOAc. The organic layer was separated, washed with water (20 ml) and brine (20 ml) and dried over Na_2SO_4. After the solvent was evaporated, the residue was purified by flash chromatography on silica gel with petroleum ether/ethyl acetate (20:1) as eluent to give the desired aryl β-ketoesters **5a-b**.

3.2.3. [1,2,4]-Triazolo[1,5-a]pyrimidin-7(4H)-one motif 6a–h

To a solution of **4a-d** (5.3 mmol) in n-BuOH (20 ml) was added **5a-b** (10.6 mmol) and TsOH (0.53 mmol), and the reaction mixture was stirred at 125 °C for 24 h. The solids were collected by filtration and washed with methanol (10 ml) to afford [1,2,4]triazolo[1,5-a]pyrimidin-7(4H)-one **6a-h**.

3.2.3.1. 2,5-Diphenyl-[1,2,4]triazolo[1,5-a]pyrimidin-7(4H)-one (6a). White solid, yield 98%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 8.14 (t, $J = 6.5$Hz, 2H), 7.89 (d, $J = 6.5$Hz, 2H), 7.57–7.53 (m, 6H), 6.34 (s, 1H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 156.3, 152.2, 131.7, 130.9, 129.4 (6C), 127.9 (3C), 127.1 (3C), 98.1. HRESIMS: m/z 289.1094 $[M+H]^+$ (calcd for $C_{17}H_{13}N_4O$, 289.1089).

3.2.3.2. 5-Phenyl-2-(p-tolyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7(4H)-one (6b). White solid, yield 95%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 13.55 (s, 1H), 8.03 (d, $J = 7.5$HZ, 2H), 7.90 (d, $J = 6.0$Hz, 2H), 7.57 (t, $J = 7.0$Hz, 3H), 7.36 (d, $J = 7.5$Hz, 2H), 6.34 (s, 1H), 2.39 (s, 3H); ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 156.3, 152.1, 140.7, 131.6, 130.0 (3C), 129.4 (3C), 127.9 (3C), 127.0 (3C), 98.1, 21.5. HRESIMS: m/z 303.1247 $[M+H]^+$ (calcd for $C_{18}H_{15}N_4O$, 303.1246).

3.2.3.3. 2-(4-Methoxyphenyl)-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidin-7(4H)-one (6c). White solid, yield 97%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 8.08 (d, $J = 8.5$Hz, 2H),

7.91 (d, $J = 6.0$Hz, 2H), 7.56 (d, $J = 6.5$Hz, 3H), 7.11 (d, $J = 8.5$Hz, 2H), 6.33 (s, 1H), 3.84 (s, 3H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 161.6, 156.2, 152.2, 131.5, 129.4 (3C), 128.7 (3C), 127.9 (3C), 114.8 (3C), 97.7, 55.8. HRESIMS: *m/z* 319.1198 $[M + H]^+$ (calcd for $C_{18}H_{15}N_4O_2$, 319.1195).

3.2.3.4. 2-(2-Fluorophenyl)-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidin-7(4H)-one (6d). Light yellow solid, yield 71%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 13.64 (s, 1H), 8.13 (t, $J = 7.5$Hz, 1H), 7.90 (d, $J = 7.0$Hz, 2H), 7.57 (t, $J = 7.5$Hz, 3H), 7.43–7.38 (m, 2H), 6.36 (s, 1H). ^{13}C-NMR:(DMSO,125MHz) δ (ppm) 161.5, 159.3, 158.1, 156.2, 151.9, 132.7, 132.2, 131.7, 130.8, 129.4 (2C), 128.0 (2C), 125.2, 118.4, 117.2, 98.1. HRESIMS: *m/z* 307.0998 $[M + H]^+$ (calcd for $C_{17}H_{12}N_4OF$, 307.0995).

3.2.3.5. 5-(4-Chlorophenyl)-2-phenyl-[1,2,4]triazolo[1,5-a]pyrimidin-7(4H)-one (6e). White solid, yield 98%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 8.14 (d, $J = 6.0$Hz, 2H), 7.92 (d, $J = 8.0$Hz, 2H), 7.63 (d, $J = 8.0$Hz, 2H), 7.55 (s, 3H), 6.38 (s, 1H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 156.2, 152.1, 136.4, 131.0, 129.79 (3C), 129.41 (6C), 127.05 (3C), 98.47. HRESIMS: *m/z* 323.0702 $[M + H]^+$ (calcd for $C_{17}H_{12}N_4OCl$, 323.0700).

3.2.3.6. 5-(4-Chlorophenyl)-2-(p-tolyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7(4H)-one (6f). White solid, yield 94%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 8.03 (d, $J = 8.0$Hz, 2H), 7.93 (d, $J = 8.5$Hz, 2H), 7.63 (d, $J = 8.5$Hz, 2H), 7.37 (d, $J = 8.0$Hz, 2H), 6.39 (s, 1H), 2.39 (s, 3H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 156.2, 152.1, 140.9, 136.3, 130.0 (3C), 129.8 (3C), 129.4 (3C), 127.0 (3C), 98.5, 21.5. HRESIMS: *m/z* 337.0858 $[M + H]^+$ (calcd for $C_{18}H_{14}N_4OCl$, 337.0856).

3.2.3.7. 5-(4-Chlorophenyl)-2-(2-fluorophenyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7(4H)-one (6g). Light yellow solid, yield 71%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 8.11 (t, $J = 7.0$Hz, 1H), 7.91 (d, $J = 8.5$Hz, 2H), 7.64–7.57 (m, 3H), 7.43-7.37 (m, 2H), 6.39 (s, 1H).^{13}C-NMR (DMSO, 125MHz) δ (ppm): 161.4, 159.4, 157.8, 156.1, 151.9, 136.5, 132.8, 131.4, 130.8, 129.8 (2C), 129.4 (2C), 125.2, 118.2, 117.4, 98.4. HRESIMS: *m/z* 341.0607 $[M + H]^+$ (calcd for $C_{17}H_{11}N_4OFCl$, 341.0605).

3.2.3.8. 5-(4-Chlorophenyl)-2-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7(4H)-one (6h). White solid, yield 98%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 8.08 (d, $J = 8.5$Hz, 2H), 7.93 (d, $J = 8.0$Hz, 2H), 7.63 (d, $J = 8.0$Hz, 2H), 7.12 (d, $J = 8.5$Hz, 2H), 6.4 (s, 1H), 3.84 (s, 3H), ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 161.7, 156.2, 152.0, 136.3, 129.7 (3C), 129.4 (3C), 128.7 (3C), 114.9 (3C), 98.5, 55.8. HRESIMS: *m/z* 353.0808 $[M + H]^+$ (calcd for $C_{18}H_{14}N_4O_2Cl$, 353.0805).

3.2.4. [1,2,4]-Triazolo[1,5-a]pyrimidine amine 8a–h

To a solution of **6b** or **6e** (2.7 mmol) in $POCl_3$ (6 ml) was added 0.1 ml DMF, the resulting mixture was stirred at 90 °C for 5 h. After cooling to room temperature, ice was added to quench the reaction, the water phase was extracted with ethyl acetate 2–3 times. The organic phase was combined and dried with anhydrous

sodium sulfate, and the organic layer was evaporated to afford the yellow solid **7a-b**. To a solution of **7a-b** (0.44 mmol) in EtOH, was added Et_3N and amine (0.88 mmol), the resulting mixture was stirred at room temperature overnight. The resulting mixture was concentrated under vacuum and water was added, the solid was filtered, and the filter cake was washed with water and dried under vacuum to afford yellow solid **8a-h**.

3.2.4.1. N-Ethyl-5-phenyl-2-(p-tolyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (8a). White solid, yield 80%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 8.21 (d, $J = 5.0$Hz, 2H), 8.11 (d, $J = 8.0$Hz, 2H), 7.52 (s, 3H), 7.36 (d, $J = 7.5$Hz, 2H), 6.97 (s, 1H), 3.62–3.58 (m, 2H), 2.37 (s, 3H), 1.27 (t, $J = 7.0$Hz, 3H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 163.5, 160.6, 156.4, 148.1, 140.4, 137.9, 130.8, 129.8 (2C), 129.1 (2C), 128.4, 127.8 (2C), 127.2 (2C), 85.4, 37.0, 21.5, 14.7. HRESIMS: *m/z* 330.1722 $[M + H]^+$ (calcd for $C_{20}H_{20}N_5$, 330.1719).

3.2.4.2. 5-Phenyl-7-(piperidin-1-yl)-2-(p-tolyl)-[1,2,4]triazolo[1,5-a]pyrimidine (8b). Light yellow solid, yield 75%; ^{1}H-NMR ($CDCl_3$, 500MHz) δ (ppm): 8.26 (d, $J = 8.0$Hz, 2H), 8.17–8.15 (m, 2H), 7.49–7.48 (m, 3H), 7.29 (d, $J = 8.0$Hz, 2H), 6.7 (s, 1H), 3.92 (t, $J = 5.4$Hz, 4H), 2.42 (s, 3H), 1.89-1.87 (m, 4H), 1.82–1.81 (m, 2H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 163.3, 160.4, 158.4, 150.6, 140.5, 137.8, 130.9, 129.9 (2C), 129.2 (2C), 128.4, 127.9 (2C), 127.1 (2C), 91.8, 55.3 (2C), 25.6 (2C), 24.3, 21.5. HRESIMS: *m/z* 370.2032 $[M + H]^+$ (calcd for $C_{23}H_{24}N_5$, 370.2030).

3.2.4.3. N,5-Diphenyl-2-(p-tolyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (8c). Light yellow solid, yield 70%; ^{1}H-NMR (DMSO, 500MHz) δ (ppm): 8.18 (d, $J = 8.0$Hz, 2H), 8.0–7.9 (m, 2H), 7.57–7.48 (m, 7H), 7.38 (d, $J = 8.0$Hz, 2H), 7.33 (t, $J = 7.0$Hz, 1H), 6.86 (s, 1H), 2.38 (s, 3H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 164.2, 160.8, 156.9, 146.9, 140.6, 137.8, 137.3, 130.9, 130.1 (2C), 129.9 (2C), 129.3 (2C), 128.3, 127.6 (2C), 127.4 (2C), 126.7, 124.9 (2C), 86.8, 21.3. HRESIMS: *m/z* 378.1718 $[M + H]^+$ (calcd for $C_{24}H_{20}N_5$, 378.1719).

3.2.4.4. N-Benzyl-5-phenyl-2-(p-tolyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (8d). White solid, yield 78%; ^{1}H-NMR ($CDCl_3$, 500MHz) δ (ppm): 8.23 (d, $J = 8.0$Hz, 2H), 8.11–8.09 (m, 2H), 7.47–7.38 (m, 8H), 7.29 (d, $J = 8.0$Hz, 2H), 6.6 (s, 1H), 6.57 (s, 1H), 4.72 (d, $J = 6.0$Hz, 2H), 2.42 (s, 3H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 164.1, 160.4, 156.7, 148.2, 140.4, 138.4, 137.9, 130.8, 129.9 (2C), 129.3, 129.1 (2C), 129.0 (3C), 128.5, 127.7 (3C), 127.2 (2C), 85.7, 45.1, 21.5. HRESIMS: *m/z* 392.1874 $[M + H]^+$ (calcd for $C_{25}H_{22}N_5$, 392.1875).

3.2.4.5. 5-(4-Chlorophenyl)-N-ethyl-2-phenyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (8e). Light yellow solid, yield 59%; ^{1}H-NMR ($CDCl_3$, 500MHz) δ (ppm): 8.36–8.34 (m, 2H), 8.12 (d, $J = 8.5$Hz, 2H), 7.50–7.46 (m, 5H), 6.51 (s, 1H), 3.62–3.58 (m, 2H), 1.51 (t, $J = 7.5$Hz, 3H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 163.9, 159.2, 156.7, 148.1, 136.9, 135.6, 131.3, 130.7, 129.6 (2C), 129.2 (2C), 129.1 (2C), 127.2 (2C), 85.2, 37.0, 14.7. HRESIMS: *m/z* 350.1173 $[M + H]^+$ (calcd for $C_{19}H_{17}N_5Cl$, 350.1172).

3.2.4.6. 5-(4-Chlorophenyl)-2-phenyl-7-(piperidin-1-yl)-[1,2,4]triazolo [1,5-a] pyrimidine (8f). Light yellow solid, yield 60%; ^{1}H-NMR ($CDCl_3$, 500MHz) δ (ppm): 8.37 (d, $J = 8.0$Hz, 2H), 8.12 (d, $J = 8.0$Hz, 2H), 7.48 (t, $J = 8.0$Hz, 5H), 6.60 (s, 1H), 3.94 (t, $J = 4.5$Hz, 4H), 1.89–1.57 (m, 6H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 163.2, 159.1, 158.3, 150.6, 136.5, 135.8, 131.0, 130.8, 129.7 (2C), 129.3 (2C), 129.2 (2C), 127.2 (2C), 91.7, 49.6 (2C), 25.7 (2C), 24.3. HRESIMS: *m/z* 390.1486 $[M + H]^+$ (calcd for $C_{22}H_{21}N_5Cl$, 390.1485).

3.2.4.7. 5-(4-Chlorophenyl)-N,2-diphenyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (8g). White solid, yield 60%; ^{1}H-NMR ($CDCl_3$, 500MHz) δ (ppm): 8.40 (d, $J = 6.0$Hz, 2H), 8.05 (d, $J = 8.5$Hz, 2H), 7.97 (s, 1H), 7.58–7.39 (m, 10H), 6.91 (s, 1H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 164.3, 159.6, 157.0, 147.0, 137.2, 136.6, 135.7, 131.1, 130.9, 130.1 (2C), 129.4 (2C), 129.3 (4C), 127.4 (2C), 126.7, 124.9 (2C), 86.80. HRESIMS: *m/z* 398.1175 $[M + H]^+$ (calcd for $C_{23}H_{17}N_5Cl$, 398.1172).

3.2.4.8. N-Benzyl-5-(4-chlorophenyl)-2-phenyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (8h). Light yellow solid, yield 75%; ^{1}H-NMR ($CDCl_3$, 500MHz) δ (ppm): 8.34 (t, $J = 3.7$Hz, 2H), 8.04 (d, $J = 8.5$Hz, 2H), 7.49–7.38 (m, 10H), 6.65 (s, 1H), 6.52 (s, 1H), 4.73 (d, $J = 5.5$Hz, 2H). ^{13}C-NMR (DMSO, 125MHz) δ (ppm): 163.8, 159.2, 156.4, 148.4, 138.3, 136.6, 135.7, 131.0, 130.8, 129.5 (2C), 129.2 (2C), 129.1 (2C), 129.0 (2C), 127.7 (3C), 127.3 (2C), 85.9, 45.1. HRESIMS: *m/z* 412.1330 $[M + H]^+$ (calcd for $C_{24}H_{19}N_5Cl$, 412.1329).

3.3. Pharmacology

3.3.1. Primary neocortical neuron culture

All animal experiment protocols were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) approved by the China Pharmaceutical University Institutional Animal Care and Use Committee. Animals were treated humanly and with regard for alleviation of suffering. Neocortical neuron cultures were obtained from embryonic day 16 C57 BL/6 mice. Briefly, neocortices were collected, stripped off meninges, minced by trituration with a Pasteur pipette, and treated with trypsin for 25 min at 37 °C. The cells were then dissociated by two successive trituration and sedimentation steps in soybean trypsin inhibitor and DNase I-containing isolation buffer, centrifuged, and resuspended in neurobasal complete medium (neurobasal medium supplemented with 2% NS21 [24], 1 mM L-glutamine, 1% HEPES with 5% fetal bovine serum), Cells were planted onto poly-L-lysine-coated clear-bottomed, black-wall, 96-well plates (Corning Life Sciences, Acton, MA) at a density of 5.6×10^5 cells/cm^2 and incubated at 37 °C in a 5% CO_2 and 95% humidity atmosphere. A final concentration of ARA-C (10 μM) was added to the culture medium after planting for 24–36 h to prevent the astrocytes proliferation. The culture medium was half-replaced on days 5 and 7 with serum-free growth medium containing neurobasal medium supplemented with 2% NS21, 1% HEPES, and 0.2 mM L-glutamine.

3.3.2. Intracellular Ca^{2+} concentration determination

Neocortical neurons at 8–9 d *in vitro* (DIV) were used to investigate the alteration of compounds on intracellular Ca^{2+} dynamics (Huang QL, PhytoChemistry, 2018) [25]. Briefly, the growth medium was removed and replaced with dye loading buffer (100 μl/well) containing 4 μM Fluo-8/AM and 0.5% BSA in Locke's buffer (in mM: HEPES 8.6, KCl 5.6, NaCl 154, D-glucose 5.6, $MgCl_2$ 1.0, $CaCl_2$ 2.3, and glycine 0.1, adjust pH to 7.4). After 45 min incubation in dye loading buffer, the neurons were washed four times with fresh Locke's buffer (200 μl/well) and placed in a Fluorescent Imaging Plate Reader (FLIPRTetra; Molecular Devices, Sunnyvale, CA) incubation chamber. Basal fluorescence levels were acquired in Locke's buffer for 5 min at a sampling rate of 1 Hz followed by an addition of vehicle or compounds using a programmable 96-channel pipetting robotic system, and the fluorescent signals were recorded for an additional 8 min. To investigate influence of compounds on 4-AP-induced Ca^{2+} response, a second addition of 4-AP or was made and the fluorescent signals were recorded for an additional 8 min.

The background fluorescence of the plate was determined from a sister well without Fluo-8/AM loading, and all the fluorescence signals were corrected by subtracting the background fluorescence. Data were presented as F/F_0, where F is the fluorescent signal at different time point minus background fluorescence, whereas F_0 is the basal fluorescence minus the background fluorescence. To determine the compounds response on SCOs, the SCO frequency from an epoch of 300 s after addition of vehicle (0.1% DMSO) or compounds for 60 s was manually counted. All the data were presented as % vehicle. To determine the compounds response on 4-AP-induced Ca^{2+} oscillation, the Ca^{2+} oscillation frequency was manually counted from an epoch of 300 s in the presence or absence of compounds after addition of 4-AP for 120 s. The data were presented as % of 4-AP. Events having $F/F_0 > 0.05$ units were included in the analyses of SCO frequency.

3.3.3. Molecular docking studies

The crystal protein of GABA receptor protein was from Protein Data Bank database (PDB), PDB code: 4COF. Autodock 4.2 (http://autodock.Scripps.edu/) was used as molecular docking software and the software for PDB crystal protein processing was Autodock tools 1.5.6 (http://autodock.scripps.edu/) during docking process. The render software package was Discovery Studio 4.5 Client (http://accelrys.com/) and algorithm was Genetic Algorithm in autodock 4.2.

4. Conclusion

In this work, eight [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one derivatives were synthesized and their anti-epileptic activities were evaluated on the intracellular calcium ion concussion of mouse neuronal cells and 4-AP induced hyper excitability model in primary cultured neocortical neurons. Five of [1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one derivatives show remarkable inhibitory effects on these two models. The most effective compounds **6b** and **6e** were obviously superior to marine natural product essramycin and positive control CBZ. The preliminary SAR was studied by modifying

the compounds **6b** and **6e** to [1,2,4]-triazolo[1,5-a]pyrimidine amine **8a-h** or chloride [1,2,4]-triazolo[1,5-a]pyrimidine **7a-b** and the results indicated that the pyrimidin-7(4H)-one motif in the active compounds of **6a-6e** is the necessary "active core" of anti-epileptic activity. The molecular docking revealed that compounds **6b** and **6e** have strong binding affinity with $GABA_A$ receptor, the result is agreement with the experimental observations.

Disclosure statement

No potential conflict of interests was reported by the authors.

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