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The design and synthesis of novel positive allosteric modulators of α7 nicotinic acetylcholine receptors with the ability to rescue auditory gating deficit in mice

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ABSTRACT

A series of novel thiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (**3aa**–**3ep**) were designed, synthesized and evaluated as the type I positive allosteric modulators (PAMs) of human α7 nAChR expressed in *Xenopus* ooctyes by two-electrode voltage clamp. The structure-activity relationship (SAR) analysis identified the compound **3ea** as a potent and efficacious PAM with the maximum activation effect of the α7 current of over 1633% in the presence of acetylcholine (100 μM) and an EC_{50} = 1.26 μM. It is highly specific to α7 nAChR over other subtypes of nAChR, 5-HT_{3A}, NMDA and $GABA_A$ receptors. **3ea** showed an elimination half-life ($T_{1/2}$) of 10.8 ± 1.5 h for 3 mg/kg, i.v. and 7.4 ± 1.1 h for 60 mg/kg, i.g. in rat. It also exhibited sufficient blood-brain barrier penetration with no significant effect on hERG channel. Most importantly, compound **3ea** dose-dependently (0.1–1 mg/kg, i.p.) reversed the prepulse inhibition (PPI) deficit induced by MK-801 in the mouse schizophrenia model.

Keywords: α7 nAChR; Positive allosteric modulators; Thiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones; Structure−activity relationship; Prepulse inhibition; Schizophrenia disease.

INTRODUCTION

The α7-subtype of nicotinic acetylcholine receptors (α7 nAChR) has been well recognized as a potential pharmacological target in central nervous system (CNS) for the treatment of neuropsychiatric disorders, such as schizophrenia and Alzheimer's disease.[1] It is a homopentamer that can form ligand-gated calcium permeable ion channels upon the activation with acetylcholine, exhibiting extremely fast desensitization as exposed to agonists.[2] Clinical studies have shown that targeting α7 nAChR with selective agonists or partial agonists can effectively improve the cognitive deficits in schizophrenia.[3] Positive allosteric modulators (PAMs) have been demonstrated with numerous advantages over agonists because it can maintain the normal temporal and spatial patterns of neurotransmission due to the lack of activity in the absence of an endogenous agonist.[4] Based on their channel kinetics and desensitization characteristics, the PAMs of nicotinic receptors are classified into type I and II.[5] Figure 1 shows the chemical structures of representative type I PAMs including macrolide **1a** (ivermectin)[6], anthridine alkaloid **1b** (galantamine)[7], indole derivative **1c** (5-hydroxyindole, 5-HI)[8], isoflavones **1d** (genistein)[9], acrylamide **1e** (AVL3288, CCMI)[10], ureas **1f** (NS-1738)[11] and **1g** (SB-206553)[12], and (2-amino-5-keto)thiazole compound **1h** (LY-2087101)[13]. The type II PAMs including urea **2a** (PNU-120596)[14], thiazole compound **2b** (JNJ-1930942)[15], and sulfonamides **2c** (TQS)[16] and **2d** (A-867744)[17] are shown in Figure 2.

Both types of PAMs have been found with *in vivo* efficacy in animal models of cognition,[18] whereas type II PAMs are less prone to induce tolerances after the chronic administration of nicotinic agonists. Type I PAMs can maintain the rapid channel kinetics, and thus may be more beneficial than type II PAMs to minimizing potential Ca^{2+}-induced cytotoxicity.[19] α7 nAChR and

other subtypes including α4β2 and α3β4 of nAChR are also expressed in the cortex, hippocampus and cerebellum of brain,[20] whose functions can be activated with acetylcholine and enhanced with nAChR PAMs. Therefore, the design and synthesis of novel selective α7 nAChR PAMs with high potency and efficacy are highly desired to achieve ideal therapeutic indexes and reduce side-effects.

Figure 1

Figure 2

To identify novel PAMs, we initially synthesized a series of fused pyrimidin-ones **3** using 2-ethylsulfanyl-5-amino-thiazines as the key intermediate. A small library of these compounds and intermediates was construed and screened for the PAM activity on the human α7 nAChR expressed in *Xenopus* oocytes by the two-electrode voltage clamp assay. Compound **3aa** (LD486)[21], one of the intermediates, exhibited the type I PAM activity on α7 nAChR with the EC_{50} of 3.20 ± 0.30 μM (n=6) and the maximum effect of 320 ± 20 % in the presence of 100 μM ACh. Based on the novel structure and good efficacy of compound **3aa**, we envisaged that more efficient compounds could be synthesized by alternative modifications. Herein, we designed and synthesized a series of thiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones **3** and evaluated their biological activities as novel PAMs of α7 nAChR.

Figure 3

Aiming to improve the efficacy and selectivity of the hit compound **3aa**, we first studied the

SAR of thiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones. The substitutes on N^2- and/or N^6- of thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one motif were first examined (Figure 3). The 6-Cl-2-Me-pyrimidin-4-yl group of **3aa** was modified with heterocyclic aromatic (**3ab**–**3ae**), aliphic (**3af**–**3ag**), and aromatic (**3ba**–**3bs**) groups, respectively, to explore the tolerance of N^2-R^2 group in the PAM activity. Compounds of amide **3ca**, sulfamide **3cb**, sulfide **3da**, and other type linkers **3cc**, **3cd**, **3db** and **3ea**–**3ek** were designed to examine the effects of space between aromatic group and thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one on the activity. Compounds **3el**–**3eq** were synthesized to evaluate the tolerance of N^6- of thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one. Compounds **16** and **19** with different skeletons were synthesized to explore the key effects of thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one.

RESULTS AND DISCUSSIONS

Scheme 1

The synthesis route of thiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones **3** is outlined in Scheme 1. The reaction of aromatic amines with chloroacetyl chloride gave 2-chloro-*N*-phenyl acetamide **4** that was treated with dipotassium cyanodithioimidocarbonate, followed by the addition of iodoethane, to produce the key intermediate [(4-amino-2-ethylthio)-thiazolo]-5-carboxmide **5** via the Thorpe-Ziegler cyclization.[22a, 22b] Intermediate **5** was refluxed in HCOOH for 1 h to afford a crude product[22c] that, without further purification, was stirred with $Na_2WO_4 \cdot H_2O$ in 30% hydrogen peroxide for 1 h at room temperature, and heated at 50°C for another 1 h to afford the key intermediate **6**. The nucleophilic substitution of the ethylsulfonyl in **6** with different aromatic and aliphatic amines yielded the corresponding products **3aa**–**3ah** and **3ba**–**3bs**, respectively.[21b]

Scheme 2

Scheme 3

Scheme 4

The nucleophilic substitution of ethylsulfonyl **6a** with the substituted amide **7**, sulfamide **8**, *N*, 4-dimethylanine, 4-methylbenzylamine and 4-methyl-thiophenol **9** (Scheme 2) gave the corresponding products **3ca**–**3cd**, respectively. Compound **3db** was synthesized according to the procedure described in Scheme 3. Intermediate **5** was refluxed in HCOOH for 1 h to afford key intermediate **6**. Then, desulfurative palladium-catalyzed Liebeskind–Srögl coupling reaction of **6** and 4-methylphenyl boric acid provided the product **3db** in presence of CuTC.[22d] Compounds **3ea**–**3eq** were prepared via an alternative method as shown in Scheme 4. Different substituted benzylsulfonyl, 2-phenylethylsulfonyl, and 3-phenylpropyl groups were introduced to afford 2-chloro-*N*-2-choloro-6-methylphenyl acetamides **4a**–**4g** that were subjected to the thorpe-Ziegler cyclization with dipotassium cyanodithioimidocarbonate, followed by the addition of chloride or bromide **11**, to produce thiazoles **10a**–**10q**. The thiazoles were treated with formic acid to yield target products **3ea**–**3eq**.

Scheme 5

2-(Benzylthio)-*N*-(2-chloro-6-methylphenyl)thiazolo[4,5-*d*]pyrimidin-7-amine **16** was synthesized according to the reference procedure shown in Scheme 5.[22e] The reaction of dipotassium cyandithiomidocarbonate **12** with benzyl bromide in acetone-water afforded

compound **13** without further purification. Certain amounts of chloroacetonitrile and Et_3N were then added to the solution of **13** and heated at 70 ^oC for 4 h to produce the key intermediate, 4-amino-2-(benzylthio)thiazole- 5-carbonitrile, **14** that was concentrated with DMF·DMA at 100 ^oC to yield intermediate **15** as a grey solid. The reaction of **15** with 2-chloro-6-methylaniline in acetic acid at a higher temperature gave the desired product **16** via a one-pot reaction.

Scheme 6

The synthesis route of compound **19** with another new skeleton is described in Scheme 6. 6-iodo-benzo[*d*]pyrimidin-4(3*H*)-one **18** was obtained via the one-pot condensation of 5-iodoanthranilic acid **17**, 2-chloro-6-methylaniline and trimethylorthoformate at 100^oC in acetic acid.[22f] The copper-catalyzed Ullmann reaction of intermediate **18** with 4-methylaniline produced the target product **19**.

The synthesized compounds were tested for the *in vitro* activity in *Xenopus* laevis oocytes expressing human α7 nAChR at room temperature as described in our previous work.[23] The compounds were inactive in eliciting α7 current in the absence of direct agonists. Adding acetylcholine (ACh, 100 μM) to 10 μM test compounds resulted in the activation of α7 currents, suggesting that the compounds were positive allosteric modulators of α7 nAChR. The concentration-response curves of the compounds are listed in Tables 1, 2, 3, and 4. The maximum modulation (at 10 μM) and EC_{50} where half maximum modulation was achieved for the compounds with the maximum effect > 400% were also determined.

Table 1

The substitution of 6-chloro-2-methyl-pyrimidin-4-yl with 6-chloro-pyrimidin-4-yl (**3ab**, Table 1), 6-chloro-pyridazin-3-yl (**3ac**, Table 1), pyridin-2-yl (**3ad**, Table 1) or pyrimidin-4-yl (**3ae**, Table 1) caused either lost or reduced activity. Substituting 6-chloro-2-methyl-pyrimidin-4-yl (**3aa**, Table 1) with aliphic groups, such as propyl (**3af**, Table 1) and benzyl (**3ag**, Table 1), resulted in completely lost activity. These results indicate that suitable heterocyclic aromatic and aliphic groups are detrimental for the allosteric modulation activity and compounds **3af**–**3ag** are inactive for allosteric modulation.

Based on the *in vitro* activities of **3aa**–**3ag** shown in Table 1, we examined the effects of the substitutes (**3ba**–**3bs**) on the phenyl group in R^1. The substitutions of R^1 in **3aa** with 3-chloro-5-methylphenyl (**3ba**) and 3-chloro-phenyl (**3bb**), and 3-methyl-phenyl (**3bc**) (Table 1) resulted in similar modulation effects. Compound **3bc** (max effect of 1540%, EC_{50} = 8.39 μM) with 3-methyl exhibited a 5-fold higher modulation effect than **3aa**. Although the EC_{50} of **3bc** was not as high as that of **3aa** (3.20 μM), it encouraged us to further modify the phenyl group to achieve better activities. Changing the 3-methyl of **3bc** to the 2-position and 4-position gave compounds **3bd** and **3be**, respectively. **3bd** showed the maximum effect of 300% at 10 μM as the modulator of α7 nAChR, and **3be** exhibited the highest maximum modulation effect of 1622% with the EC_{50} of 8.45 μM. The substitutions of 4-Me in **3be** with H, 4-Cl, 4-Br, and 4-F (**3bf**–**3bi**) dramatically reduced the activity. The isomeric 2- (**3bj**) and 3- fluorophenyl (**3bk**) compounds showed significant activities. Compound **3bk** exhibited a potency with EC_{50} = 7.65 μM and the maximum modulation effect of 450%. Moving the fluorine atom to the 2-position (**3bj**) dramatically reduced the activity to the maximum modulation effect of 200%. The substitutions of

the 4-methyl in **3be** (max effect of 1622%) with 4-ethyl (**3bl**, max effect of 250%) and 4-methoxy (**3bm**, max effect of 200%) resulted in almost 10-fold loss in the maximum effect. Similar results were obtained when 4-methyl was substituted with 4-OCF_3 (**3bn**, with the max effect of 200%).

Based on the maximum effects acquired from 3-fluoro, 3-methyl and 4-methyl substitutes, we envisaged that the introduction of methyl and /or fluoro to the meta- and para-positions of phenyl group could improve the activity. Therefore, compounds **3bo**−**3bs** were designed and synthesized, which, to our delightfulness, resulted in the maximum effects ranging from 300% to 600%.

Compound **3be** (R = 4-Me-Ph) exhibited the highest activity with 5-fold higher maximum effect than that of the lead compound **3aa**. In order to further explore the SAR of the linker between the aromatic group and key pharmacophore motif, changing –NH– (**3be**, Max mod 1622%) with different type of amide (**3ca**, 450%), sulfamide (**3cb**, 97%), –NMe– (**3cc**, 107%), –CH_2NH– (**3cd**, 111%), –S– (**3da**, 226%), or bond (**3db**, 185%) resulted in a much decrease of activity, indicating the high influence of the space on the activity (Table 2).

Table 2

Attempts to extend the linker of -S- atom to -CH_2S- group gave compounds **3ea**–**3ei** with the maximum effects and EC_{50} listed in Table 2. The SAR study revealed that **3ea** with no substitution on its phenyl group had a higher activity than the mono-substituted derivatives **3eb**–**3eh**. In addition, the activity of **3ea** is much higher than that of **3ag** (Table 1) with -CH_2NH- as the linker. Mono-substituted fluorine derivatives **3eb**–**3ed** exhibited similar effects compared to the di-fluoro derivative **3ei** (max mod of 1046% and EC_{50} = 3.54 μM), acceptable EC_{50} values in the range of 2.05–7.65 μM were achieved. Further extending the linker of -S- atom to -$(CH_2)_2$S- or -$(CH_2)_3$S-

groups produced **3ej**–**3ek** with completely lost activities. Based on these results, it can be deduced that further modifying the 2-position of thiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones may give more efficient α7 nAChR PAMs.

The modifications of the 2-chloro-6-methyl group (**3ea**) in R^2 with 2-fluoro (**3el**), 3-fluoro (**3em**), 4-fluoro (**3en**), 4-chloro (**3eo**), 2-chloro (**3ep**) and 2-methyl (**3eq**) led to dramatically reduced activity or even complete loss of effect (Table 3), indicating that N^6-(2-chloro-6-methyl)-phenyl group was the key pharmacophore for this novel series of compounds. In addition, the low activities of **16** and **19** suggest that thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one is the key biological skeleton (**16**: max mod of 33%, Table 4 *vs.* **3ea**: 1633%, Table 4; **19**: max mod of 63% Table 6 *vs.* **3be**: 1622%, Table 1).

Table 3

Table 4

Based on the results presented above, it can be concluded that, among the synthesized analogs, **3ea** is the optimal PAM of α7 nAChR. A 2-minute preincubation of oocytes expressing α7 nAChR with **3ea**, followed by the application of 100 μM ACh, remarkably increased the peak current and slightly prolonged the current duration (Figure 4A). In addition, such enhanced ACh-evoked current by 10 μM **3ea** was completely inhibited with 10 nM MLA, a selective α7 nAChR antagonist. To evaluate the specificity of **3ea** for α7 nAChR, its effects on other nAChRs

and 5-HT_{3A} receptor expressed in oocytes were investigated. As shown in Figure 4B, **3ea** was only able to cause potentiation to α7 nAChR. The inspection of Figure 4C and D suggests that **3ea** slightly affected the desensitization of α7 nAChR, causing a minor increase in $\tau_{desensitization}$ (ACh: $\tau_{desensitization}$ = 5.56 ± 1.27; ACh + **3ea**: $\tau_{desensitization}$ = 6.54 ± 1.31; $P > 0.05$). We also tested the effect of **3ea** on NMDA and $GABA_A$ channels. The results show that **3ea** had little effect on NR1/NR2B and several subtypes of $GABA_A$ channels (Figure S1). Based on these results, it can be concluded that **3ea** is a selective type I α7 nAChR PAM.

Figure 4

The activation of α7 nAChR with 100 μM ACh alone was firstly conducted as the control. **3ea** was then co-applied with 100 μM ACh after 2-min pre-incubation at the initial concentration of 0.1 μM to activate α7 current. The **3ea** concentration was increased at the 10-min intervals to 0.3, 1, 3, 10, and 30 μM. Figure 5A shows α7 currents evoked with different concentrations of **3ea** in the presence of 100 μM ACh. The **3ea** concentration-dependent activation of α7 current was plotted (Figure 5B) and fitted to the Hill equation, which gave EC_{50} = 1.26 ± 0.18 μM, E_{max} = 1633 ± 87% and Hill coefficient (n_H)= 1.85 ± 0.24. To further confirm the activity of **3ea**, the ACh concentration-response curves were generated in the absence and presence of 10 μM **3ea.** A 2-min pre-incubation with **3ea** increased the E_{max} 15-fold from 109 ± 2% to 1545 ± 59%, reduced the EC_{50} of ACh from 256.4 ± 20.1 μM to 186.8 ± 14.5 μM, and slightly increased of n_H from 1.14 ± 0.10 to 1.57 ± 0.16 (Figure 5C).

Figure 5

The voltage protocol was run for at least 1 min and perfused continuously (0.5 mL/min) with an extracellular bath solution containing compound **3ea** (1, 3, 10, or 30 μM) and 100 nM cisapride, a hERG classical inhibitor, followed by washing with the bath solution. The peak tail currents at -40mV in the presence and absence of drugs were normalized to their respective control values and plotted as the relative current amplitude vs. **3ea** concentration. As shown in Figure 6, the hERG current remained stable with the increase of **3ea** concentration, suggesting that **3ea** had no cardiac toxicity *in vitro*.

Figure 6

The assay that we previously developed for rat plasma was used to determine the pharmacokinetic profile of **3ea**.[23] Mean half-life for the iv dose and oral administration (60 mg/kg) was 10.8 and 7.4 h respectively. Considering the experimental error in vivo as well as the clearance results from two administration pathways, these $t_{1/2}$ values are likely in the acceptable range. Low clearance (0.005 L/h/kg) for compound **3ea** and the metabolic stability from the i.v. dose in rat are translated well to gavaged dose (60 mg/kg). Since the bioavailability of the compound **3ea** is around 8%, the CL/F estimates for gavaged doses (0.06 L/h/kg) are reasonable. The mean residence time (MRT) for gavage (9.5 h) is longer than that for intravenous injection (7.4 h), indicating the mean absorption time is nearly 2 h. The low bioavailability of **3ea** could be explained by precipitation in the rat gut. These results suggest that suitable drug formulation of oral administration needs further exploration during its pre-clinical studies. The pharmacokinetic parameters were evaluated in each individual rat by a non-compartmental approach using the software DAS2. The administration doses of 60 mg/kg (i.g.) and 3 mg/kg (i.v.) resulted in the maximum concentrations (C_{max}) of 200 ± 80 ng/mL and 700 ± 200 ng/mL and the areas under the

curve (AUC_{Inf}) of 1100 ± 200 ng·h/mL and 680 ± 40 ng·h/mL, respectively (Table 5).

Table 5

A frequently confronted challenge in developing CNS targeted drugs is the insufficient blood-brain barrier (BBB) penetration. Table 6 lists the results of the CNS exposure of **3ea** in mice. The compound was administered intraperitoneally (ip) to C57BL/6J mice at 1mg/kg and its brain and plasma concentrations were assayed at 0.5, 2 and 8 h after the administration. The brain/plasma concentration ratios were measured to be 2.5, 2.7 and 2.3 at 0.5, 2.0 and 8.0 h post-administration, respectively, suggesting the excellent brain penetration of **3ea**. Therefore, **3ea** can robustly activate central receptors, while minimizing possible side effects caused by the interactions with the peripheral systems.

Table 6

To further evaluate the modulation effects of **3ea**, the mouse model of schizophrenia with auditory gating deficit induced by NMDA antagonist MK-801 was measured for the prepulse inhibition the acoustic startle chambers before and after the administration of **3ea**. Three 20-ms prepulses of different intensities (8, 12 and 16 above 68 dB background noise) at 80 ms delay intervals appeared before the application of the startle stimulus (pulse alone) at 120 dB for 40 ms (Figure 7A). The intraperitoneal injection of MK-801 (0.1 mg/kg) dramatically reduced the PPI from 36% to 11%. The administration of different doses of **3ea** (0.1–1.0 mg/kg) reversed the reduced PPI to 22%, 26% and 28%, respectively, while clozapine (1 mg/kg, i.p.), the positive

control, reversed the reduced PPI to 32% (Figure 7B). These results indicate that type I PAM **3ea** can rescue the auditory gating deficit in schizophrenia-like behavior of mice.

Figure 7

In order to verify whether the *in vivo* activity is directly related to the activation of alpha7 nACh receptors, we redesigned the experimental group, as shown below in Figure 8.[3d] Intraperitoneal injection of MK-801 (0.1 mg/kg, i.p.) 30 minutes before the test resulted in a significant reduction of PPI from 41% to 8.7%, administration of **3ea** (1 mg/kg, i.p.) 60 minutes before the test reversed the reduction of PPI to 30%. Pretreatment with MLA (3 mg/kg, i.p.) 30 minutes prevented the reversal by **3ea** of MK-801-induced auditory gating impairment to 14%. Injection of **3ea** or MLA alone did not significantly alter the percentage of prepulse inhibition compared with control group. These results suggest that *in vivo* activity of compound **3ea** is directly related to the activation of alpha7 nACh receptors.

Figure 8

CONCLUSIONS

A series of novel thiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (**3aa**−**3eq**) were designed, synthesized and evaluated as the type I positive allosteric modulators (PAMs) of human α7 nAChR expressed in *Xenopus* ooctyes in two-electrode voltage clamp assay. The structure-activity relationship (SAR) analysis revealed that N^6-(2-chloro-6-methyl)-phenyl was the key pharmacophore for the activity of these compounds. Compound **3ea** exhibited high selectivity for α7 nAChR over other subtypes

of α4β2, α3β4 nAChRs and 5-HT_{3A}, NMDA, $GABA_A$ receptors with an excellent pharmacokinetic profile and good brain tissue distributions. It was able to successfully reverse the PPI impairments induced by MK-801 in mouse acoustic startle model of schizophrenia. Our findings demonstrate that the novel compound **3ea**, which is suggestive as type I PAM, is a potential drug candidate for improvement of cognitive function in neurodegenerative models of rodents.

EXPERIMENTAL SECTION

General: Commercial reagents were purchased from Ouhe, Aladdin and J&K Scientific and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AVIII-400 spectrometer at ambient temperature with $CDCl_3$ or DMSO-d_6 as the solvent. Chemical shifts were referenced to the residual peaks of the solvent [$CDCl_3$: δ = 7.26 (^{1}H), 77.16 ppm (^{13}C); DMSO-d_6: δ = 2.50 (^{1}H), 39.52 ppm (^{13}C)]. High-resolution mass spectra were recorded with a Bruker Apex IV Fourier transform ion cyclotron resonance mass spectrometer. Flash column chromatography was performed with 200–300 mesh silica gel. All the final compounds were tested by HPLC and the purity in every case was $\geqslant$95%. The reverse phase HPLC was conducted on Agilent Technologies 1260 Infinity, which was equipped with C18 column (Aglilent Eclipse plus C18, 3.5 μm, 4.6 mm × 100 mm). The mobile phase A was water, and mobile phase B was acetonitrile. The gradient of 70% B was run at a fl T rate of 1.0 mL/min over 10 min.

1.1 2-((6-Chloro-2-methylpyrimidin-4-yl)amino)-6-(2-chloro-6-methylphenyl)thiazolo[4,5-*d*] pyrimidin-7(6*H*)-one (3aa).

Compound **6a** (370 mg, 1.0 mmol) and 4-amino-6-chloro-2-methylpyrimidine (287 mg, 2.0 mmol) were dissolved in anhydrous THF (3 mL). Then, NaH (48 mg, 2.0 mmol) was added to the solution, the reaction was held at reflux for 0.5h with stirring, then cooled and quenched with ethanol. The reaction mixture was concentrated under reduce pressure. The crude material was purified by silica gel chromatography (CH_2Cl_2/MeOH = 50:1) to afford the compound **3aa**. Yield: 64%. Yellow solid, m.p.: > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 12.80 (bs, 1H), 8.44 (s, 1H), 7.58 (d, J = 7.2 Hz, 1H), 7.51 (t, J = 8 Hz, 1H), 7.46 (d, J = 7.2 Hz, 1H), 6.99 (s, 1H), 2.56 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 167.8, 164.0, 159.5, 158.1, 155.9, 150.9, 139.1, 134.0, 132.3, 131.4, 130.3, 128.1, 112.0, 104.6, 25.5, 18.2. HRMS: m/z calcd. for $C_{17}H_{13}Cl_2N_6OS$ $[M + H]^+$ 419.0243; found 419.0252. HPLC purity: 97.8%, retention time = 1.63 min.

1.2 6-(2-Chloro-6-methylphenyl)-2-((3-chloro-5-methylphenyl)amino)thiazolo[4,5-*d*] pyrimidin-7(6*H*)-one (3ba).

Compound **6a** (370 mg, 1.0 mmol) was dissolved in acetic acid (7 mL). Then, 3-chloro-5-methylaniline (284 mg, 2.0 mmol) was added to the solution. The reaction was held at 40 °C for 6 h with stirring. After completion of the reaction (TLC), the reaction mixture was poured into ice water. The precipitated solid was collected and filtered. The product was washed with water, dried under vacuum. The crude material was recrystallized to afford the compound **3ba**. Yield: 89%. White solid, m.p.: 260–262 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.26

(bs, 1H), 8.42 (s, 1H), 7.82 (s, 1H), 7.57 (d, J = 7.7 Hz, 1H), 7.43–7.52 (m, 2H), 7.34 (s, 1H), 7.01 (s, 1H), 2.33 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 167.0, 165.7, 154.7, 150.4, 140.8, 140.6, 138.6, 133.5, 133.3, 131.8, 130.9, 129.8, 127.6, 123.6, 117.4, 115.2, 108.3, 20.9, 17.7. HRMS: m/z calcd. for $C_{19}H_{15}Cl_2N_4OS$ $[M + H]^+$ 417.0338; found 417.0342. HPLC purity: 97.2%, retention time = 1.36 min.

6-(2-Chloro-6-methylphenyl)-2-((3-chlorophenyl)amino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3bb).

The title compound was prepared from compound **6a** and 3-chloroaniline following the general procedure of **3ba**. Yield: 81%. White solid, m.p.: 248–251 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.33 (bs, 1H), 8.41 (s, 1H), 8.02 (s, 1H), 7.37–7.60 (m, 5H), 7.16 (d, J = 7.9 Hz, 1H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 167.4, 166.2, 155.2, 150.9, 141.4, 139.1, 134.0, 134.0, 132.3, 131.4, 131.3, 130.2, 128.1, 123.4, 118.4, 117.5, 108.9, 18.2. HRMS: m/z calcd. for $C_{18}H_{13}Cl_2N_4OS$ $[M + H]^+$ 403.0181; found 403.0169. HPLC purity: 99.6%, retention time = 2.34min.

6-(2-Chloro-6-methylphenyl)-2-(m-tolylamino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3bc).

The title compound was prepared from compound **6a** and *m*-toluidine following the general procedure of **3ba**. Yield: 78%. White solid, m.p.: 203–206 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.09 (bs, 1H), 8.37 (s, 1H), 7.42–7.61(m, 5H), 7.29 (t, J = 8.0 Hz, 1H), 6.95 (d, J = 7.4 Hz, 1H), 2.34 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 168.0, 166.5, 155.2, 150.8, 140.0, 139.1, 134.1, 132.4, 131.4, 130.3, 129.6, 128.1, 124.9, 119.7, 116.5, 108.2, 21.8,

18.2. HRMS: m/z calcd. for $C_{19}H_{16}ClN_4OS$ $[M + H]^+$ 383.0727; found 383.0719. HPLC purity: 98.9%, retention time = 2.01 min.

6-(2-Chloro-6-methylphenyl)-2-(o-tolylamino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3bd).

The title compound was prepared from compound **6a** and *o*-toluidine following the general procedure of **3ba**. Yield: 75%. White solid, m.p.: 268–270 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.53 (bs, 1H), 8.33 (s, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.56 (dd, J = 7.7, 1.2 Hz, 1H), 7.41–7.51 (m, 2H), 7.26–7.37 (m, 2H), 7.22 (dd, J = 10.7, 4.0 Hz, 1H), 2.30 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 171.3, 166.7, 155.0, 150.7, 139.1, 138.0, 134.1, 132.6, 132.3, 131.5, 131.3, 130.2, 128.0, 127.4, 126.9, 125.0, 107.9, 18.2, 18.2. HRMS: m/z calcd. for $C_{19}H_{16}ClN_4OS$ $[M + H]^+$ 383.0727; found 383.0717. HPLC purity: 99.7%, retention time = 1.70min.

6-(2-Chloro-6-methylphenyl)-2-(p-tolylamino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3be).

The title compound was prepared from compound **6a** and *p*-toluidine following the general procedure of **3ba**. Yield: 88%. White solid, m.p.: 241–243 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.10 (s, 1H), 8.39 (s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.57 (dd, J = 7.8, 1.7 Hz, 1H), 7.50 (t, J = 7.7 Hz, 1H), 7.44–7.47 (m, 1H), 7.23 (d, J = 8.4 Hz, 2H), 2.31 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 168.0, 166.5, 155.1, 150.7, 139.1, 137.5, 134.0, 133.2, 132.3, 131.4, 130.2, 130.1, 128.0, 119.4, 108.0, 20.9, 18.2. HRMS: m/z calcd. for $C_{19}H_{16}ClN_4OS$ $[M + H]^+$ 383.0727; found 383.0717. HPLC purity: 96.9%, retention time = 2.16min.

6-(2-Chloro-6-methylphenyl)-2-((3,4-dimethylphenyl)amino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3bo).

The title compound was prepared from compound **6a** and 3,4-dimethylaniline following the general procedure of **3ba**. Yield: 87%. White solid, m.p.: 226–228 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.03 (bs, 1H), 8.38 (s, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.43–7.55 (m, 4H), 7.17 (d, J = 7.8 Hz, 1H), 2.25 (s, 3H), 2.21 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 168.2, 166.6, 155.1, 150.7, 139.1, 137.7, 137.6, 134.1, 132.3, 131.4, 130.6, 130.2, 128.1, 120.6, 117.0, 107.9, 20.2, 19.3, 18.2. HRMS: m/z calcd. for $C_{20}H_{18}ClN_4OS$ $[M + H]^+$ 397.0884; found 397.0876. HPLC purity: 99.6%, retention time = 2.27min.

6-(2-Chloro-6-methylphenyl)-2-((3,5-dimethylphenyl)amino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3bp).

The title compound was prepared from compound **6a** and 3,5-dimethylaniline following the general procedure of **3ba**. Yield: 95%. White solid, m.p.: 152–154 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.03 (bs, 1H), 8.37 (s, 1H), 7.57 (d, J = 7.6 Hz, 1H), 7.41–7.52 (m, 2H), 7.32 (s, 2H), 6.78 (s, 1H), 2.30 (s, 6H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 168.0, 166.4, 155.1, 150.7, 139.8, 139.1, 138.9, 134.1, 132.3, 131.4, 130.2, 128.0, 125.8, 117.0, 108.1, 21.6, 18.2. HRMS: m/z calcd. for $C_{20}H_{18}ClN_4OS$ $[M + H]^+$ 397.0884; found 397.0872. HPLC purity: 97.6%, retention time = 2.43 min.

6-(2-Chloro-6-methylphenyl)-2-((3,4,5-trimethylphenyl)amino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3bq).

The title compound was prepared from compound **6a** and 3,4,5-trimethylaniline following the general procedure of **3ba**. Yield: 82%. White solid, m.p.: 260–262 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.97 (bs, 1H), 8.37 (s, 1H), 7.56 (d, J = 7.2 Hz, 1H), 7.49 (d, J = 7.4 Hz, 1H), 7.45 (d, J = 7.0 Hz, 1H), 7.31 (s, 2H), 2.26 (s, 6H), 2.13 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 168.4, 166.6, 155.1, 150.7, 139.1, 137.4, 136.9, 134.1, 132.3, 131.4, 131.0, 130.2, 128.0, 118.8, 107.8, 21.0, 18.2, 15.2. HRMS: m/z calcd. for $C_{21}H_{20}ClN_4OS$ $[M + H]^+$ 411.1040; found 411.1035. HPLC purity: 99.3%, retention time = 2.71 min.

6-(2-Chloro-6-methylphenyl)-2-((3,4-difluorophenyl)amino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3br).

The title compound was prepared from compound **6a** and 3,4-difluoroaniline following the general procedure of **3ba**. Yield: 67%. White solid, m.p.: 255–258 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.35 (s, 1H), 8.43 (s, 1H), 8.00 (ddd, J = 13.0, 7.2, 2.5 Hz, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.45–7.54 (m, 3H), 7.42 (d, J = 8.9 Hz, 1H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 167.4, 166.1, 155.1, 150.9, 150.9, 150.8, 147.0, 144.6, 144.5, 139.1, 137.0, 133.9, 132.3, 131.4, 130.2, 128.0, 118.5, 118.3, 115.4, 108.8, 108.3, 108.0, 18.2. HRMS: m/z calcd. for $C_{18}H_{12}ClF_2N_4OS$ $[M + H]^+$ 405.0382; found 405.0376. HPLC purity: 99.7%, retention time = 2.00min.

6-(2-Chloro-6-methylphenyl)-2-((3-fluoro-4-methylphenyl)amino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3bs).

The title compound was prepared from compound 6**a** and 3-fluoro-4-methylaniline following

the general procedure of **3ba**. Yield: 91%. White solid, m.p.: 222–225 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.27 (bs, 1H), 8.41 (s, 1H), 7.74 (d, J = 12.6 Hz, 1H), 7.57 (dd, J = 7.7, 1.0 Hz, 1H), 7.43–7.53 (m, 2H), 7.28–7.34 (m, 2H), 2.22 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 167.5, 166.3, 161.0 (d, J = 241 Hz), 155.2, 150.9, 139.3 (d, J = 11 Hz), 139.1, 134.0, 132.4, 132.3, 131.4, 130.2, 128.1, 119.3, 119.1, 114.8, 108.5, 106.0 (d, J = 27 Hz), 18.2, 14.1. HRMS: m/z calcd. for $C_{19}H_{15}ClFN_4OS$ $[M + H]^+$ 401.0633; found 401.0622. HPLC purity: 98.4%, retention time = 2.25min.

1.3 *N*-(6-(2-Chloro-6-methylphenyl)-7-oxo-6,7-dihydrothiazolo[4,5-*d*]pyrimidin-2-yl)-4-methylbenzamide (3ca).

Compound **6a** (370 mg, 1.0 mmol) and *p*-tolylamide (270 mg, 2.0 mmol) were dissolved in anhydrous THF (3 mL). Then, NaH (48 mg, 2.0 mmol) was added to the solution, the reaction was held at reflux for 0.5 h with stirring, then cooled and quenched with ethanol. The reaction mixture was concentrated under reduce pressure. The crude material was purified by silica gel chromatography (CH_2Cl_2/MeOH = 50:1) to afford the compound **3ca**. Yield: 47%. White solid, m.p.: 272–274 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 13.41 (bs, 1H), 8.49 (s, 1H), 8.08 (d, J = 8.0 Hz, 2H), 7.43–7.61 (m, 3H), 7.37 (d, J = 8.0 Hz, 2H), 2.38 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 166.6, 165.0, 163.8, 156.1, 151.0, 144.3, 139.1, 134.0, 132.3, 131.5, 130.3, 129.8, 129.0, 128.7, 128.1, 111.7, 21.6, 18.2. HRMS: m/z calcd. for $C_{20}H_{16}ClN_4O_2S$ $[M + H]^+$ 411.0677; found 411.0680. HPLC purity: 98.4%, retention time = 1.85min.

***N*-(6-(2-Chloro-6-methylphenyl)-7-oxo-6,7-dihydrothiazolo[4,5-*d*]pyrimidin-2-yl)**

-4-methylbenzenesulfonamide (3cb).

The title compound was prepared from compound **6a** and 4-methylbenzenesulfonamide following the general procedure of **3ca**. Yield: 84%. White solid, m.p.: 263–264 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.38 (s, 1H), 7.75 (d, J = 8.2 Hz, 2H), 7.53–7.57 (m, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.43–7.44 (m, 1H), 7.35 (d, J = 8.0 Hz, 2H), 2.36 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 167.7, 153.7, 151.6, 142.8, 138.7, 138.6, 133.1, 131.7, 131.1, 129.8, 129.5, 127.6, 126.1, 104.5, 21.0, 17.6. HRMS: m/z calcd. for $C_{19}H_{15}ClN_4O_3S_2$ $[M + H]^+$ 447.0346; found 447.0350. HPLC purity: 97.4%, retention time = 0.97 min.

6-(2-Chloro-6-methylphenyl)-2-(methyl(p-tolyl)amino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3cc).

The title compound was prepared from compound **6a** and *N*,4-dimethylaniline following the general procedure of **3af**. Yield: 89%. Yellow solid, m.p.: >300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.87 (s, 1H), 7.40 (d, J = 7.9 Hz, 1H), 7.33 (t, J = 6.0 Hz, 1H), 7.30 (m, 4H), 7.27 (d, J = 7.2 Hz, 1H), 3.67 (s, 3H), 2.41 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 174.0, 166.5, 155.2, 149.0, 141.9, 138.8, 138.6, 133.8, 132.9, 131.0, 130.6, 129.4, 127.9, 125.8, 109.5, 40.9, 21.2, 18.3. HRMS: m/z calcd. for $C_{20}H_{18}ClN_4OS$ $[M + H]^+$ 397.0884; found 397.0885. HPLC purity: 95.7%, retention time = 2.38min.

6-(2-Chloro-6-methylphenyl)-2-((4-methylbenzyl)amino)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3cd).

The title compound was prepared from compound **6a** and *p*-tolylmethanamine following the

general procedure of **3af**. Yield: 83%. Yellow solid, m.p.: >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.38 (s, 1H), 8.27 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.42 (d, J = 7.2 Hz, 1H), 7.28 (d, J = 8.0 Hz, 2H), 7.18 (d, J = 7.9 Hz, 2H), 4.57 (s, 2H), 2.29 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 166.4, 154.5, 150.0, 138.6, 136.5, 134.8, 133.7, 131.9, 130.8, 129.7, 129.1, 128.8, 127.6, 127.2, 106.7, 20.7, 17.7. HRMS: m/z calcd. for $C_{20}H_{18}ClN_4OS$ $[M + H]^+$ 397.0884; found 397.0881. HPLC purity: 97.9%, retention time = 1.81 min.

1.4 6-(2-Chloro-6-methylphenyl)-2-(p-tolylthio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3da).

Compound **6a** (370 mg, 2.0 mmol) was dissolved in THF (10 mL). Then, 4-methylbenzenethiol (250 mg, 3.0 mmol) and Et_3N (2 mL) were added to the solution. The reaction was held at 65 °C for 6 h with stirring. After completion of the reaction (TLC), the reaction mixture was concentrated under reduce pressure. The crude material was purified by silica gel chromatography (petroleum ether/EtOAc = 10:1) to afford the compound **3da**. Yield: 64%. White solid, m.p.: 266–268 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.92 (s, 1H), 7.62 (d, J = 8.1 Hz, 2H), 7.39–7.44 (m, 1H), 7.32–7.38 (m, 3H), 7.28 (d, J = 7.0 Hz, 1H), 2.45 (s, 3H), 2.16 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 181.6, 166.9, 155.1, 149.4, 142.4, 138.5, 135.9, 133.4, 132.8, 131.5, 130.9, 129.7, 128.1, 124.8, 117.9, 21.6, 18.3. HRMS: m/z calcd. for $C_{19}H_{15}ClN_3OS_2$ $[M + H]^+$ 400.0339; found 400.0341. HPLC purity: 96.9%, retention time = 3.93 min.

1.5 6-(2-Chloro-6-methylphenyl)-2-(4-methyl-phenyl)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3db)

Compound **5** (984 mg, 3 mmol) was refluxed in HCOOH (5mL) for 1 h to afford key intermediate **6** (839 mg), which was purified via a column chromatography (petroleum ether: ethyl acetate = 10: 1), in a yield of 83 % as a white solid. MP: 170–171 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.94 (s, 1H), 7.27–7.46 (m, 3H), 3.41 (q, J = 7.4 Hz, 2H), 2.18 (s, 3H), 1.51 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.6, 166.0, 155.1, 149.2, 138.4, 133.3, 132.7, 130.8, 129.6, 128.0, 117.2, 28.3, 18.2, 14.3.

Then, **6** (169 mg, 0.5 mmol) and 4-methylphenyl boric acid (135 mg, 1.5equiv) was stirred in a sealed tube with THF (5mL) in presence of $Pd(Ph_3P)_4$ (29 mg, 5 mol%) and CuTC (285 mg, 1.5equiv) for 16h . After the reaction, the crude product was purified by column chromatography (petroleum ether: ethyl acetate = 30: 1) to provide the product **3db** (126 mg) as a white solid in a yield of 69%. MP: 287–288 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.04 (d, J = 8.2 Hz, 2H), 8.01 (s, 1H), 7.45 (d, J = 7.9, 1.0 Hz, 1H), 7.38 (t, J = 7.8 Hz, 1H), 7.35 – 7.30 (m, 3H), 2.44 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 175.4, 166.9, 155.9, 149.3, 143.1, 138.5, 133.4, 132.8, 130.9, 129.9, 129.9, 129.6, 128.1, 127.4, 117.9, 21.6, 18.3. HRMS: m/z calcd. for $C_{19}H_{15}ClN_3OS$ $[M + H]^+$ 368.06183; found: 368.06188. HPLC: purity: >99.9%, retention time = 4.33min.

1.6 6-(2-Chloro-6-methylphenyl)-2-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3ea).

Compound **5a** (1.10 g, 5 mmol) was dissolved in acetone (10 mL). Then, the solution of dipotassium cyandithiomidocarbonate (970 mg, 5 mmol, 10ml) and 2 mol/L NaOH aqueous solution (2.5 mL) were added to the solution. The reaction mixture was stirred for 2 h at room temperature, then heated to 80 ^{o}C for 0.5 h. Afterward, the reaction mixture was cooled to room

temperature. To the mixture Benzyl bromide (855 mg, 5 mmol) was added drop wise with continuous stirring. After completion of the reaction (TLC), the reaction mixture was poured into ice water. The precipitated solid was collected and filtered. The product was washed with water. The crude product so obtained was dried under vacuum to obtain the intermediate compound **10a**. Compound **10a** (1.20 g, 3 mmol) was dissolved in HCOOH (2 mL). The reaction was held at reflux for 1 h with stirring, then cooled and quenched with water. The reaction mixture was extracted with ethyl ether (20 mL×5), dried with Na_2SO_4, and concentrated under reduce pressure. The crude material was purified by silica gel chromatography (petroleum ether/EtOAc = 10:1) to afford the compound **3ea**. Yield: 75%. Yellow solid, m.p.: 134–138 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.52 (s, 1H), 7.56–7.60 (m, 1H), 7.53–7.56 (m, 2H), 7.48–7.53 (m, 1H), 7.44–7.48 (m, 1H), 7.35–7.41 (m, 2H), 7.31 (ddd, J = 7.3, 3.7, 1.2 Hz, 1H), 4.70 (s, 2H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 175.2, 165.7, 154.5, 150.8, 138.6, 135.7, 133.1, 131.7, 131.1, 129.8, 129.1, 128.6, 127.8, 127.6, 116.3, 37.0, 17.6. HRMS: m/z calcd. for $C_{19}H_{15}ClN_3OS_2$ $[M + H]^+$ 400.0339; found 400.0340. HPLC purity: 99.5%, retention time = 3.15 min.

6-(2-Chloro-6-methylphenyl)-2-((2-fluorobenzyl)thio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3eb).

The title compound was prepared from compound **5a** and 2-fluorobenzyl bromide (compound **10b**) following the general procedure of **3ea**. Yield: 44%. Yellow solid, m.p.: 184–186 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.53 (s, 1H), 7.64 (d, J = 6 Hz, 1H), 7.58 (d, J = 8 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 7.33–7.42 (m, 1H), 7.18–7.27 (m, 2H), 4.73 (s,

2H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 174.6, 165.7, 160.5 (d, J = 247 Hz), 154.6, 150.9, 138.6, 133.1, 131.7, 131.5 (d, J = 3 Hz), 131.2, 130.3 (d, J = 8 Hz), 129.9, 127.6, 124.7 (d, J = 4 Hz), 122.8 (d, J = 14 Hz), 116.5, 115.6 (d, J = 21 Hz), 30.8, 17.7. HRMS: m/z calcd. for $C_{19}H_{14}ClFN_3OS_2$ $[M + H]^+$ 418.0245; found 418.0248. HPLC purity: 95.2%, retention time = 3.31min.

6-(2-Chloro-6-methylphenyl)-2-((3-fluorobenzyl)thio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3ec).

The title compound was prepared from compound **5a** and 3-fluorobenzyl bromide (compound **10c**) following the general procedure of **3ea**. Yield: 32%. Yellow solid, m.p.: 177–179 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.53 (s, 1H), 7.58 (d, J = 8 Hz, 1H), 7.48 (t, J = 8 Hz, 1H), 7.44 (d, J = 6.8 Hz, 1H), 7.38–7.42 (m, 3H), 7.14 (m, 1H), 4.71 (s, 2H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 174.9, 165.7, 160.8, 154.6, 150.9, 138. 9 (d, J = 8 Hz), 138.6, 133.1, 131.7, 131.1, 130.6 (d, J = 8 Hz), 129.9, 127.6, 125.3 (d, J = 3 Hz), 116.4, 115.9 (d, J = 22 Hz), 114.7 (d, J = 21 Hz), 36.3, 17.7. HRMS: m/z calcd. for $C_{19}H_{14}ClFN_3OS_2$ $[M + H]^+$ 418.0245; found 418.0246. HPLC purity: 97.8%, retention time = 3.12min.

6-(2-Chloro-6-methylphenyl)-2-((4-fluorobenzyl)thio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3ed).

The title compound was prepared from compound **5a** and 4-fluorobenzyl bromide (compound **10d**) following the general procedure of **3ea**. Yield: 25%. Yellow solid, m.p.: 193–195 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.96 (s, 1H), 7.46 (dd, J = 8.7, 5.4 Hz, 3H), 7.38 (t, J = 7.8 Hz,

1H), 7.31 (d, *J* = 7.5 Hz, 1H), 7.04 (t, *J* = 8.6 Hz, 2H), 4.64 (s, 2H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 175.6, 166.0, 162.5 (d, *J* = 247.2 Hz), 155.2, 149.5, 138.6, 133.4, 132.8, 131.4 (d, *J* = 3.2 Hz), 131.1, 131.0 (d, *J* = 8.3 Hz), 129.8, 128.2, 115.9 (d, *J* = 21.7 Hz), 37.3, 18.4. HRMS: m/z calcd. for $C_{19}H_{14}ClFN_3OS_2$ $[M + H]^+$ 418.0245; found 418.0261. HPLC purity: 95.4%, retention time = 3.11min.

6-(2-Chloro-6-methylphenyl)-2-((4-methylbenzyl)thio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3ee).

The title compound was prepared from compound **5a** and 4-methylbenzyl bromide (compound **10e**) following the general procedure of **3ea**. Yield: 63%. Yellow solid, m.p.: 238–240 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.52 (s, 1H), 7.58 (d, *J* = 8 Hz, 1H), 7.51 (t, *J* = 8 Hz, 1H), 7.46 (d, *J* = 6.8 Hz, 1H), 7.42 (d, *J* = 8 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 4.65 (s, 2H), 2.28 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 175.4, 165.8, 154.6, 150.8, 138.6, 137.2, 133.1, 132.6, 131.7, 131.2, 129.9, 129.3, 129.1, 127.6, 116.3, 36.9, 20.7, 17.7. HRMS: m/z calcd. for $C_{20}H_{17}ClN_3OS_2$ $[M + H]^+$ 414.0496; found 414.0500. HPLC purity: 99.3%, retention time = 4.20 min.

6-(2-Chloro-6-methylphenyl)-2-((4-methoxybenzyl)thio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3ef).

The title compound was prepared from compound **5a** and 4-methoxybenzyl bromide (compound **10f**) following the general procedure of **3ea**. Yield: 27%. Yellow solid, m.p.: 296–298 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.52 (s, 1H), 7.58 (d, *J* = 7.2 Hz, 1H), 7.42–7.55 (m,

4H), 6.94 (d, J = 8.6 Hz, 2H), 4.64 (s, 2H), 3.74 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 175.4, 165.8, 158.9, 154.6, 150.8, 138.6, 133.1, 131.7, 131.1, 130.5, 129.9, 127.6, 127.3, 114.1, 104.5, 55.1, 36.7, 17.7. HRMS: m/z calcd. for $C_{20}H_{17}ClN_3O_2S_2$ $[M + H]^+$ 430.0445; found 430.0451. HPLC purity: 98.5%, retention time = 3.00 min.

6-(2-Chloro-6-methylphenyl)-2-((4-chlorobenzyl)thio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3eg).

The title compound was prepared from compound **5a** and 4-chlorobenzyl bromide (compound **10g**) following the general procedure of **3ea**. Yield: 84%. White solid, m.p.: 284–286 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.96 (s, 1H), 7.39–7.47 (m, 3H), 7.34–7.39 (m, 1H), 7.28–7.34 (m, 3H), 4.62 (s, 2H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 175.5, 165.9, 155.3, 149.5, 138.6, 134.3, 134.0, 133.4, 132.9, 131.1, 130.7, 129.8, 129.1, 128.3, 117.8, 37.2, 18.4. HRMS: m/z calcd. for $C_{19}H_{14}Cl_2N_3OS_2$ $[M + H]^+$ 433.9949; found 433.9952. HPLC purity: 96.1%, retention time = 4.27 min.

6-(2-Chloro-6-methylphenyl)-2-((4-bromobenzyl)thio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3eh).

The title compound was prepared from compound **5a** and 4-bromobenzyl bromide (compound **10h**) following the general procedure of **3ea**. Yield: 68%. White solid, m.p.: 294–296 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.96 (s, 1H), 7.46 (t, J = 10.4 Hz, 3H), 7.38 (t, J = 9.2 Hz, 3H), 7.31 (d, J = 7.5 Hz, 1H), 4.60 (s, 2H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 175.4, 165.9, 155.3, 149.5, 138.6, 134.8, 133.4, 132.9, 132.1, 131.1, 131.1, 129.8, 128.3, 122.2, 117.8, 37.3,

18.4. HRMS: m/z calcd. for $C_{19}H_{14}BrClN_3OS_2$ $[M + H]^+$ 477.9444; found 477.9459. HPLC purity: 99.3%, retention time = 4.61min.

6-(2-Chloro-6-methylphenyl)-2-((2,4-difluorobenzyl)thio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3ei).

The title compound was prepared from compound **5a** and 2,4-difluorobenzyl bromide (compound **10i**) following the general procedure of **3ea**. Yield: 74%. Yellow solid, m.p.: 249–251 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.97 (s, 1H), 7.58 (td, J = 8.7, 6.4 Hz, 1H), 7.42–7.46 (m, 1H), 7.38 (t, J = 7.8 Hz, 1H), 7.29–7.33 (m, 1H), 7.27 (s, 1H), 6.86 (dddd, J = 11.1, 9.1, 4.6, 2.8 Hz, 2H), 4.67 (s, 2H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 175.3, 165.8, 155.2, 149.5, 138.5, 133.4, 132.8, 132.4, 132.4, 132.3, 131.0, 129.8, 128.2, 119.3, 117.8, 111.8, 111.7, 111.6, 111.5, 104.5, 104.3, 104.0, 30.7, 30.6, 18.3. HRMS: m/z calcd. for $C_{19}H_{13}ClF_2N_3OS_2$ $[M + H]^+$ 436.0151; found 436.0161. HPLC purity: 97.7%, retention time = 3.49min.

6-(2-Chloro-6-methylphenyl)-2-(phenethylthio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3ej).

The title compound was prepared from compound **5a** and (2-bromoethyl)benzene (compound **10j**) following the general procedure of **3ea**. Yield: 91%. White solid, m.p.: 122–124 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.96 (s, 1H), 7.44 (d, J = 7.5 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.22–7.36 (m, 6H), 3.67 (t, J = 7.6 Hz, 2H), 3.17 (t, J = 7.6 Hz, 2H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.5, 166.1, 155.3, 149.4, 139.2, 138.6, 133.5, 132.9, 131.1, 129.8, 128.8, 128.8, 128.2, 126.95, 117.5, 35.4, 35.3, 18.4. HRMS: m/z calcd. for $C_{20}H_{17}ClN_3OS_2$ $[M + H]^+$ 414.0496; found 414.0505. HPLC purity: 99.1%, retention time = 3.85min.

6-(2-Chloro-6-methylphenyl)-2-((3-phenylpropyl)thio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3ek).

The title compound was prepared from compound **5a** and (3-bromopropyl)benzene (compound **10k**) following the general procedure of **3ea**. Yield: 91%. White solid, m.p.: 142–144 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.97 (s, 1H), 7.44–7.48 (m, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.29–7.35 (m, 3H), 7.22 (dd, J = 10.0, 4.1 Hz, 3H), 3.44 (t, J = 7.2 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.17–2.28 (m, 5H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.7, 166.1, 155.2, 149.3, 140.6, 138.5, 133.4, 132.8, 131.0, 129.7, 128.6, 128.5, 128.2, 126.3, 117.3, 34.7, 33.3, 30.5, 18.3. HRMS: m/z calcd. for $C_{21}H_{19}ClN_3OS_2$ $[M + H]^+$ 428.0652; found 428.0644. HPLC purity: 99.6%, retention time = 5.01 min.

1.7 2-Chloro-*N*-(2-chloro-6-methyphenyl)acetamide (4a).

2-Chloro-6-methylaniline (14.20 g, 100 mmol) was dissolved in glacial acetic acid (100 mL) containing saturated solution of sodium acetate (80 mL). Then, the solution was cooled in an ice-bath with stirring. To the ice cold solution chloroacetyl chloride (14.18 g, 150 mmol) was added dropwise with continuous stirring to avoid the vigorous reaction. After 6 hours, a white colored product was separated out and filtered. The product was washed with water. The crude product so obtained was dried under vacuum to obtain the white solid compound **4a**. Yield: 58%.

1.8 4-Amino-*N*-(2-chloro-6-methylphenyl)-2-(enthylthio)thiazole-5-carboxamide (5).

The compound **4a** (10.90 g, 50 mmol) was dissolved in acetone (100 mL). Then, the solution

of dipotassium cyandithiomidocarbonate (9.70 g, 50 mmol, 100mL) and 2 mol/L NaOH aqueous solution (25 mL) were added to the solution. The reaction mixture was stirred for 2h at room temperature, then heated to 80 ^{o}C for 0.5h. Afterward, the reaction mixture was cooled to room temperature. To the mixture Iodoethane (7.80 g, 50 mmol) was added drop wise with continuous stirring. After completion of the reaction (TLC), the reaction mixture was poured into ice water. The precipitated solid was collected and filtered. The product was washed with water. The crude product so obtained was dried under vacuum to obtain the compound **5**. Yield: 81%. Yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.32 (s, 1H), 7.13–7.23 (m, 2H), 6.62 (s, 1H), 6.14 (s, 2H), 3.22 (d, J = 6.9 Hz, 2H), 2.33 (s, 3H), 1.46 (t, J = 6.6 Hz, 3H).

1.9 6-(2-Chloro-6-methylphenyl)-2-(enthylsulfonyl)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (6a).

The compound **5** (6.60 g, 20 mmol) was dissolved in HCOOH (15 mL). The reaction was held at reflux for 1h with stirring, then cooled and quenched with water. The reaction mixture was extracted with ethyl ether (20mL×5), dried with Na_2SO_4, and concentrated under reduce pressure to afford a crude product. Then, the intermediate compound, $Na_2WO_4 \cdot H_2O$ (1.00 g, 3 mmol) were dissolved in ethanol (100 mL). To the mixture 30% hydrogen peroxide solution (20 mL) was added drop wise with continuous stirring. The reaction mixture was stirred for 1h at room temperature, then heated to 50 ^{o}C for 1 h. Afterward, the reaction mixture was cooled to room temperature, poured into ice water. The precipitated solid was collected and filtered. The product was washed with water. The crude product so obtained was dried under vacuum to obtain the compound **6a**.

1.10 *N*-(2-Chloro-6-methyl-phenyl)-2-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-amine (16)

To a solution of dipotassium cyandithiomidocarbonate **12** (5.8 g, 30 mmol) in a solution of acetone (30 mL) and water (30 mL) at 0 ^{o}C, benzyl bromide (5.1 g, 30 mmol) was added slowly. The mixture was warmed to room temperature and stirred for another 6 hours. The reaction mixture was evaporated under vacu and 120 mL acetone was added, and the filtrate was dried to provide the compound **13** (7.20 g, yield 97%) without further purification. Chloroacetonitrile (1.50 g, 20 mmol) and Et_3N were added to the solution of **13** (4.90 g, 20 mmol) in ethanol (50 mL) and acetone (30 mL). The reaction mixture was held at 70 ^{o}C for 4 h produced the key 4-amino-2-(benzylthio)thiazole-5-carbonitrile **14** as a solid of 4.40 g in a yield of 72%. A solution of 4-amino-2-(benzylthio)thiazole-5-carbonitrile **14** (1.50 g, 6 mmol) in DMF-DMA (1.70 g, 14.4 mmol) was refluxed at 100 ^{o}C for 6 h. After cooling down, the mixture was filtered, washed with EtOAc, recrystalized with ethanol and dried to afford **15** as a light brown solid (2.16 g, 59% yield).

A solution of **15** (456 mg, 1.5 mmol) and 2-chloro-6-methyl-aniline (283 mg, 2 mmol) in acetic acid (3 mL) was refluxed at 110 ^{o}C for 3 h. When completed, the reaction mixture was allowed to attain room temperature. Water was added, then filtered. The precipitate was washed with water and ether and dried. The crude product was purified by chromatography to provide **16** a white solid (408mg, 68% yield). M.p.: 267–269 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.04 (s, 1H), 8.57 (s, 1H), 7.39 (t, J = 8.0 Hz, 3H), 7.36 – 7.24 (m, 5H), 4.61 (s, 2H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 176.1, 169.8, 157.5, 155.9, 141.2, 136.0, 135.7, 132.4, 130.1, 129.5, 129.3, 128.8, 128.0, 127.9, 107.5, 37.6, 18.9. HRMS (ESI+) m/z calcd for

$C_{19}H_{16}ClN_4S_2$ $[M + H]^+$ 399.0499, found 399.0489. HPLC purity: 99.7%, retention time = 3.94min.

1.11 3-(2-Chloro-6-methylphenyl)-6-(p-tolylamino)quinazolin-4(3*H*)-one (19)

2-amino-5-iodobenzoic acid **17** (1.40 g, 5.5 mmol), 2-chloro-6-methylaniline (1.00 g, 7.5 mmol) and trimethoxymethane (636 mg, 6.0 mmol) was added to toluene (25 mL), acetic acid (30 mg, 0.5 mmol) was added dropwise. The mixture was refluxed at 100 ^oC for 24 hours. After cooling down, the solution was concentrated under reduced pressure and quenched with EtOAc and brine. The organic layer was evaporated under vacu and purified by chromatography to provide **18** as yellow oil (1.30 g, 65% yield).

18 (1.10 g, 2.7 mmol), p-toluidine (535 mg, 5.0 mmol), K_2CO_3 (690 mg, 5.0 mmol), CuI (95 mg, 0.8 mmol), L-proline (92 mg, 0.8 mmol) and DMSO (5 mL) were added to a 35 mL sealed tube and stirred at 80 ^oC for 24 hours under argon atmosphere. After cooling down, the mixture was diluted with EtOAc (25 mL) and filtered through celite with EtOAc (50 mL). The crude product was purified by flash column chromatography on a silica gel column to provide **19** as a yellow solid (203 mg, 20% yield). M.p.: 205–209 ^oC. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.90 (d, J = 2.8 Hz, 1H), 7.72 – 7.60 (m, 2H), 7.46 (dd, J = 8.8, 2.7 Hz, 1H), 7.41 (dd, J = 8.0, 1.5 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.29 – 7.25 (m, 1H), 7.08 (d, J = 8.0 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 6.42 (s, 1H), 2.31 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 159.9, 144.5, 142.7, 141.3, 138.9, 138.5, 134.4, 132.9, 132.3, 130.4, 130.0, 129.5, 128.8, 128.0, 123.6, 123.4, 120.2, 110.5, 20.8, 18.3. HRMS (ESI+) m/z calcd for $C_{22}H_{18}ClN_3O$ $(M + H)^+$ 376.1217, found 376.1219. HPLC purity: 99.7%, retention time = 4.98min.

2. Two-electrode voltage clamp (TEVC) recording in *Xenopus* oocytes

Oocytes were harvested from *Xenopus laevis* female clawed frogs after anesthesia, washed twice in the Ca^{2+}-free OR2 solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.4) before transferred to ~25 mL tubes, and treated with 2 mg/mL collagenase in OR2 solution (Sigma type II, Sigma-Aldrich Inc, St Louis, MO, USA) for 20 min at 20–25 °C under gentle rotation. The stage V and VI oocytes were selected for microinjections. For two-electrode voltage clamp recordings in oocytes, capped cRNAs were transcribed *in vitro* using the T3 mMESSAGEmMACHINE Kit (Ambion, Austin, TX, USA) following the linearization of plasmids in pBluescript KSM vectors. The oocytes were injected with 46 nL of cRNA solution containing approximately 20 ng human α7 nAChR cRNA or approximately 1 ng human 5-HT_{3A} cRNA using a microinjector (Drummond Scientific, Broomall, PA, USA). For the expression of heteromeric rat α3β4 and rat α4β2 nAChRs, approximately 2 ng total cRNAs were injected in a 1:1 combination of each subunit into oocytes that were incubated at 16 °C in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), pH 7.4 adjusted with NaOH). Recordings were made 24–72 hours post-injection. Oocytes were impaled with two microelectrodes (0.5–1.0 MΩ) filled with 3 M KCl in a 40-μL recording chamber. The membrane potential was held at -90 mV using standard voltage clamp procedures. Currents were recorded in Ringer's solution (115 mM NaCl, 2.5 mM KCl, 10 mM HEPES, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 0.0005 mM atropine) at room temperature (22 ± 1 °C) using a GeneClamp 500B amplifier (Axon Instruments, Union City, CA, USA)

3. Culture and whole-cell patch clamp recordings of HEK293 cells stably expressing hERG channel

Human embryonic kidney HEK 293 cells were stably transfected with hERG cDNA, passaged in Dulbecco's Modified Eagles Medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL) and geneticin (200 μg/mL), seeded on 8 mm × 8 mm glass cover slips in 35 mm^2 diameter dishes (containing 2 mL of medium) at a density that enabled the cells to be isolated for whole cell voltage-clamp recordings, and cultured at 37 ºC in a humidified 5% CO_2 environment.

For whole-cell patch clamp recordings, currents were recorded at room temperature using an EPC 10 UBS patch clamp amplifier in combination with Patchmaster (HEKA). The total resistance of patch electrodes was measured to be 3-5 megaohms. The extracellular solution contained 140 mM, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose and 10 mM HEPES with pH adjusted to 7.4 with NaOH. The internal pipette solution contained 130 mM KCl, 1 mM $MgCl_2$, 5 mM ethylene glycol-bis (beta-aminoethyl ether) *N*,*N*,*N*,*N*' -tetra acetic acid salt (EGTA), 5 mM MgATP and 10 mM HEPES with pH adjusted to 7.2 with KOH. Cells were clamped from the holding potential at -80 mV to +20 mV for 2 s, before repolarization to -40 mV for 1.6 s, and then back to the holding potential. The voltage protocol was run once every 10 s. Data were acquired and analyzed using the PatchMaster software (HEKA).

4. Liquid chromatograph-mass spectrometry (LC-MS) assay for pharmacokinetic analysis of 3ea

All blood samples were stored at -20 ºC and thawed at room temperature before use. Rat

plasma samples were respectively added with 20 μL 1.0 μg/mL internal standard (IS), and vortex-mixed for 15 s before addition of 280 μL of methanol for 1 min to precipitate proteins. The supernatant was collected by centrifuge at 13000 r/min for 15 min at 4 ºC, filtered through a 0.22 μm filter membrane, and injected with 5 μL into the liquid chromatography tandem mass spectrometry (LC-MS/MS) system for the analysis.

LC-MS/MS was performed on a Waters Acquity HPLC system coupled to TQ detector. Chromatographic separations were achieved with a Luna C18 (2) column (150 mm×4.6 mm, 5 μm) at 40 ºC using acetonitrile and 0.1% formic acid in H_2O (80:20, v:v) at a flow rate of 0.5 mL/min as the mobile phase. The temperature of auto-sampler was set to 4 ºC. The mass spectrometer was operated in positive ion mode using an electrospray ionization (ESI) source. The multiple reaction monitoring (MRM) mode was used to detect the specific precursor ion to product ion transitions of m/z 400.0->129.9 for **3ea** and m/z 419.0->169.0 for LD486 (IS). The optimal ESI-MS/MS parameters were as follows: positive electrospray ionization with capillary voltage of 3.0 kV, cone voltage of 44 V, desolvation temperature of 400 ºC, source temperature of 150 ºC, desolvation gas flow of 600 L/h, and cone gas flow of 50 L/h

Data analysis

All data are expressed as the means ± SEM. Statistical significance was assessed by Student's t-test and one-way ANOVA using the Prism version 5.0 software. $P<0.05$ was considered statistically significant. In the two-electrode voltage clamp and whole-cell patch clamp recordings, currents were quantified by measuring peak current amplitudes and analyzed using PatchMaster and Origin 9.0 software. The values of $\tau_{desensitization}$ were obtained by fitting the decaying portion of

the current traces to a monoexponentially decaying function ($I = I_{infinity} + I_0 \times e^{-t/\tau}$). All concentration-response curves were fitted to the Hill equation as follows: $I_{normalized} = E_{max}/(1 + (EC_{50}/C)^{nH})$. The pharmacokinetic parameters were evaluated in each individual rat by a non-compartmental approach using the software DAS2.

5. Pharmacokinetics of 3ea in plasma and brain.

The ability of **3ea** to penetrate the blood−brain barrier (BBB) *in vivo* was determined by the HPLC-MS-MS analysis as described below. The **3ea** solution of 0.4 mg/mL was prepared in a clear solution of 10%DMSO+50%PEG400+40%SBECD, and administered to mice via i.p. at the dose of 1 mg/kg. The plasma, cortex and hippocampus of the mice (three mice in each group) were collected 1 h after the administration.

The collected plasma samples (20 μL) were respectively mixed with 2 μL MeOH and 200 μL of 50 ng/mL IS in methanol/acetonitrile (1:1, v/v), vortexed for 1 min, and centrifuged at 4000 rpm for 15 min. The supernatant was collected and diluted 10x with methanol : water (1:1, v/v) containing 0.1%FA for HPLC-MS-MS analysis.

The brain samples (50 μL) were respectively mixed with 5 μL MeOH and 200 μL of 50 ng/mL ISTD in methanol/acetonitrile (1:1, v/v), vortexed for 1 min, and centrifuged at 4000 rpm for 15 min. The supernatant was collected and diluted 10x with methanol: water (1:1, v/v) containing 0.1%FA for the HPLC-MS-MS analysis.

The calibration standards were prepared in the blank sample on the day of analysis by adding the appropriate volumes of diluted **3ea** (0.4 mg/mL).

Data were analyzed by one-way ANOVA, followed by Dunnett's test for the multiple

comparisons or by Student's *t*-test for the single comparison. $P<0.05$ was considered to be statistically significant.

6. Prepulse Inhibition (PPI) Test[24]

Adult C57BL/6J males (n = 8−11 for each group) were group-housed (four to five per cage) in a temperature-controlled (23 ± 2°C) and humidity-controlled (50 ± 5%) environment on a 12 h/12 h light/dark cycle (light 7:00 AM to 7:00 PM) with ad libitum access to food and water. The animal experimental protocols were approved by the Animal Use and Care Committee of Qingdao University and were consistent with the Ethical Guidelines of the International Association. Test (**3ea**) and tool (MK-801) compounds were respectively diluted in 40% polyethylene glycol (PEG) or 0.9% saline to desired concentrations 12 h before the experiments and stored at -20°C. The compound solutions of different concentrations were administered to mice intraperitoneally at 5 ml/kg body weight.

PPI was measured in four standard startle chambers. A mouse holder rested on the platform in each sound–attenuated chamber. The motor response was monitored with a vibration sensor on the platform connected to a computer and recorded using the Pan Lab software. Mice were placed in the laboratory for at least 2 days before the test to adapt to the environment. One day before the test, mice were habituated to the environment in a plexiglass cylinder for 15 minutes under 68 dB background noise. In the next day, following a 5 min-acclimatization period, the mice were exposed to 50 test trials including 10 pulse-only trials, 30 prepulse-pulse trials, and 10 null trials under 68 dB background noise. The pulse-only trial consisted of a single 40 ms 120 dB white noise burst. Prepulse-pulse trial consisted of a 20 ms white noise prepulse 8, 12, or 16 dB above

the 68-dB background, followed by an 80 ms 120 dB pulse. Only white noise background was used as the stimulus for the null-only trials. All trials were conducted in the pseudo random order at the intervals ranging from 8–22 s with an average value of 15s. The percent PPI for each prepulse intensity was calculated as the ratio of the average to prepulse-pulse response to the average pulse-only response subtracted from 1, followed by the multiplication by 100. Null activity and pulse-only responses were measured by the Panlab startle response system and reported in arbitrary units (AU).

■ ABBREVIATIONS USED

PAMs: Positive allosteric modulators; α7 nAChR: α7-Subtype of nicotinic acetylcholine receptors; SAR: Structure−activity relationship; EC_{50}: Median effect concentration; CNS: Central nervous system; Et_3N: Triethylamine; AcOH: Acetic acid; NaOH: Sodium hydroxide; THF: Tetrahydrofuran; rt: Room temperature; PhMe: Toluene; DMSO: Dimethyl sulfoxide; K_2CO_3: Potassium carbonate; Ar: Argon; DMF·DMA: *N*,*N*-Dimethylformamide dimethyl acetal; Ach: Nicotinic acetylcholine; MLA: Methyllycaconitine; hERG: Human Ether-a-go-go-Related Gene; HEK: Human embryonic kidney; PK: Pharmacokinetic; $t_{1/2}$: Half-life; C_{max}: Maximum concentration; $T_{max:}$ When occurs time Cmax; AUC_{last}: AUC from time 0 to last measurable concentration; AUC_{Inf}: AUC from time 0 to time infinity; AUC: Area under curve; MRT: Mean residence time; Vz: Volume of distribution during terminal phase; CL: Clearance; F: Oral bioavailability; CNS: Central nervous system; BBB: Blood-brain barrier; CSF: Cerebro-spinal

fluid; NMDA: *N*-Methyl-D-aspartic acid; GABA: Gamma aminobutyric acid; PPI: Prepulse inhibition; cDNA: Complementary deoxyribonucleic acid; cRNA: Complementary ribonucleic acid; HEPES: 4-(2-Hydroxyethyl)-1- piperazineethanesulphonic acid; EGTA: Ethylene glycol-bis (beta-aminoethyl ether) *N*,*N*,*N*,*N*' -tetra acetic acid salt; LC-MS: Liquid chromatograph-mass spectrometer; HPLC: High performance liquid chromatography; SEM: Standard error of mean; ANOVA: Analysis of variance; i.g.: Intragastric administration; i.v.: Intravenous injection; i.p.: Intraperitoneal injection. ISTD: Internal standard. FA: Fomic acid. PEG: Polyethylene glycol. SBECD: sulphobutylether-beta-cyclodextrin.

ANCILLARY INFORMATION

Supporting Information

Selectivity assessments of **3ea** on subtypes of NMDA and $GABA_A$ receptors; Figure of mean plasma concentration-time profiles of **3ea**; Table of pharmacokinetic parameters of **3ea** and **3ei**; Figure of mean plasma concentration-time profiles of **3ea** and **3ei**; *In vitro* activities of compounds **3ce**−**3cl** and **3dc**−**3di**; Chemical data of compounds **3ab–3ag**, **3bf–3bn**, **3ce**−**3cl**, **3dc**−**3di**, and **3el–3eq**; NMR spectra and HPLC reports of the target compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxxxxxxxxxxxxx. (PDF)

Molecular formula strings of the target compounds (CSV)

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Notes

All authors declare no competing financial interest.

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GROUPED TABLES AND ARTWORK

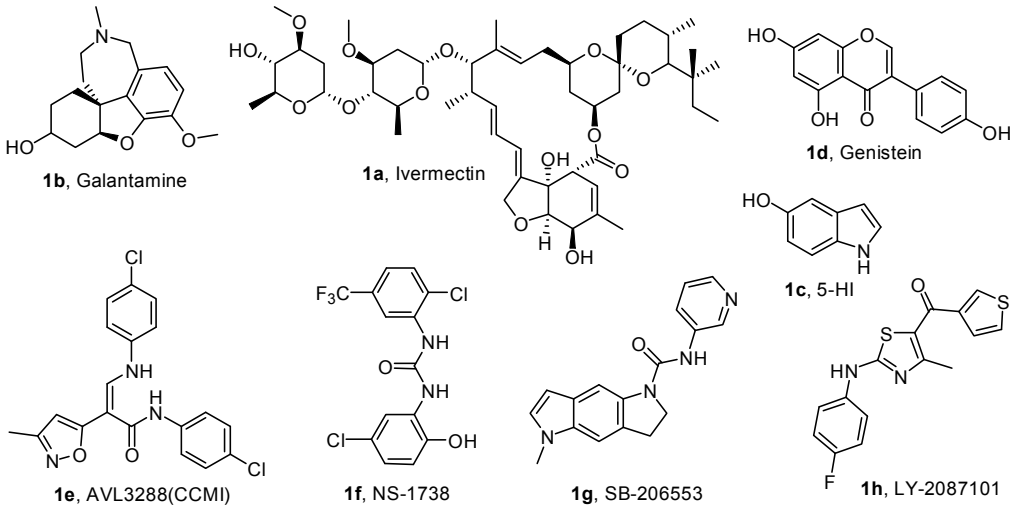


Figure 1. Representative type I α7 nAChR PAM

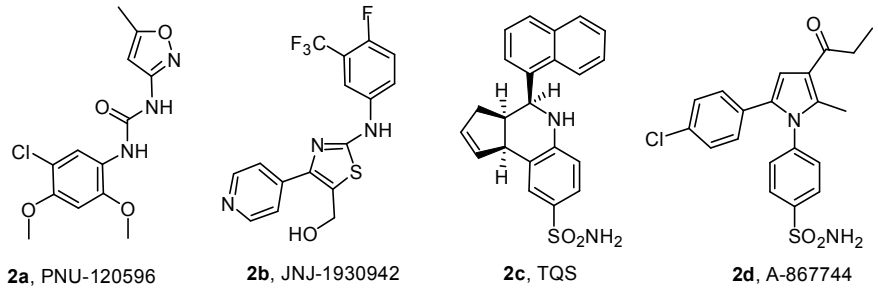


Figure 2. Representative type II α7 nAChR PAM

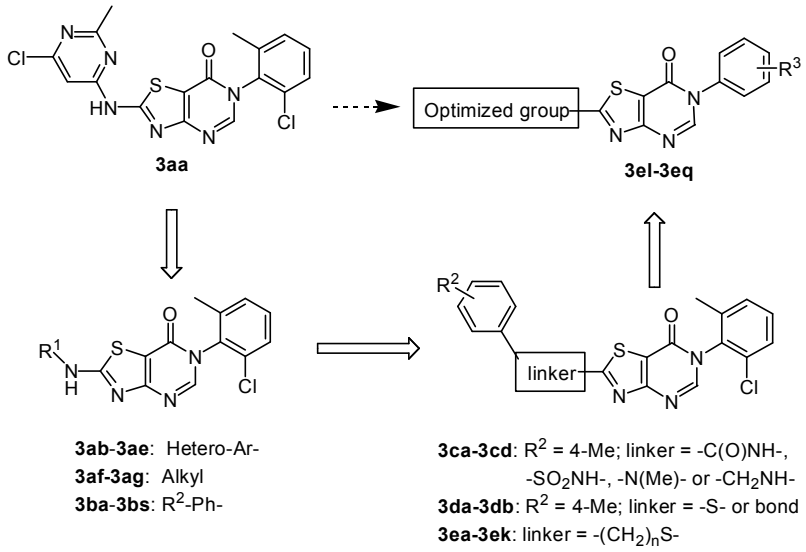


Figure 3. Strategy for optimizing active compound structures

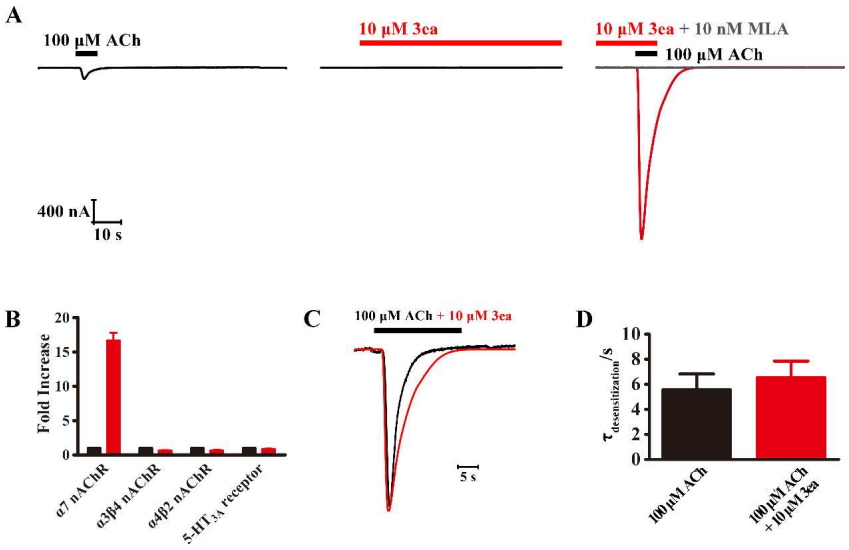


Figure 4. Selective enhancement of human α7 nAChR expressed in *Xenopus* oocytes by **3ea**. (A) Currents evoked with 100 μM ACh alone (left) and in the presence of **3ea** (right). The currents activated by **3ea** were recorded in the absence (red trace) and presence (gray trace) of 10 nM MLA (right); (B) Fold-increases of α7 nAChR (evoked by 100 μM ACh, n = 5), α3β4 nAChR (100 μM ACh, n = 5), α4β2 nAChR (100 μM ACh, n = 5) and 5-HT_{3A} receptors (10 μM 5-HT, n = 5) after incubation with 10 μM **3ea**; (C) Superimposition of scaled α7 current traces evoked with 100 μM ACh in the absence (black trace) and presence (red trace) of 10 μM **3ea**; (D) Comparison between the desensitization time constants ($\tau_{desensitization}$) in the absence and presence of **3ea** (n=5).

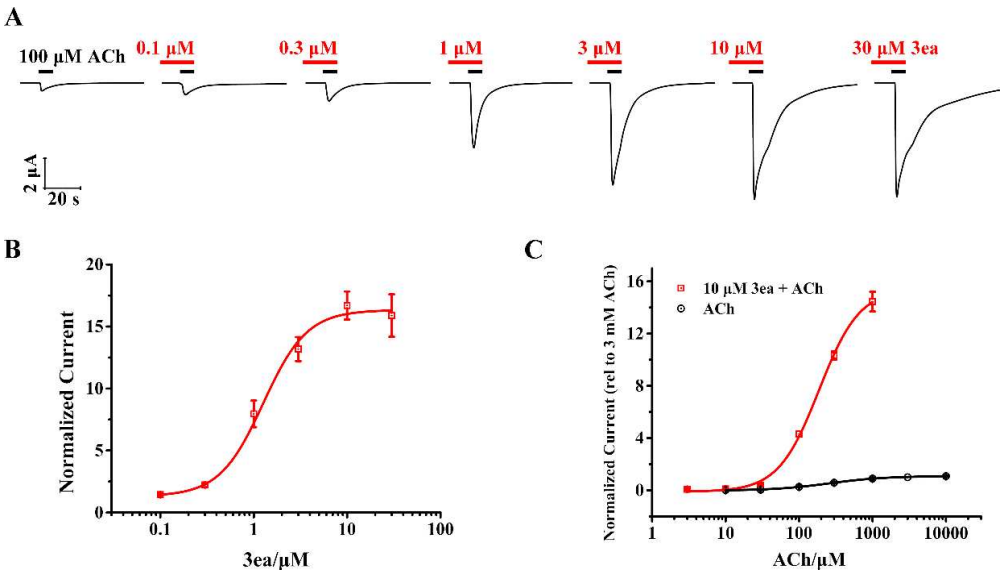


Figure 5. Concentration-dependent enhancement of α7 current by **3ea**. (A) Representative α7 currents evoked by 100 μM ACh in the absence and presence of **3ea** at various concentrations. *Xenopus* oocytes were pre-incubated with **3ea** for 2min, followed by the co-application with 100 μM ACh (10s); (B) Relationship between **3ea** concentration and the response of α7 nAChR. *Xenopus* oocytes expressing α7 nAChR were stimulated with 100 μM ACh in the absence and presence of increasing concentrations of **3ea**. Peak currents were measured and normalized with the amplitude of currents elicited by 100 μM ACh alone. The maximum efficacy of **3ea** was determined to be 1633 ± 87% with an EC_{50} of 1.26 ± 0.18 μM and Hill coefficient (n_H) of 1.85 ± 0.24 (n=5 for all data points). (C) ACh concentration-response curves in the absence and presence of 10 μM **3ea**. Peak currents were measured and normalized with the amplitude of currents elicited by 3 mM ACh alone. The curve parameters were determined as follows: ACh alone: E_{max} = 109 ± 2%, EC_{50} = 256.4 ± 20.1 μM, n_H = 1.14 ± 0.10; ACh + **3ea**: E_{max}=1545 ± 59%, EC_{50} = 186.80 ± 14.49 μM, n_H = 1.57 ± 0.16 (n=5 for each data point).

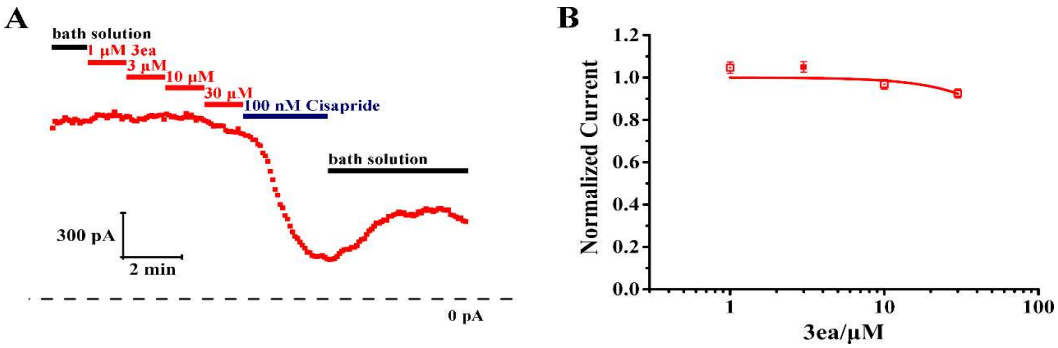
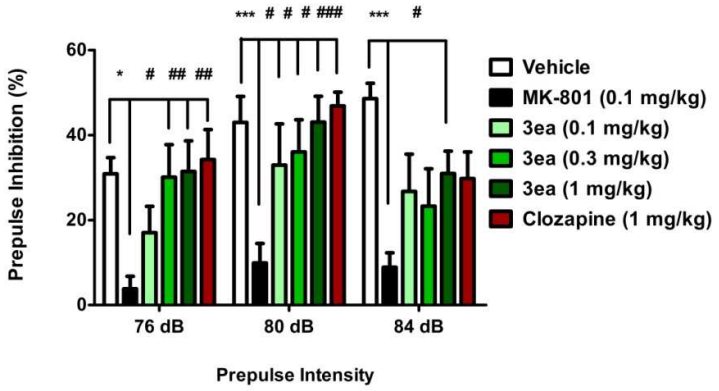
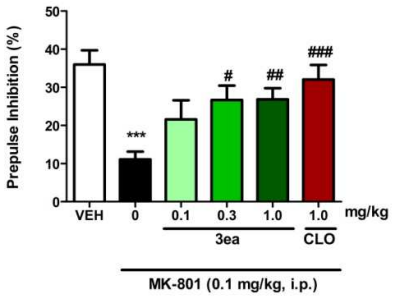


Figure 6. Effects of **3ea** on hERG channel stably expressed in HEK293 cells. (A) Time course of hERG tail currents in the presence of different concentrations (1, 3, 10, 30 μM) of **3ea** and 100 nM cisapride as the positive control. (B) hERG peak tail currents at various **3ea** concentrations (n = 4 for each data point).



A



B

Figure 7. **3ea** reversed the prepulse inhibition impairment induced by MK-801 in mice. (A) Peritoneal injection of **3ea** (0.1, 0.3, 1 mg/kg) significantly attenuated the auditory gating deficits induced by MK-801 (0.1 mg/kg) at three prepulse intensities (8, 12 and 16 dB above background noise). Clozapine was used as the positive control. The columns are presented as means ± S.E.M. (n = 5-10/group). *$p < 0.05$ and ***$p < 0.001$, compared with the vehicle-treated control; #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$, compared with MK-801-treated group, two-way ANOVA. (B) Averaged percentages of PPI across each prepulse level. **3ea** dose-dependently reversed the MK-801-induced deficits in PPI. All data are presented as means± S.E.M. (n = 9–13/group). ***$p < 0.001$ versus vehicle; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ versus the MK-801-treated group, one-way ANOVA and Dunnett's test. VEH, vehicle; CLO, clozapine; and PPI, prepulse inhibition.

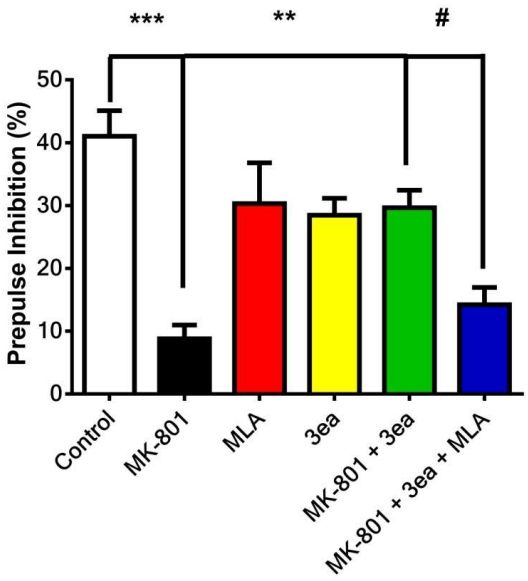
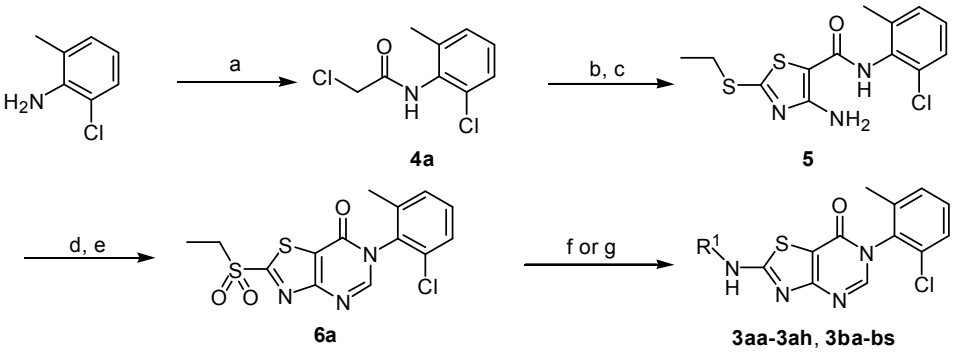


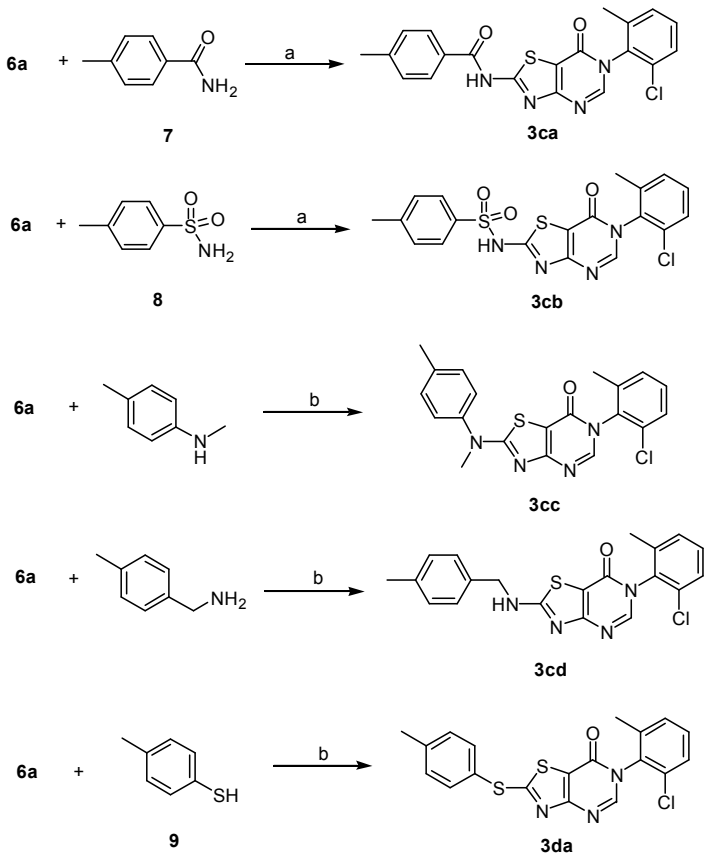
Figure 8. In vivo administration of α7 nACh antagonist MLA reverses the effect of **3ea** on enhanced PPI across all prepulse levels. MK-801 (0.1 mg/kg, i.p.) caused auditory gating impairment in the PPI task. MLA alone had no effect on PPI as comparted with **3ea** alone. Co-administration of MLA (3 mg/kg, i.p., 30 min prior) and **3ea** (1 mg/kg, i.p.) almost abolished the **3ea** effect on improvement of PPI. All data are expressed as the means ± S.E.M. **p<0.01, ***p < 0.001 versus MK-801-treated group; #p<0.05, versus MK-801 + **3ea**-treated group, one-way ANOVA.



3aa: R^1 = 6-Chloro-2-methylpyrimidin-4-yl
3ab: R^1 = 6-Chloropyrimidin-4-yl
3ac: R^1 = 6-Chloropyridazin-3-yl
3ad: R^1 = Pyridin-2-yl
3ae: R^1 = Pyrimidin-4-yl
3af: R^1 = Propyl
3ag: R^1 = Benzyl
3ba: R^1 = 3-Cl-5-Me-Ph
3bb: R^1 = 3-Cl-Ph
3bc: R^1 = 3-Me-Ph
3bd: R^1 = 2-Me-Ph
3be: R^1 = 4-Me-Ph
3bf: R^1 = Ph
3bg: R^1 = 4-Cl-Ph
3bh: R^1 = 4-Br-Ph
3bi: R^1 = 4-F-Ph
3bj: R^1 = 2-F-Ph
3bk: R^1 = 3-F-Ph
3bl: R^1 = 4-Et-Ph
3bm: R^1 = 4-OMe-Ph
3bn: R^1 = 4-OCF_3-Ph
3bo: R^1 = 3,4-diMe-Ph
3bp: R^1 = 3,5-diMe-Ph
3bq: R^1 = 3,4,5-triMe-Ph
3br: R^1 = 3,4-diF-Ph
3bs: R^1 = 3-F-4-Me-Ph

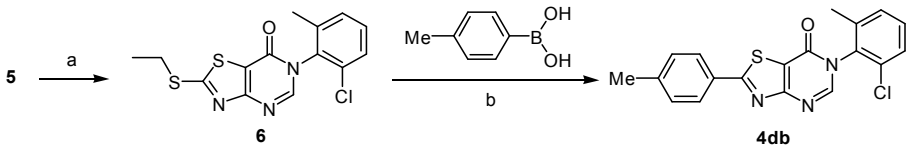
[a] Reagents and conditions: (a) $ClCH_2COCl$, AcONa, AcOH, H_2O, 0 °C then rt; (b) Dipotassium cyanodithioimidocarbonate, NaOH, acetone, H_2O, rt then 80 °C; (c) CH_3CH_2I, rt; (d) HCOOH, reflux; (e) H_2O_2, $Na_2WO_4 \cdot 2H_2O$, EtOH, rt then 50 °C; (f) R^1NH_2 NaH, anhydrous THF, reflux; (g) R^1NH_2, Et_3N, anhydrous THF, rt.

SCHEME 1



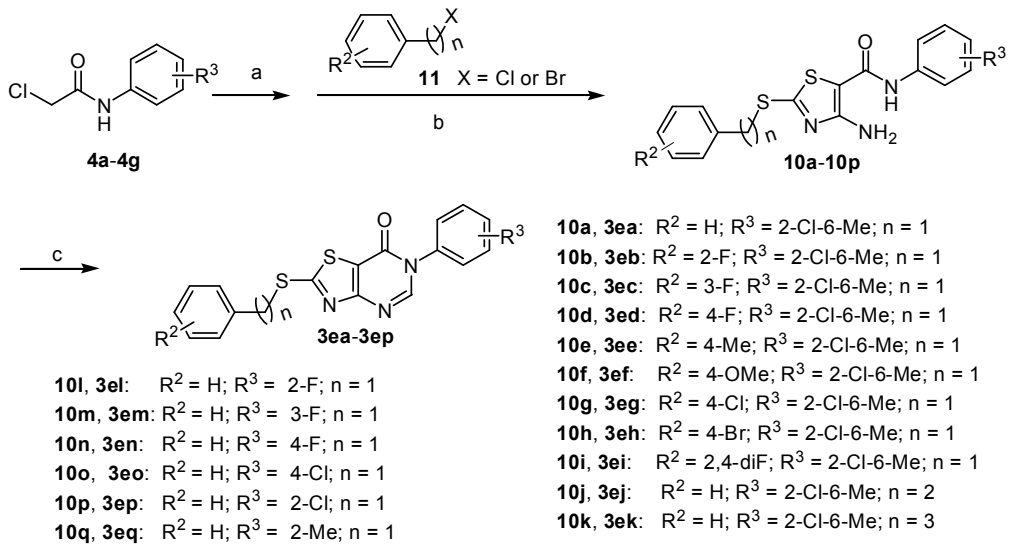
[a] Reagents and conditions: (a) NaH, anhydrous THF, reflux. (b) Et_3N, anhydrous THF, rt.

SCHEME 2



[a] Reagents and conditions: a) HCOOH, reflux. b) $Pd(Ph_3P)_4$, CuTC, THF, 80 ^oC. CuTC = copper(I) thiophene-2-carboxylate.

SCHEME 3

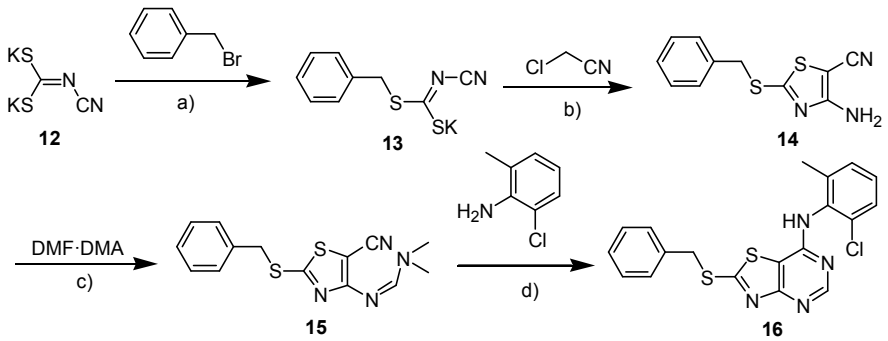


10a, **3ea**: R^2 = H; R^3 = 2-Cl-6-Me; n = 1
10b, **3eb**: R^2 = 2-F; R^3 = 2-Cl-6-Me; n = 1
10c, **3ec**: R^2 = 3-F; R^3 = 2-Cl-6-Me; n = 1
10d, **3ed**: R^2 = 4-F; R^3 = 2-Cl-6-Me; n = 1
10e, **3ee**: R^2 = 4-Me; R^3 = 2-Cl-6-Me; n = 1
10f, **3ef**: R^2 = 4-OMe; R^3 = 2-Cl-6-Me; n = 1
10g, **3eg**: R^2 = 4-Cl; R^3 = 2-Cl-6-Me; n = 1
10h, **3eh**: R^2 = 4-Br; R^3 = 2-Cl-6-Me; n = 1
10i, **3ei**: R^2 = 2,4-diF; R^3 = 2-Cl-6-Me; n = 1
10j, **3ej**: R^2 = H; R^3 = 2-Cl-6-Me; n = 2
10k, **3ek**: R^2 = H; R^3 = 2-Cl-6-Me; n = 3

10l, **3el**: R^2 = H; R^3 = 2-F; n = 1
10m, **3em**: R^2 = H; R^3 = 3-F; n = 1
10n, **3en**: R^2 = H; R^3 = 4-F; n = 1
10o, **3eo**: R^2 = H; R^3 = 4-Cl; n = 1
10p, **3ep**: R^2 = H; R^3 = 2-Cl; n = 1
10q, **3eq**: R^2 = H; R^3 = 2-Me; n = 1

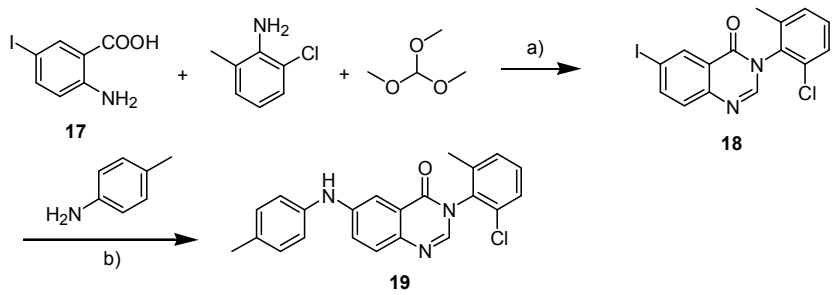
[a] Reagents and conditions: (a) Dipotassium cyanodithioimidocarbonate, NaOH, acetone, H_2O, rt then 80 °C; (b) rt; (c) HCOOH, reflux.

SCHEME 4



[a] Reagents and conditions: (a) acetone, H_2O, 0 °C then r.t.; (b) EtOH, acetone, Et_3N, 70 °C; (c) 100 °C; (d) AcOH, 110 °C.

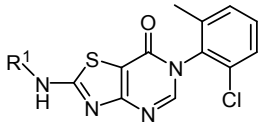
SCHEME 5



[a] Reagents and conditions: (a) AcOH, PhMe,100 ^{o}C, 24h; (b) CuI, L-poline, K_2CO_3, DMSO, Ar, 80^{o}C.

SCHEME 6

Table 1. *In vitro* activities of compounds **3aa**–**3ag** and **3ba**–**3bs** in oocytes expressing human α7 nAChR[a]



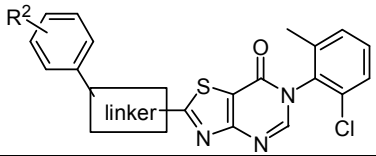
3aa-3ag, **3ba-bs**

Compd	R^1	EC_{50} (μM)	Max effect (% at 10 μM)
3aa	6-Cl-2-Me-pyrimidin-4-yl	3.20 ± 0.30	320 ± 20
3ab	6-Cl-Pyrimidin-4-yl	ND	200
3ac	6-Cl-Pyridazin-3-yl	ND	IA
3ad	Pyridin-2-yl	ND	IA
3ae	Pyrimidin-4-yl	ND	101 ± 6
3af	Propyl	ND	IA
3ag	Benzyl	ND	IA
3ba	3-Cl-5-Me-Ph	ND	305
3bb	3-Cl-Ph	ND	300
3bc	3-Me-Ph	8.39 ± 2.49	1540 ± 180
3bd	2-Me-Ph	ND	300
3be	4-Me-Ph	8.45 ± 0.07	1622 ± 106
3bf	Ph	ND	300
3bg	4-Cl-Ph	ND	200
3bh	4-Br-Ph	ND	150

3bi	4-F-Ph	ND	250
3bj	2-F-Ph	ND	200
3bk	3-F-Ph	7.65 ± 0.08	450 ± 2
3bl	4-Et-Ph	ND	250
3bm	4-OMe-Ph	ND	200
3bn	4-OCF_3-Ph	ND	200
3bo	3,4-diMe-Ph	6.99 ± 0.49	650 ± 20
3bp	3,5-diMe-Ph	5.02 ± 0.14	1100 ± 13
3bq	3,4,5-triMe-Ph	3.15 ± 0.99	600 ± 80
3br	3,4-diF-Ph	ND	300
3bs	3-F-4-Me-Ph	5.65 ± 0.67	387 ± 44

[a]Data were collected from 2–5 individual oocytes expressing α7 current recorded by two-electrode voltage clamp. EC_{50} is the compound concentration where the half of maximum activation effect (max effect) was achieved. ND-not determined; IA-inactive.

Table 2. *In Vitro* Activities of Compounds **3ca**–**3cd**, **3da**–**3db**, **3ea**–**3ek** in Oocytes Expressing Human α7 nAChR[a]

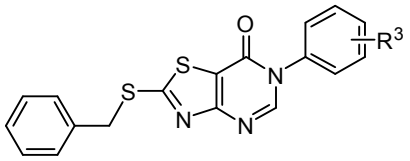


Compd	R^2	Linker	EC_{50} (μM)	Max mod (% at 10 μM)
3ca	4-Me	-C(O)NH-	20.43 ± 7.66	450 ± 40
3cb	4-Me	-SO_2NH-	ND	97 ± 12
3cc	4-Me	-N(Me)-	ND	107
3cd	4-Me	-CH_2NH-	ND	111
3da	4-Me	-S-	ND	226
3db	4-Me	bond	ND	185
3ea	H	-CH_2S-	1.26±0.18	1633±87
3eb	2-F	-CH_2S-	2.05±0.84	558±67
3ec	3-F	-CH_2S-	7.65±0.36	1136±28
3ed	4-F	-CH_2S-	2.17 ± 0.71	788 ± 54
3ee	4-Me	-CH_2S-	ND	321
3ef	4-OMe	-CH_2S-	ND	292
3eg	4-Cl	-CH_2S-	1.19 ± 0.15	444±103
3eh	4-Br	-CH_2S-	ND	320
3ei	2,4-diF	-CH_2S-	3.54 ± 0.94	1046±80

3ej	H	-$(CH_2)_2$S-	ND	112
3ek	H	-$(CH_2)_3$S-	ND	174

[a]Same as that in Table 1.

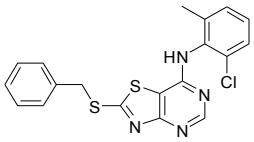
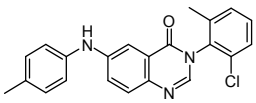
Table 3. *In Vitro* Activities of Compounds **3el**–**3eq** in Oocytes Expressing Human α7 nAChR[a]



Compd	R^3	EC_{50} (μM)	Max mod (% at 10 μM)
3el	2-F	ND	127
3em	3-F	ND	119
3en	4-F	ND	75
3eo	4-Cl	ND	104
3ep	2-Cl	ND	217
3eq	2-Me	ND	171

[a] Same as that in Table 1.

Table 4. *In Vitro* Activities of Compounds **16** and **19** in Oocytes Expressing Human α7 nAChR[a]

Compd	Structures	EC_{50} (μM)	Max effect (% at 10 μM)
16		ND	33
19		ND	63

[a] Same as that in Table 1.

Table 5. Non-compartmental pharmacokinetic parameters of **3ea** in rat plasma after intravenous and oral administrations[a]

Parameter	Administration dose	
	3 mg/kg (i.v.)	60 mg/kg (i.g.)
$t_{1/2}$(h)	10.8±1.5	7.4 ±1.1
T_{max}(h)	-	1.3±0.3
C_{max}(ng/mL)	700±200	200±80
AUC_{last}(ng·h/mL)	600 ± 70	-
AUC_{Inf}(ng·h/mL)	680 ± 40	1100 ± 200
MRT(h)	7.0 ± 1.8	9.5 ± 1.1
Vz(L/kg)	0.069 ± 0.011	-
Vz/F(L/kg)	-	0.60 ± 0.09
CL(L/h/kg)	0.005 ± 0.001	-
CL/F (L/h/kg)	-	0.06 ± 0.01
Oral bioavailability	-	8.0%

[a]Each measurement was repeated 3 times (n = 3).

Table 6. Plasma and brain concentrations of **3ea** in mice at 0.5, 2, and 8 h after 1mg/kg *i.p* administration [a]

Time Point (hr)	Average Conc. in Plasma (ng/mL)	Average Conc. in Brain (ng/g)	Average Brain/Plasma Ratio
0.50	34.3±8.9	84.7±21.0	2.5
2	9.8±0.92	26.3±1.8	2.7
8	0.9±0.1	3.7±1.0	2.3

[a]Each measurement was repeated 3 times (n = 3)

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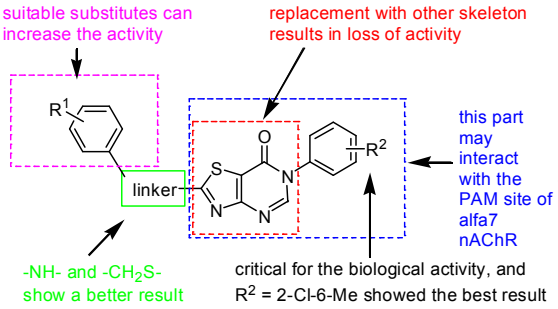

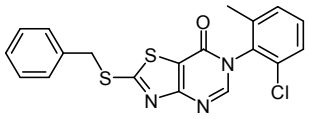

Maximum efficacy (at 100 μM ACh): 1633 ± 87%;
EC_{50} =1.26 ± 0.18 μM;
T1/2 = 10.8 h, 3 mg/kg (rat, i.v.);
High subtype selectivity on α7 nAChR;
Brain/plasma concentration ratio: ~2.5 (i.p., mice);
No significant effect on hERG channel;
Reversion of the reduced PPI similar to clozapine (mice, 1 mg/kg, i.p.)