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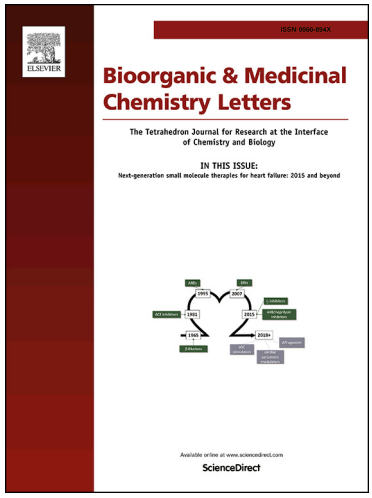

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Chemical Conversion of Nicotinamide into Type I Positive Allosteric Modulator of α7 nAChRs

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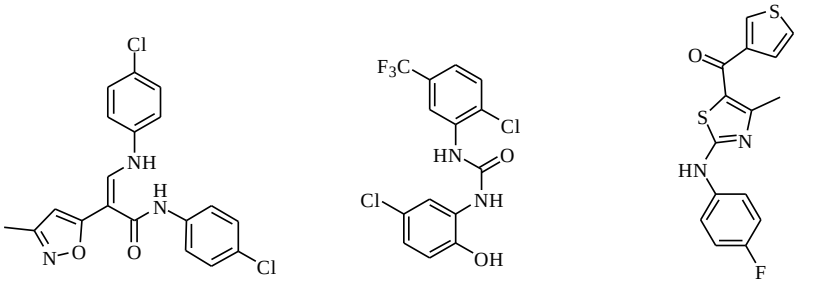
Abstract: Structural modifications of nicotinamide, a form of vitamin B3, gave rise to a series of compounds (**8aa**−**8ce**) that exhibit activities as type I positive allosteric modulators (PAMs) of human α7 nAChR expressed in *Xenopus* ooctyes in two-electrode voltage clamp assay. The compound **8ai** was a potent and efficacious PAM with an EC_{50} = 3.34 ± 1.13 μM and the maximum activation effect of α7 current over 1474 ± 246% in the presence of acetylcholine (100 μM). It is highly specific to α7 nAChR over other subtypes of nAChR and 5-HT_{3A} receptors. The structure−activity relationship analysis identified a key skeleton of nicotinamide nucleus critical for biological activity. Taken together, the **8ai** as a type I PAM of α7 nAChR may be beneficial for improvement of cognitive deficit.

Keywords: Nicotinamide; α7 nAChR; Positive allosteric modulators; Cognitive impairment; Alzheimer's disease

The α7-subtype of nicotinic acetylcholine receptors (α7 nAChR) has been well recognized as a potential target in the central nervous system (CNS) for treatment of neuropsychiatric disorders, such as Alzheimer's disease (AD).[1] Several studies have shown that coincident compromises in cholinergic activity and cognition in early AD may be mediated by the α7 nAChR, suggesting that the activation of α7 nAChR may represent an effective treatment strategy for the cognitive impairments associated with early AD.[2] The α7 homomeric receptor demonstrates a wide-spread localization in the brain and is characterized by a high calcium ion permeability and a fast

desensitization rate.[3] Clinical studies have shown that targeting α7 nAChR with selective agonists or partial agonists can effectively improve the cognitive deficits in Alzheimer's disease.[4] Positive allosteric modulators (PAMs) have been demonstrated numerous advantages over agonists for their maintenance of the normal temporal and spatial patterns of neurotransmission and high selectivity. [5] They can also improve the safety compared with orthosteric drugs.[6] For instance, MDL-800 was reported to be a well-characterized allosteric activator with good selectivity and high activity of SIRT6.[7] Based on their channel kinetics and desensitization characteristics, the PAMs of nicotinic receptors are classified into types I and II.[8] Both types of PAMs have been found with *in vivo* efficacy in animal models of cognition deficit,[9] whereas type I PAMs can maintain the rapid channel kinetics, which may be more beneficial than type II PAMs at minimizing potential Ca^{2+}-induced cytotoxicity.[10] α7 nAChR and other subtypes including α4β2 and α3β4 of nAChR are also expressed in the cortex, hippocampus, and cerebellum,[11] whose functions can be activated by acetylcholine or enhanced by α7 nAChR PAMs. Therefore, the design and synthesis of novel selective α7 nAChR PAMs with high potency and efficacy are expected to achieve desired therapeutic indexes with little side effects.

The chemical structures of representative type I PAMs includes acrylamide **1** (AVL-3288, CCMI, Figure 1),[12] ureas **2** (NS-1738, Figure 1)[13] and (2-amino-5-keto)thiazole compound **3** (LY-2087101, Figure 1).[14] Among these type I PAMs, only AVL-3288 is currently in the clinical phase I development stage. Therefore, it is necessary to discover and develop more potent type I PAMs with high selectivity.



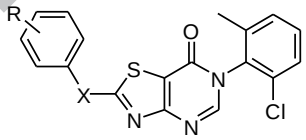
1, AVL-3288 (CCMI) **2**, NS-1738 **3**, LY-2087101

Figure 1. Representative type I α7 nAChR PAMs.

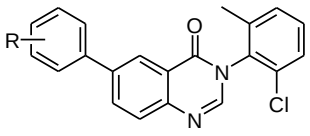
Nicotinamide is amide form of vitamin B3, which has recently been shown to be

effective in restoring cognition and memory. Nicotinamide can prevent necrosis and apoptosis in the brains of MPTP-treated mice and promote learning and memory.[15] Nicotinamide improves sevoflurane-induced cognitive impairment and has an anti-inflammatory and anti-apoptotic effect against sevoflrane-induced damages.[16] It has also been shown that nicotinamide can forestall the pathology and cognitive decline in mice.[17] Therefore, rational use and modification of nicotinamide structure may lead to discovering novel α7 nAChR PAMs compounds that can better improve cognitive impairment.

We have previously synthesized a series of thiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (Figure 2) that as type I PAMs exhibit good activity on α7 nAChR expressed in *Xenopus* oocytes in two-electrode voltage clamp assay.[18] Among them, compound **4** shows as a potent PAM with the maximum activation effect of α7 current over 1622 ± 106% in the presence of acetylcholine (100 μM) and an EC_{50} = 8.45 ± 0.078 μM. Compound **5** exhibits the type I PAM activity on α7 nAChR with an EC_{50} of 1.26 ± 0.18 μM (n = 6) and enhancement rate of 1633 ± 87% (10 μM) in the presence of 100 μM ACh. A series of 3*H*-quinazolin-4-one derivatives **6** were designed and synthesized on the basis of compound **4** and **5** (Figure 2). However, they show a weak negative activity (**6a**: enhancement rate of 76% at 10 μM; **6b**: 65%) rather than activating effect. [19]



4: R = 4-Me, X = NH
enhance rate at 10 μM = 1622 ± 106 %,
EC_{50} = 8.45 ± 0.078 μM
5: R = 4-H, X= -CH_2S-
enhance rate at 10 μM = 1633 ± 87%,
EC_{50} = 1.26 ± 0.18 μM



6a: R = 4-F
enhance rate at 10 μM = 76 ± 9 %
6b: R = 4-OMe
enhance rate at 10 μM = 65 ± 1%

Figure 2. The representative structures reported by our research work.

In this work, we envisaged that more efficient compounds could be produced by introducing the structure of nicotinamide to molecular **6**.[20] To test this hypothesis, we started unclosing the pyrimidinone ring of **6** to obtain the compound **7** with a weak

positive activity (136%, Figure 3). Then, further modification of **7** was performed with nicotinamide instead of benzamide to provide compound **8aa** that showed a 5-fold improvement of activity (604%, Figure 3). Herein, we report the synthesis of a new series of nicotinamides **8ac**–**8ce** and evaluation of their biological activities as novel PAMs of α7 nAChRs.

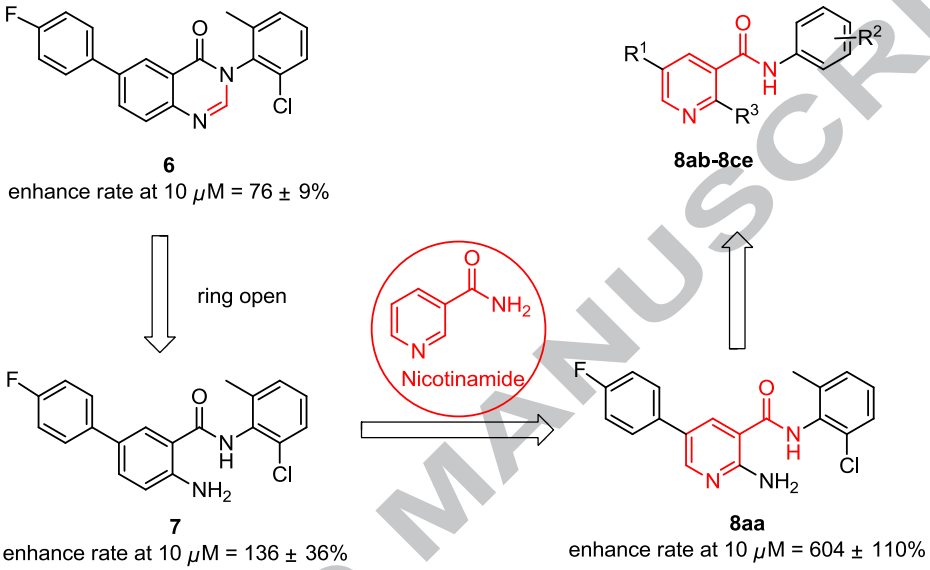
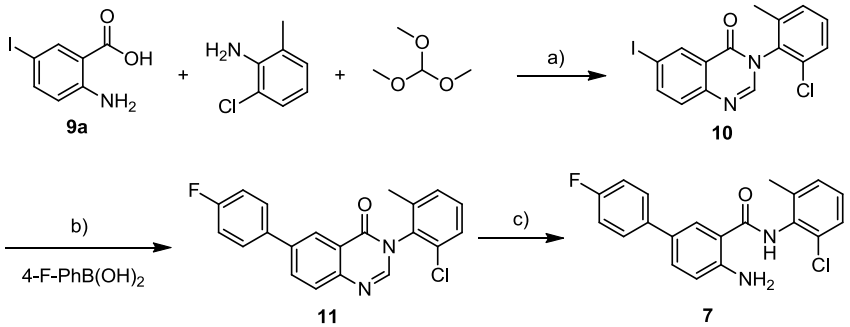


Figure 3. The optimization of α7 nAChR modulators

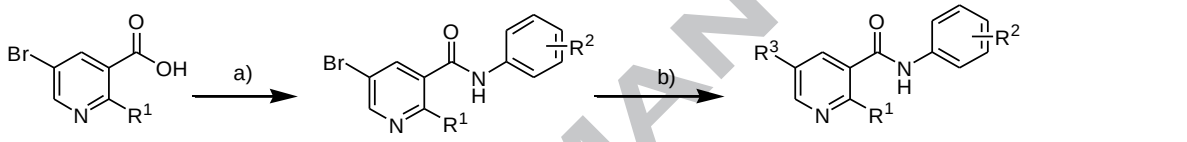
The synthesis route of benzamide derivative **7** is outlined in Scheme 1. The key intermediate **10** was obtained via one pot reaction of 2-amino-5-iodo-benzoic acid **9a**, 2-chloro-6-methyl-phenylamine and trimethoxymethane in toluene in the presence of catalytic acetic acid at 110 ºC for 24h.[21] Then palladium-catalyzed Suzuki coupling reaction of **10** with 4-fluro-phenylboronic acid provided **11** in the presence of 0.05 equiv $Pd(PPh_3)_4$ and 2 equiv K_2CO_3 with good yield.[22] Finally, **11** was dissolved in absolute ethyl alcohol in the presence of 5N NaOH at 73 ºC for 2 h to produce benzamide derivative product **7**.



Scheme 1.

Reagents and conditions: a) AcOH(cat.), Toluene, 110 °C (yield: 65%); b) $Pd(PPh_3)_4$, K_2CO_3,THF, 90 °C (yield: 75%); c) 5N NaOH, C_2H_5OH, 73 °C (yield: 66%).

The nicotinamide derivatives **8aa**–**8ce** were synthesized from the commercially available nicotinic acid derivatives **9b** and **9c** as outlined in Scheme 2. **9b** and **9c** reacted with $SOCl_2$ in toluene at 110 °C for 2 h gave two nicotinoyl chloride intermediates. The nicotinoyl chlorides were stirred with corresponding anilines in THF at 0 °C for 0.5 h, then reflux at 90 °C for another 4 h produced **12a**–**12d**, which were treated with corresponding boronic acids in the presence of 0.05 equiv $Pd(PPh_3)_4$ and 2 equiv K_2CO_3 in THF at 90 °C to afford target nicotinamide derivatives **8aa**–**8ce** with 18–68 % yields.



9b: R^1 = -NH_2
9c: R^1 = -H

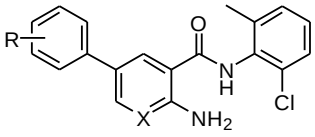
12a: R^1 = -NH_2; R^2 = 2-Cl-6-Me
12b: R^1 = -NH_2; R^2 = 4-H
12c: R^1 = -NH_2; R^2 = 2-Me
12d: R^1 = -H; R^2 = 2-Cl-6-Me

8aa-**8aw**: R^1 = -NH_2; R^2 = 2-Cl-6-Me;R^3 = R-Ph
8ba-**8bc**: R^1 = -NH_2; R^2 = 2-Cl-6-Me; R^3 = Het-Ar
8ca: R^1 = -NH_2; R^2 = 4-H; R^3 = 4-F-3-Me-Ph
8cb: R^1 = -NH_2; R^2 = 2-Me; R^3 = 4-F-3-Me-Ph
8cc-**8ce**: R^1 = -H; R^2 = 2-Cl-6-Me; R^3 = R-Ph

Scheme 2. Reagents and conditions: a) $SOCl_2$, Toluene, 110 °C; Corresponding anilines R^2NH_2, THF, 0 °C–90 °C(38 %–43 %); b) $R^3B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, THF, 90 °C (18 %–68 %).

The target compounds were tested for the *in vitro* activities in *Xenopus laevis* oocytes expressing human α7 nAChR with method described in our previous work.[23] Those compounds were inactive in eliciting α7 current in the absence of direct agonists, then 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 maximum modulation (at 10 μM) and EC_{50} (max effect > 400%) were determined and listed in Tables 1–3.

Table 1. *In vitro* activities of compounds **7** and **8aa**−**8aw** for enhancement of human α7 nAChR[a]



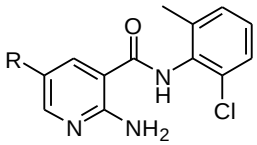
Compd.	R	X	EC_{50} (μM)	Max effect (%, at 10 μM)
7	4-F	CH	ND	136 ± 36
8aa	4-F	N	4.57 ± 1.43	604 ± 110
8ab	3-F	N	ND	86 ± 3
8ac	2-F	N	ND	119 ± 7
8ad	4-Cl	N	ND	278 ± 47
8ae	4-CF_3	N	ND	164 ± 7
8af	4-OCF_3	N	ND	153 ± 22
8ag	4-NO_2	N	ND	135 ± 2
8ah	4-F-2-Me	N	ND	225 ± 67
8ai	4-F-3-Me	N	3.34 ± 1.13	1474 ± 246
8aj	2-Cl-4-F	N	ND	186 ± 5
8ak	3-Cl-4-F	N	5.49 ± 0.45	495 ± 88
8al	4-Cl-3-F	N	ND	229 ± 82
8am	3-F-4-Me	N	3.31 ± 1.22	609 ± 123
8an	2-F-4-Me	N	ND	372 ± 14
8ao	4-OMe	N	8.50 ± 2.59	531 ± 96
8ap	3-OMe	N	ND	212 ± 8
8aq	2-OMe	N	4.32 ± 0.84	1008 ± 85
8ar	4-SMe	N	4.96 ± 2.57	774 ± 183
8as	4-OH	N	ND	257 ± 28
8at	4-Me	N	3.19 ± 1.77	539 ± 105
8au	4-Et	N	1.35 ± 0.18	455 ± 101
8av	4-$NHCOCH_3$	N	ND	109 ± 9
8aw	4-H	N	ND	273 ± 39

[a]Data were collected from 2–5 individual oocytes expressing $\alpha 7$ 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.

The substitution of the benzamide with nicotinamide (**8aa**, Table 1) resulted in an

obvious increase in activity. This result indicates that nicotinamide may be beneficial for allosteric modulation activity of α7 nAChR. Based on the *in vitro* activities of **7** and **8aa** as shown in Table 1, we examined the effects of the substitutes (**8ab**−**8aw**) on the phenyl group in R. Moving the fluorine atom of **8aa** to the 3-positon (**8ab**) and 2-position (**8ac**) dramatically reduced the activity. The substitutions of R in **8aa** with 4-Cl, 4-CF_3, 4-OCF_3, 4-NO_2 (**8ad**–**8ag**) reduced the activity. Based on the maximum effects acquired from 4-fluoro, we envisaged that the introduction of methyl or chlorine to the ortho- and meta-positions of phenyl group may improve the activity. Therefore, compounds **8ah**−**8ak** were designed and synthesized and provided the maximum effects ranging from 186% to 1474%. Among them, **8ai** (maximum effect of 1474% and EC_{50} = 3.34 ± 1.13 μM) exhibited the highest activity. Not surprisingly, when we exchanged the 4-fluoro with the 3- or 2-position substituents, the activity of two compounds decreased (maximum effect: 1474% for **8ai** *vs* 609% for **8am**, 495% for **8ak** *vs* 229% for **8al**) with one compound increased slightly (maximum effect: 225% for **8ah** *vs* 372% for **8an**). Changing the 4-F of **8aa** (604%) to 4-OMe (**8ao**, 531%), 3-OMe (**8ap**, 212%), 4-OH (**8as**, 257%), 4-Me (**8at**, 539%), 4-Et (**8au**, 455%), 4-$NHCOCH_3$ (**8av**, 109%), 4-H (**8aw**, 273%) did not obviously improve activities. However, Changing the 4-F of **8aa** (604%) to 2-OMe (**8aq**, 1008%), 4-SMe (**8ar**, 774%) can achieve better activities.

Table 2. *In vitro* activities of compounds **8ba**−**8bc** for enhancement of human α7 nAChR[a]

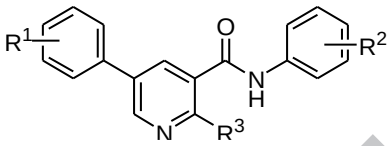


Compd.	R	EC_{50} (μM)	Max effect (%, at 10 μM)
8ba	Thiophen-3-yl	ND	153 ± 12
8bb	Furan-3-yl	ND	111 ± 6
8bc	Pyridin-3-yl	ND	138 ± 12

[a]Same as that in Table 1.

The modifications of the 4-fluorophenyl group in **8aa** to aromatic heterocycle substituents (**8ba**–**8bc**) led to dramatic reduction of activity or even complete loss of the effect (Table 2), indicating that the substituted phenyl group was the key pharmacophore for this novel series of compounds.

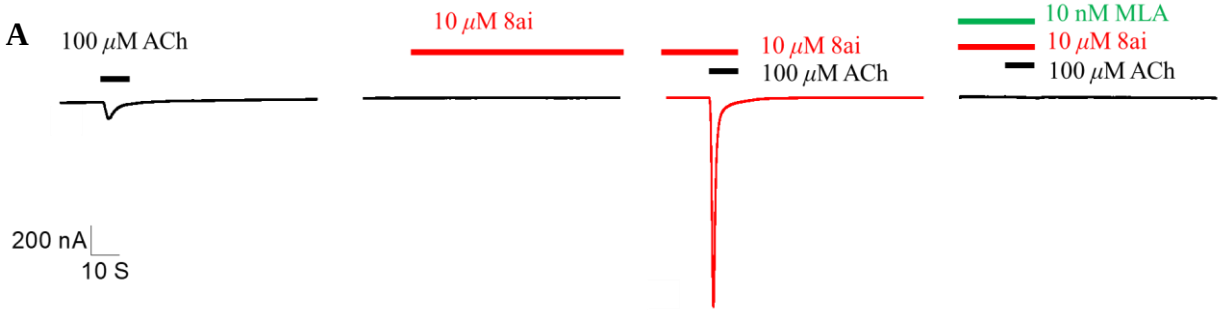
Table 3. *In vitro* activities of compounds **8ca**–**8ce** for enhancement of human α7 nAChR[a]



Compd.	R^1	R^2	R^3	EC_{50} (μM)	Max effect (%, at 10 μM)
8ca	4-F-3-Me	4-H	$-NH_2$	ND	94 ± 10
8cb	4-F-3-Me	2-Me	$-NH_2$	ND	156 ± 22
8cc	4-F	2-Cl-6-Me	-H	ND	248 ± 31
8cd	4-Me	2-Cl-6-Me	-H	1.97 ± 0.96	603 ± 191
8ce	4-Cl	2-Cl-6-Me	-H	ND	150 ± 18

[a]Same as that in Table 1.

Changing the 2-chloro-6-methyl group (**8ai**, 1474%) in R^2 to 4-H (**8ca**, 94%) or 2-Me (**8cb**, 156%) remarkably reduced the activity, indicating that the 2-chloro-6-methyl group was important for the activity. When we changed the $-NH_2$ to –H in R^3, the activity of two compounds decreased (maximum effect: 604% for **8aa** *vs* 248% for **8cc**, 278% for **8ad** *vs* 150% for **8ce**) with one compound increased (maximum effect: 539% for **8at** *vs* 603% for **8cd**).



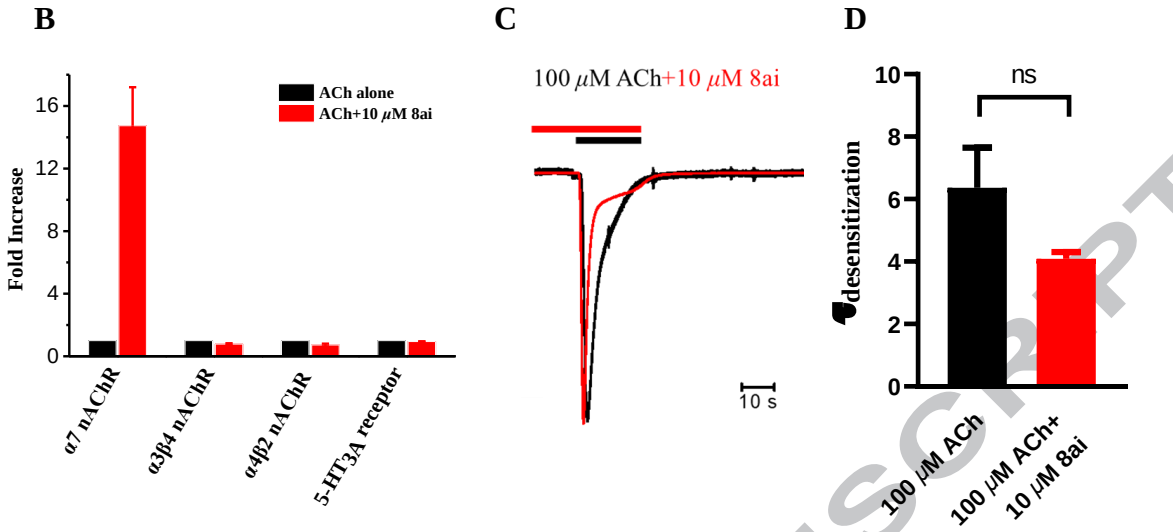
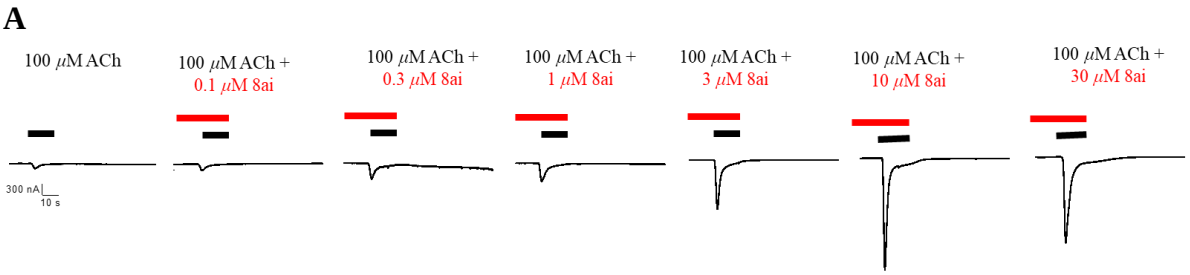


Figure 4. Selective enhancement of human α7 nAChR current by **8ai** over other subtypes of nAChRs and 5-HT_{3A} expressed in *Xenopus* oocytes. (A) α7 currents were recorded by TEVC in response to 100 μM ACh alone (first trace) or in the presence of **8ai** (second trace). The currents activated by **8ai** were recorded in the absence (third trace) and presence (fourth trace) of 10 nM **MLA** (right). (B) Fold-increases of α7 nAChR (evoked by 100 μM ACh, n = 5), $\alpha 3\beta 4$ nAChR (100 μM ACh, n = 5), $\alpha 4\beta 2$ nAChR (100 μM ACh, n = 5), and 5-HT_{3A} receptors (10 μM 5-HT, n = 5) after incubation with 10 μM **8ai**. (C) Superimposition of scaled α7 current traces evoked with 100 μM ACh in the absence (black trace) and presence (red trace) of 10 μM **8ai**. (D) Comparison between the desensitization time constants ($\tau_{desensitization}$) in the absence and presence of **8ai** (ACh, $\tau_{desensitization}$ = 6.37 ± 2.56; ACh + 8ai, $\tau_{desensitization}$ = 3.49 ± 0.50; p = 0.1092 > 0.05, n = 5).

Based on the results presented above, it can be concluded that, among the synthesized analogs, **8ai** is the optimal PAM of α7 nAChR. A preincubation of **8ai** for 2 min before application of 100 μM ACh remarkably increased the peak current of α7 nAChR (Figure 4A). The examination of the channel desensitization kinetics showed that **8ai** had a little effect the desensitization of α7 nAChR, causing a minor decrease in $\tau_{desensitization}$(Figure 4C, D).



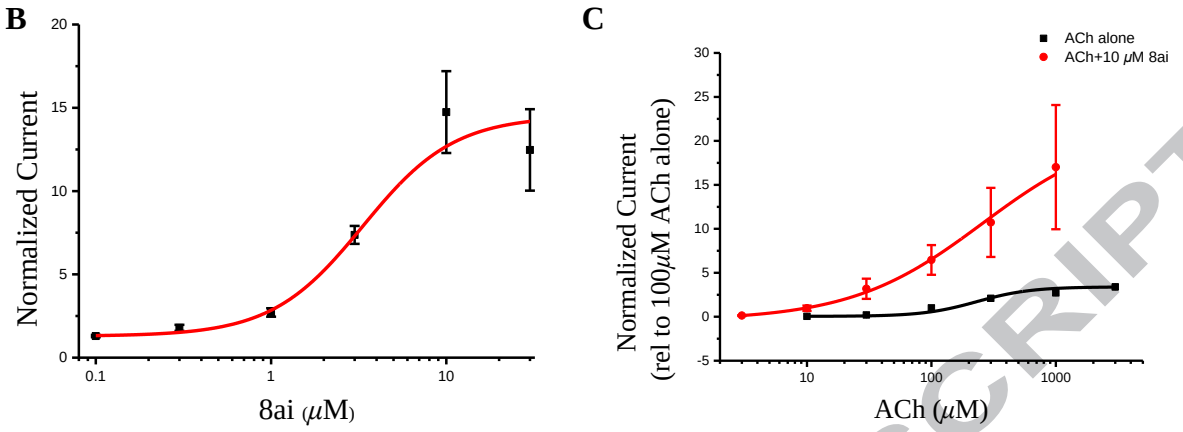


Figure 5. Concentration-dependent enhancement of α7 current by **8ai**. (A) Representative α7 currents were evoked by 100 μM ACh in the absence and presence of **8ai** at various concentrations. *Xenopus* oocytes were preincubated with **8ai** for 2 min, followed by the coapplication with 100 μM ACh (10 s). (B) Relationship between **8ai** concentration and activity of α7 nAChR. *Xenopus* oocytes expressing α7 nAChR were stimulated with 100 μM ACh in the absence and presence of increasing concentrations of **8ai**. Peak currents were measured and normalized with the amplitude of currents elicited by 100 μM ACh alone. The maximum efficacy of **8ai** for enhancement of α7 current was 1474 ± 246% with an EC_{50} of 3.34 ± 1.13 μM, and Hill coefficient (n_H) of 1.67 ± 0.67 (n = 5 for all data points). (C) ACh concentration−response curves in the absence and presence of 10 μM **8ai**. Peak currents were measured and normalized with the amplitude of currents elicited by 100 μM ACh alone. The curve parameters were determined as follows: ACh alone: E_{max} = 339 ± 0%, EC_{50} = 231.8 ± 2 μM, n_H = 2.00 ± 0.00. ACh + **8ai**: E_{max} = 2167 ± 577%, EC_{50} = 257.6 ± 162.2 μM, n_H = 0.82 ± 0.12 (n =4 for each data point).

The activation of α7 nAChR by 100 μM ACh alone was first recorded as a control before different concentrations (0.1~30 μM) of compound **8ai** were co-applied with 100 μM ACh after 2 min preincubation. As shown in Figure 5A, **8ai** enhanced α7 currents in dose-dependent manner in the presence of 100 μM ACh. Fitting the concentration-dependent activation of α7 current by **8ai** gave rise to an EC_{50} = 3.34 ± 1.13 μM, E_{max} = 1474 ± 246%, and Hill coefficient (n_H) =1.67 ± 0.37 (Figure 5B). To further confirm the activity of **8ai**, concentration-dependent activation of α7 by ACh was generated in the absence or presence of 10 μM **8ai**. Preincubation of **8ai** for 2 min increased α7 current about 7-fold from 339 ± 0% to 2167 ± 577%, without significant alteration of the EC_{50} value of ACh from 231.8 ± 2 μM to 257.6 ± 162.2 μM (Figure 5C).

In conclusion, our chemical modifications of nicotinamide identified a novel

compound **8ai** that functions as type I PAM of α7 nAChR. The structure−activity relationship (SAR) analysis revealed that the substitutions of phenyl group at the 5-position and the 2-chloro-6-methylcarbamoyl at the 3-position of the pyridine ring are important for the activity of these compounds. The compound **8ai** may serve as a lead compound for further development of potential candidate for improving cognitive function.

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Author Contributions

Xin Li[†] and Wenjun Xie[†] contributed equally to this work. The manuscript was written through contributions of all authors.

Conflict of interest

The authors declare that this study was carried out only with public funding. There is no funding or no agreement with commercial for profit firms.

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Supplementary data

Chemical data of compounds; NMR spectra, HPLC reports and HRMS spectra of the target compounds. The Supporting Information is available free of charge on the website at DOI: xxxxxxxxxxxxxxx. (PDF)

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Graphic Abstract