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Original article

Probe to Bifunctional Memantine Derivatives for Treatment of Alzheimer's Disease

Zheng Liu, Haiyun Chen, Fangcheng Luo, Baojian Guo, Xiaoyong Jing, Zaijun Zhang, Yewei Sun

Affiliation:
Institute of New Drug Research and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of Traditional Chinese Medicine, Jinan University College of Pharmacy, Guangzhou 510632 China

The name of the department(s) and institution(s) to which the work should be attributed:
Institute of New Drug Research and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of Traditional Chinese Medicine, Jinan University College of Pharmacy, Guangzhou 510632, China

Address reprint requests to
*** Pei Yu, PhD,**
Institute of New Drug Research and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of Traditional Chinese Medicine, Jinan University College of Pharmacy, Guangzhou 510632 China or at pennypeiyu@163.com. Tel.: +8620-8522-5030; Fax: +8620-8522-5030

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ABSTRACT: Alzheimer' disease (AD) is a neurodegenerative disease commonly occurring in older people. Two types of drugs, the acetylcholinesterase (AChE) inhibitor and the N-methyl-D-aspartate receptor (NMDAR) antagonist, were approved to treat AD by FDA. Of them, memantine was the only one of NMDAR antagonist. Previous studies had revealed that the carbamate grouphad a better AChE inhibitor activity. Herein, a series of new memantine derivatives with a carbamate group were designed and synthesized. They were expected to haveboth NMDAR antagonism and AChE inhibition. However, the neuroprotective effect of these new compounds against the glutamate-induced neurotoxicity was not as effective as memantine. The AChE inhibition of them wasalso lower than tacrine. The combine of memantine moiety and carbamate group attenuated the activities of the two functional groups. It may be caused by the changes of the spatial structures after combination. The introduction of large spatial structures makes the functional groups difficult to orientate to the active site.

KEYWORDS: Acetylcholinesterase (AChE) inhibitor; Alzheimer's disease (AD); Memantine derivatives; Multi-functional drugs; NMDAR antagonist..

Statement of Originality of work: The manuscript has been read and approved by all the authors, the requirements for authorship have been met, and that each author believes that the manuscript represents honest and original work.

INTRODUCTION

Alzheimer' disease (AD) is a neurodegenerative disease commonly occurring in aging people. About 36 million people worldwide are living with dementia, and the number will be double every 20 years to 66 million by 2030, and to 115 million by 2050[1]. Deaths from Alzheimer's increased 66% from 2000 to 2008, while deaths from other major diseases decreased[2]. The etiology of AD is rather complicated and a cascade of neuronal damage involves in the progression and development of AD. The abnormal of Amyloid beta protein aggregation, the hyperphosphorylation of microtubule-associated protein tau, the injury of

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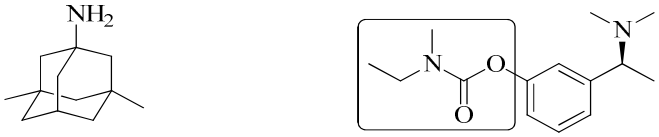
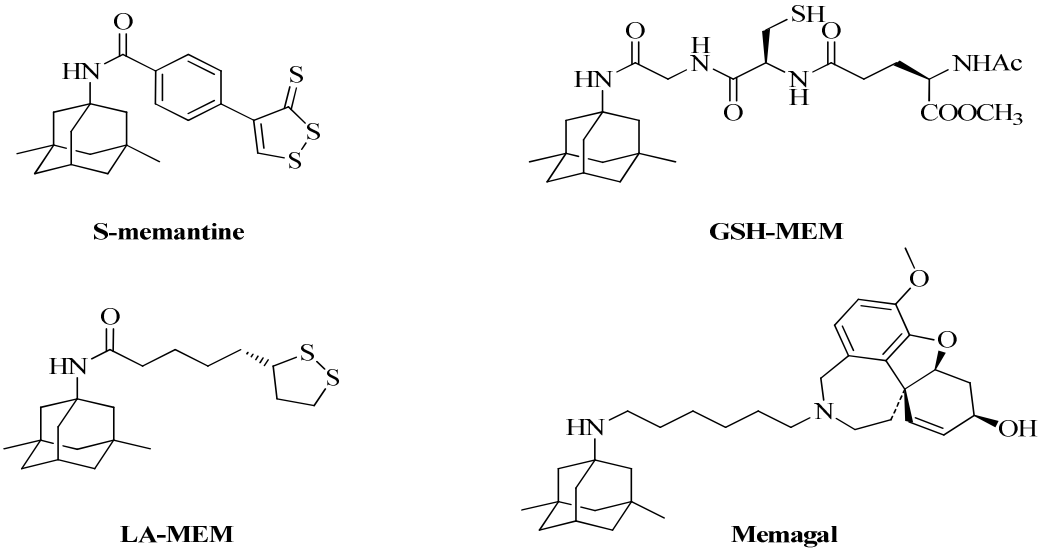
cholinergic system and oxidative stress were supposed to relate to AD[3-6]. However, excitotoxicity induced by glutamate via the NMDA receptor was one of the most important reasons. That is glutamate bound to the NMDA receptor and opened the calcium channel, triggering the calcium fluxes, breaking the cellular ion homeostasis and inducing a cascade of neuronal damage[7-10].
Memantine (1-amino-3, 5-dimethyladamantane) (Figure 1), is an open-channel, noncompetitive NMDAR blocker with low-affinity and fast receptor kinetics[11-13]. After used as clinical drugs for treatment Parkinson's diseases for more than 20 years, it was approved as a treatment for moderate to severe Alzheimer's disease in the US (2004) and Europe (2003). Memantine is also an important verified neuroprotective agentia up to now. It specifically binds to the NMDA receptor, blocks the calcium influx, reduces the ROS and affords neuronal protection[14-16]. AChE inhibitor rivastigmine **(Fig.1)** is used for the treatment of mild to moderate AD and approved by FDA as a treatment of PD in 2006[17]. It inhibits AChE activity of the central nervous system, increases the acetylcholine level and reduces aberrant motor behavior[18]. The carbamate is the key moiety of rivastigmine structure[17].



Memantine **Rivastigmine**

Figure 1.The structures of Memantine and Rivastigmine.

Treatment with memantine alone just improves the cognitive level, while rivastigmine ameliorates the behavior disorders more than cognitive level.It is reported that combinationof memantine and AChE inhibitors ameliorated both behavioral disorder and cognitive disorder[19]. In addition, a serious of compounds that contain memantine moiety and other functional groups, such as H_2S donor, glutathione, lipoic acid and galanthamine **(Fig. 2)** also showed a good activity of neuroprotection against AD[20-22]. In fact, previous studies showed that monofunctional drugs were not effective enough to treatment neurodegenerative disease, multifunctional drugs had more potential in curing these complex diseases[23]. Considering this, we designed and synthesized a series ofmemantine derivativescombinedwith carbamate moiety, hoping that they would have NMDARantagonism and cholinesterase inhibition activity concurrently**(Fig. 3)**.



S-memantine **GSH-MEM**

LA-MEM **Memagal**

Figure 2.The structures of S-Memantine, GSH-MEM, LA-MEM and Memagal.

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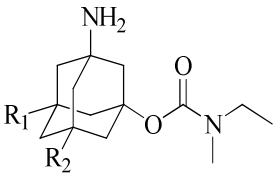
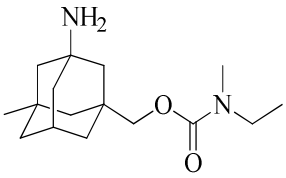


22 R_1=CH_3, R_2=H
23 R_1=R_2=CH_3
24 R_1=CH_2CH_3, R_2=H



31

Figure 3.The structures of new compounds.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Methyladamantane, ethyladamantane and 1,3-dimethyl adamantane were purchased from Xikai Chemical Co., Ltd (Zhangjiagang, China). N-ethyl-N-methyl carbamoylchloride was purchased from Energy Chemical Co.,Ltd (Shanghai, China). Other reagents were purchased from Wenrui Scientific Instrument Co., Ltd (Guangzhou, China). Solvents and reagents were reagent grade and used without further purification. The reagents used in the biological activity assay were all purchased from Sigma (St Louis, MO, USA).

BIOASSAY

CULTURES OF PRIMARY CEREBELLAR GRANULE NEURONS (CGNS)

Cerebellar granule neurons (CGNs) were prepared from 8-day-old Sprague-Dawley rats (The Experimental Animal Center of Sun Yat-sen University) as described in our previous publication[24]. Briefly, neurons were seeded at a density of 1.0-1.5×10^6 cells/mL in basal modified Eagle's (BME) medium containing 10% fetal bovine serum, 25 mM KCl, 2 mM glutamine, and penicillin (100 units/mL)/streptomycin (100 μg/mL). Cytosine arabinoside (10 μM) was added to the culture medium 24 hours after plating to limit the growth of non-neuronal cells, cultures at 8 days *in vitro* (DIV) were used for the experiments.

CULTURES OF PRIMARY CORTICAL NEURONS (CNS)

Fetal rat brains from Sprague-Dawley pregnant rats of 16-18 days gestation were used (The Experimental Animal Center of Sun Yat-sen University). Cortical neurons (CNs) were obtained as described previously with minor modifications[25]. Following enzymatic treatment (TrypLE Express) for 15 min at 37 °C, isolated neurons were suspended in commercial DMEM medium containing 0.3 g/L glutamine, 4 g/L glucose, 10% heat-inactivated iron-supplemented calf serum, 1% (v/v) penicillin/streptomycin mixture. Cells were placed on 96-well at a density of 4-5×10^4 cells/well. The plates were pre-coated with 10 μg/mL of poly-L-lysine, to allow the attachment of neurons themselves to the plates. Cortical neurons were grown at 37 °C in a humidified incubator with 5% CO_2/95% air atmosphere. After 4-6 h, unattached cells and debris were removed by replacing the initial medium with fresh Neurobasal medium containing B27 supplements. Subsequent partial medium replacement was carried out twice a week and cultures at 10-12 days *in vitro* (DIV 10-12) were used for the experiments.

MTT ASSAY

The percentage of surviving neurons was determined by the activity of mitochondrial dehydrogenases with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). The assay was performed according to the specifications of the manufacturer (MTT kit I; Roche Applied Science). Cell viability was expressed as a percentage of the value of the cells without inducer treatment.

AChE INHIBITION ACTIVITY

Ellman's method was adapted for determination of AChE activities in rat brain homogenates[26]. Brain tissue from adult Wistar rats was homogenized at 2% w/v in 0.1 M sodium phosphate buffer, pH 8.0, with added NaCl 58.5 mg/mL and Triton X-100 0.05% v/v. Aliquots of homogenate (20μL) were incubated with different concentration of drugs for 3h in phosphate buffer pH 7.0 before addition of 5, 5'-dithiobis(2-nitrobenzoic acid) and 1 mM acetylthiocholine iodide. The reaction was run at 37 °C in a final volume of 200μL in 96-well microplates and followed at 412 nm for 10 min with a plate reader.

DATA ANALYSIS

The data are expressed as the means ± SD. The statistical significance was determined by one-way

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ANOVA followed by the Tukey's test. Differences were accepted as significant at $p<0.05$.

MOLECULAR DOCKING

HARDWARE: All required software ware installed in Linux Ubuntu 12.04 LTS 32-bit system equipment with Processor Pentium (R) Dual-Core CPU T4400 2.2GHz, Intel X4500 graphics card and 2 GB of RAM.

SOFTWARE: MGLTools[27] downloaded from http://mgltools.scripps.edu/AutoDock Vina 1.1.2[28] downloaded from http://vina.scripps.edu/, MarvinSketch 15.2.23 downloaded from http://www.chemaxon.com/, UCSF Chimera 1.10.1[29] downloaded from http://www.cgl.ucsf.edu/chimera/.

MOLECULAR MODELING

LIGAND PREPARATION.

The two dimension structure of Tacrine, 22 and 31 were sketched with MavinSketch. And then, the two dimension structures were converted to 3 dimension use PRODRG2 Server[30]. The ligand structures were saved as PDB format. Then, the MGLTools was applied to add Gasteiger charges to ligands and saved as PDBQT file.

PROTEIN MOLECULE PREPARATION

The protein of AChE (PDB ID:1ACJ)[31] binding with THA (Tacrine) was applied to docking studies. The crystal structure of AChE was downloads from Protein Databank (http:// http://www.rcsb.org/)[32].The protein was modified by UCSF Chimera. The waters and co-crystallized ligands were deleted. Then hydrogens and Gasteiger charges were added by MGLToolsand saved as PDBQT file.

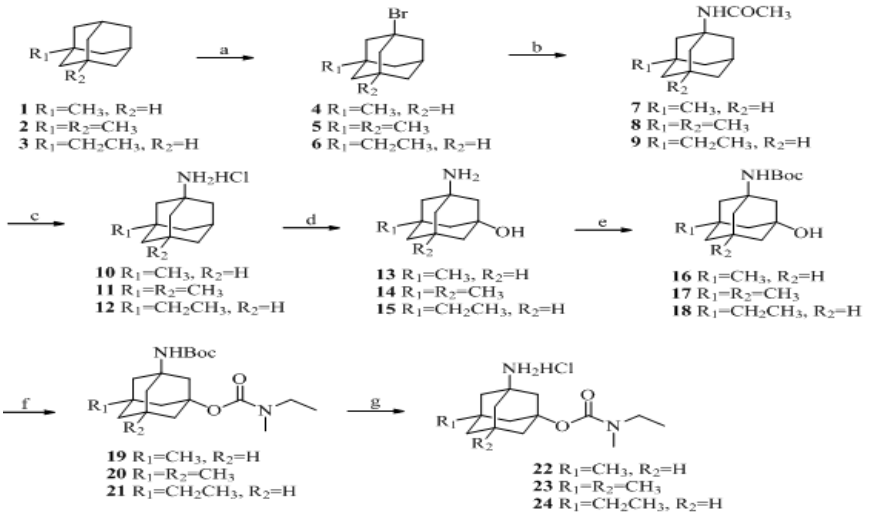
AUTODOCK VINA PARAMETERS SETTING

The search space of AChE was set as center_x = 4.636 center_y = 70.06, center_z = 65.935, size_x = 22.5, size_y = 22.5, size_z = 22.5 and other parameters were set as default.

RESULTS AND DISSCUSION

CHEMICAL SYNTHESIS

The role of amino group in chemical structure of memantine is helping to penetrate the blood-brain barrier and bind to the NMDAR, therefore the amino group was reserved in new designed molecules[33]. To evaluate the effects of the side chains of the memantine cycle, we designed and synthesized compounds **22-24**, with 1-metnyl, 1, 3-dimethyl and 1-ethyl group on the memantine moiety. The synthesis of compounds **22-24** is similar and showed in **Scheme 1.** Commercially available methyladamantane, or the 1, 3-dimethyladamantane,or the ethyladamantane were brominated with Br_2, then undergone Ritter reaction by treatment with concentrated sulfuric acid and acetonitrile to afford acetamide compounds **7-9**, respectively[34,35]. After hydrolyzed, the amideswere obtained as intermediates. Then salification of these amides with hydrogen chloride obtained salts **10-12**. These adamantanamine hydrochloride derivaties **10-12** were reacted with a mixture of nitric acid and sulfuric acid firstly, then hydroxylated by NaOH to afford compounds **13-15**.[36] With a tert-butyloxycarbonyl protecting the amino group, the alcohols **16-18** were condensed with N-ethyl-N-methyl carbamoylchloride to obtain compounds **19-21**. The BOC protective group was removed by treatment with anhydrous hydrogen chloride in ethyl acetate, affording the desired **molecules 22-24**.



Scheme 1. Synthesis of compounds 22-24.

Reagents and conditions: (a) Br_2, reflux, 4 h; rt, overnight; (b) CH_3CN, H_2SO_4, 12 h; (c) diethylene glycol, NaOH, 170 ^oC, 15 h; HCl, EA; (d) HNO_3, H_2SO_4, 0 ^oC, 2 h; rt, 30 h;

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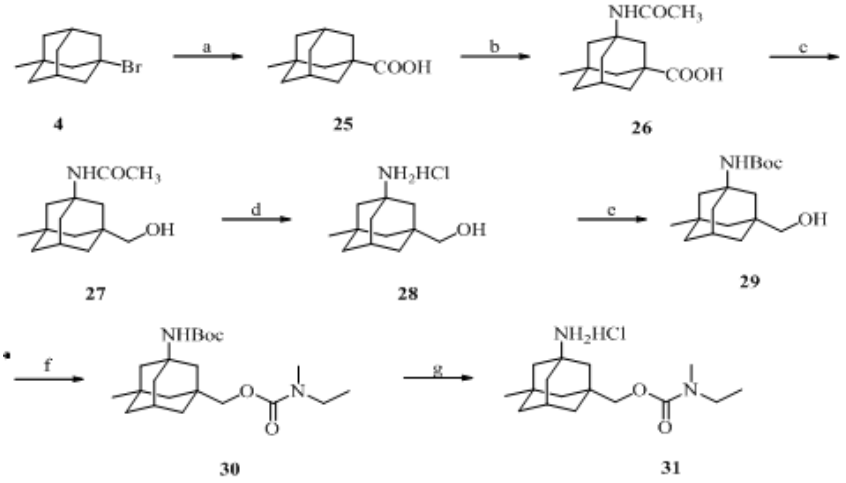
H_2O, NaOH; (e) THF, TEA, $(Boc)_2O$, DMAP, 5 h; (f) NaH, THF, reflux, 2-3 h; MeEtNCOCl, 2 h; (g) HCl, ether, rt, 0.5-1 h.

To demonstrate the influence of the space between the carbamate group and memantine moiety, compound **31** which was memantin linked with carbamate groupby methylene, was designed. It was synthesized as illustrated in **Scheme 2.** Firstly, 3-bromo-1-methyl adamantine was undergone Koch-Haff reaction by treatment with concentrated sulfuric acid and formic acid to afford adamantanecarboxylic acid compound **25**.[37] After an amidation on adamantane for compound 25, compound **26** was achieved. Then it was reduced by sodium borohydrideto give alcohol **27**.[38,39] Hydrolyzation the acetamide group of compound **27** get the amine as a hydrochloric acid salt **28**. Protected the amino group of **28** with a tert-butyloxycarbonyl (BOC group), the alcohol **29** wasesterified by N-ethyl-N-methyl carbamoylchloride to give compound **30**. Finally, cleavage of the BOC group under acidcondition afforded compound **31**.

Scheme 2. Synthesis of compound 31.



Reagents and conditions: **(a)** HCOOH, n-Hexane, H_2SO_4; **(b)** CH_3CN, H_2SO_4; **(c)** $ClCOOC_2H_5$, TEA, $NaBH_4$; **(d)** NaOH, diethylene glycol, 170 ^oC, 15 h; **(e)** THF, $(BOC)_2O$, TEA, DMAP, 5 h; **(f)** MeEtNCOCl, NaH, THF, reflux; **(g)** HCl, ether, rt, 0.5-1 h.

BIOLOGICAL EVALUATION

IN VITRO NEUROPROTECTION EFFECTS AGAINST GLUTAMATE–INDUCED EXCITOTOXICITY ON CGNS AND CNS

Most of neurodegeneration disorders accompanied with various degrees of neuronal loss, while glutamate-induced excitotoxicity was perceived to playing an important role in the process of neuronal losing[40]. Memantine specifically binds to the excessive, primarily extrasynaptic NMDA receptors and blocks the abnormal neuronal electrophysiological signals transmitting. However, it has much less effect on NMDAR-mediated physiological synaptic transmission[41,42]. Therefore, we evaluated whether these new memantine derivatives prevent glutamate-induced excitotoxicity in primary CGNs **(Fig. 4)**. At 8 days *in vitro* culture (DIV 8), CGNs were pretreated with different concentrations (0.1, 1, 10 and 100 μM) of compounds **22, 23, 24, 31 and** memantine for 2 hours, respectively. Then GCNs were exposed to 200 μM glutamate for another 24 hours. The cell viability was measured by MTT assay. As showing **Fig. 4**, all the new compounds didn't prevented glutamate-induced excitotoxicity in CGNs at a relative lower concentration (0.1-1 μM). When the concentrations of compounds were up to 10-100 μM, the viability of cells pretreated with compounds **22, 23** and **24** showed a decrease. Meanwhile, compound 31significantly increased the cell viability against glutamate-induced CGNs death ($p<0.05$ for 10 μM, $p<0.001$ for 100 μM). Memantine significantly protected CGNs from glutamate-induced excitotoxicity at the concentration of 1-100 μM ($p<0.001$). The protection of compound **31** was lower than memantine ($p<0.001$). These data indicated that the carbamate group of the new compounds attenuated the NMDAR antagonism activity of memantine nucleus.

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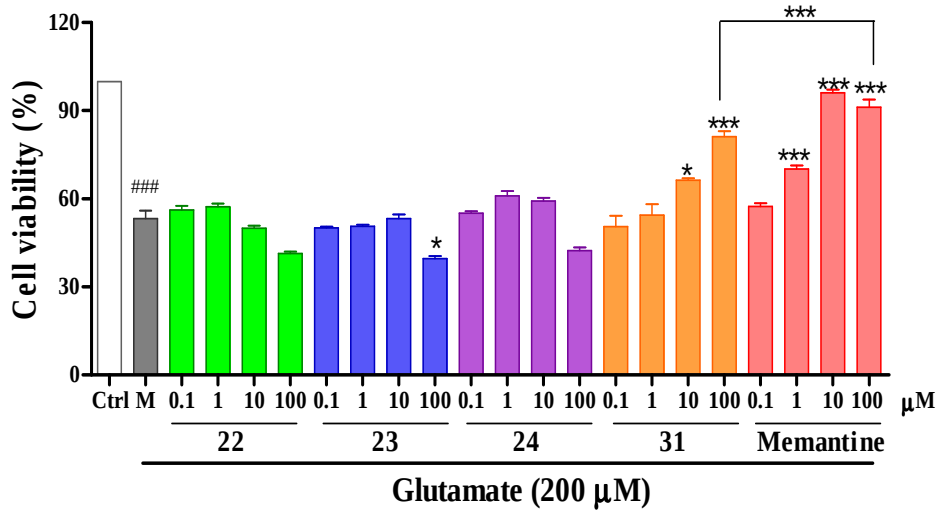

Figure 4. Protective effect against glutamate-induced excitotoxicity on cerebellar granular neurons.

We also evaluated the neuroprotective effect of these new compounds on primary CNs **(Fig. 5).** The results showed that only compound **31** at a high concentration (100 µM) could prevent glutamate-induced cell viability reduction (*p<0.001*), the other three compounds **22, 23** and **24** couldn't protect neurons against excitotoxicity. Memantine significantly increased the cell viability when the concentration was higher than 1 µM (*p<0.001*), and the protective effect is higher than compound **31** with a statistical significance (*p<0.001*).

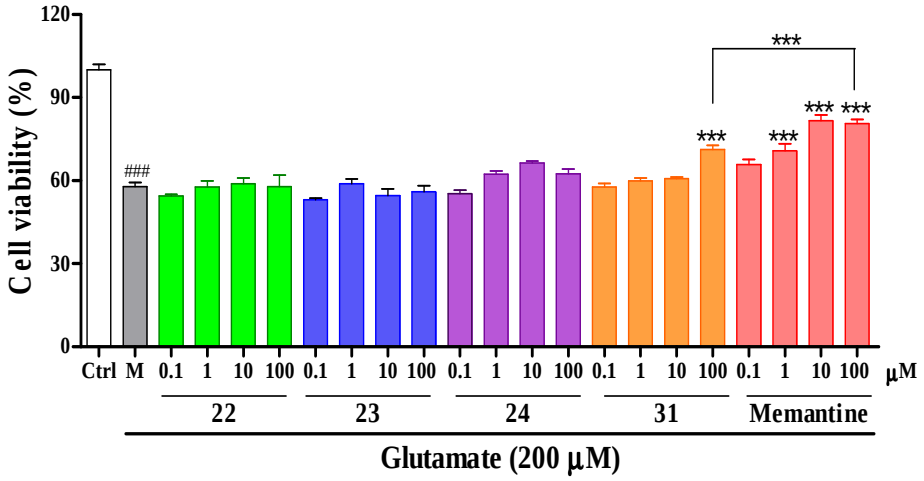

Figure 5. Protective effect against glutamate-induced excitotoxicity on E16-18 primary cortical.

We demonstrated that compounds **22, 23** and **24** had no NMDAR inhibitory activity, however, compound **31** had a significant neuroprotection against glutamate-induced neurotoxicity at a high concentration. Considering the chemical structures of these compounds, it indicated that the length of the carbon chain on the carbamate group is helpfulfor these new compounds to bind to NMDAR. The length of the carbon chain increased the elastic and decreased the steric hindrance around the memantin moiety. On the other hand, the length of the carbon chain also improved the lipid-water partition coefficient of **31** and increased the binding capacity. The NMDAR binding activity of compound **22** (5-methyl-), compound **23** (5, 7- dimethyl-), compound **24** (5-

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ethyl-) has no significant difference. These data indicated that carbamateon the amantadine cycle reduce their inhibition activity for NMDAR.

The protection effects of these new compounds against glutamate-induced excitotoxicity both on CGNs and primary CNs were low as same. There are possible two reasons responsible for the decrease of NMDAR inhibitor activity. The introduction of carbamate group to the memantine moiety increases the steric hindrance of new compounds. It is more difficult to bind to the binding site of the channel of NMDAR comparing with memantine. Moreover, among the tested five compounds, the cLogP of memantine is the maximum, compound **31** is on the second **(Table 1).** The order of the cLogP was accordance with the NMDAR antagonism. These data suggested that the lipophilicity played an important role in the activity of NMDAR inhibition of compounds. It is necessary to study further in order to reveal which reason influence more on the activity.

Table 1.The cLogP of new compounds and Memantine.

Compound	cLog p
22	1.05
23	1.52
24	1.46
31	1.76
Memantine	2.11

AChE INHIBITING ACTIVITY *IN VITRO*

One of hallmarks of AD pathology is cholinergic system impairments in neuronal system, which decreased the cholinergic neurotransmitters in neocortex, hippocampus and selected thalamic nuclei[5]. Cholinesterase inhibitors have a positive effect on cognition, psychiatric symptoms, and global function[43,44]. Previous studies revealed that by covalently carbamylating the serine residue within the active site gorge, the carbamate moiety is thought to be greatly responsible for AChE inhibitoreffect[17,45]. Therefore, the AChE inhibitory effects of new compounds bearing carbamate moieties were tested. As showed in **Fig. 6**, tacrine, the first generation of AChE inhibitor, shows a nearly 45% of AChE inhibition at the concentration of 1 μM. The new compounds, **22-24** and **31**, with carbamate moiety show a concentration-dependent moderate inhibition on AChE at the concentration of 1-100 μM (about 20%). Compound **31**, with a methylene linked the carbamate group and the memantine moiety, didn't showed an advantage than compounds **22-24**. These results indicated that the lengthening of the carbon chain linked the memantine moiety and the carbamate group may be not influence the AChE antagonism. Rivastigmine, with carbamate group on the phenyl cycle, had excellent AChE inhibition. The IC_{50} of it is low to 0.0043 μM[46]. In fact, the "active site gorge" of AChE, a deep and narrow gorge, is its remarkable features. At the gorge mouth, a peripheral site exists. Fourteen aromatic residues lined asubstantial portion of the surface of the gorge (~40%)[45].

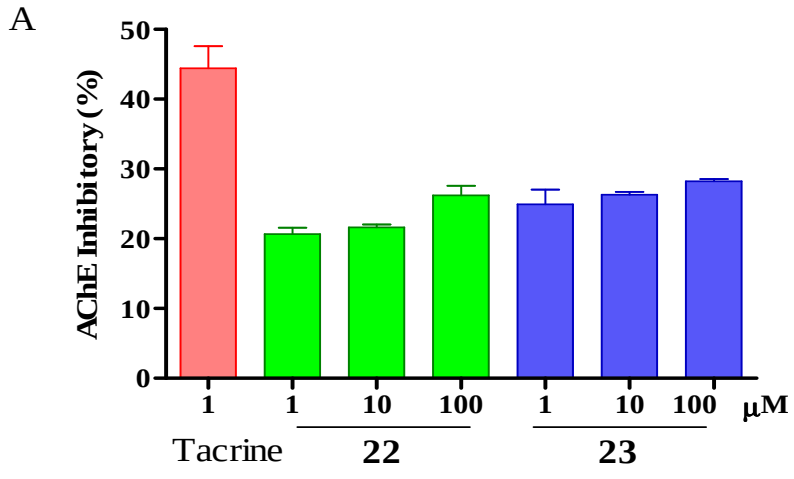

Figure 6. *In vitro* inhibitory effects of new compounds to AChE. **(A)** The effects of compounds 22 and 23; **(B)** The effects of compounds 24 and 31.

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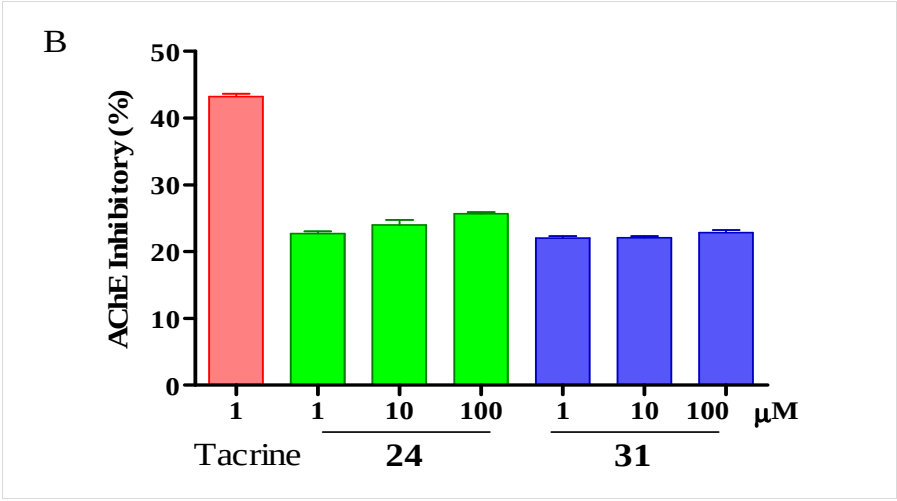
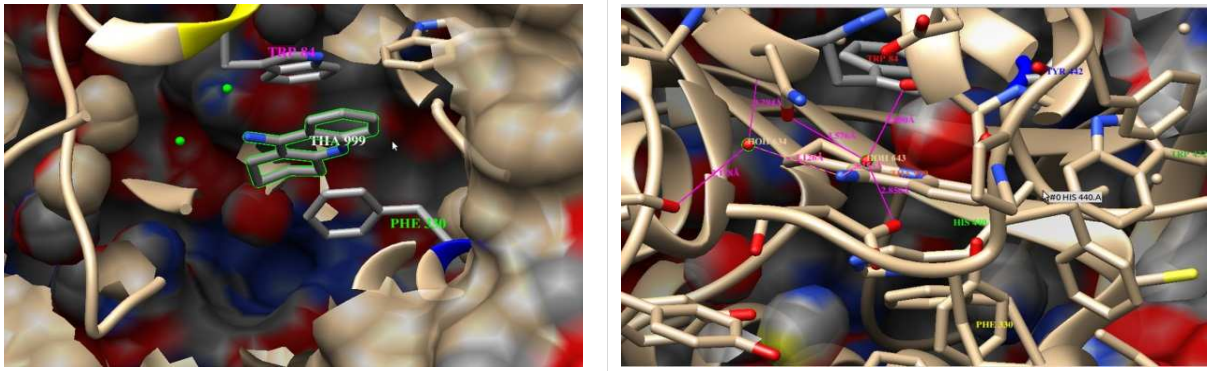


Molecular docking was used to reveal differences of these compounds binding to AChE. The AChE crystal structure (PDB ID: 1acj), which ligand with an inhibitor of THA (tacrine binding to AChE site), was chosen as docking protein. In the co-crystal structure, tactine inserted into the active-site gorge of AChE. The position is in the middle of Trp 84 and Phe 330. 3-Phenyl and pyrazole rings were stacked against to the indole ring of Trp 84 and forms π-π interactions **(Fig. 7A)**. The nitrogen in the pyrazole ring of THA forms a hydrogen-bonding to the main-chain carbonyl oxygen of His-440 (3.1Å). In addition, the amino group of THA formed a hydrogen bond to two solvent water molecules in the active site of AChE (HOH 634, 3.126Å; HOH 643, 3.155Å) **(Fig. 7B)**[31].



(A) **(B)**

Figure 7. The interactions of THA (tacrin) with site gorge of AChE (PDB ID:1acj) **(A),** π-π interactions of THA with AChE. **(B),** Hydrogen bond interactions of THA with AChE.

The docking result was displayed on **Table 2** and top-scored docking poses of tacrine, **22** and **31** were showed on the **(Fig. 8).** Similarly, the top-scored docking pose of tacrine was in the same position with THA and showed identical interactions with THA. While, the top-scored docking poses of compound **22** and **31** showed a different type of binding with AChE residues **(Fig. 9)**. Importantly, compound **22** formed four H-bonds with AChE, with the residuals Try 121 (3.165 Å), Ser 122 (2.702 Å), Glu 199 (3.357 Å) and a water molecule HOH 634 (2.068 Å). Compound 31 also had four H-bonds interacting with main chain of Ser 122 (2.900 Å), Ser 200 (3.267 Å) and tow solvent water molecule HOH 607 (1.862 Å), HOH 643 (3.102 Å). However the two compounds didn't form π-π interactions with Trp 84 and Phe 330. The molecular results of compounds **23** and **24** were similar to the compound **22** (the results didn't show). Lacking the π-π interactions may be the most important reason of lower affinity toward AChE for these memantine derivatives than tacrine.

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Table 2. The Docking Result of Tacrine, Compounds 22 and 31.

Ligand	Binding energy (kcal/mol)	H-bond interaction
Tacrine	-9.1	3
22	-8.6	4
31	-8.7	4

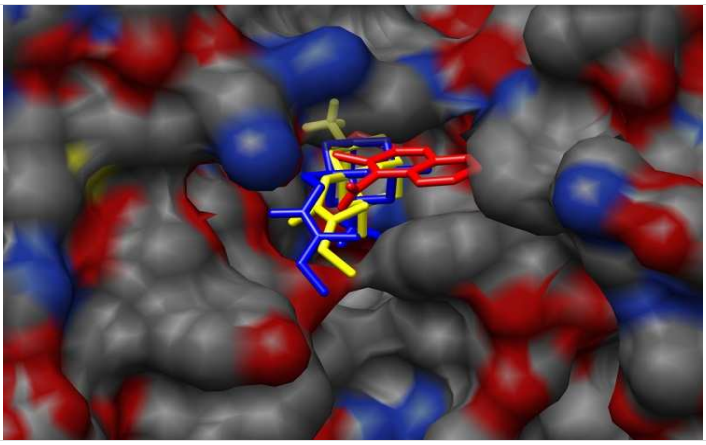
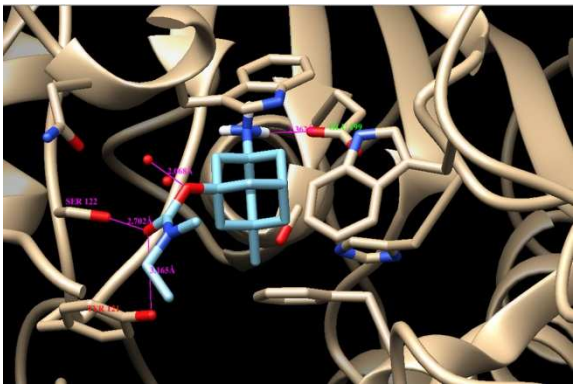
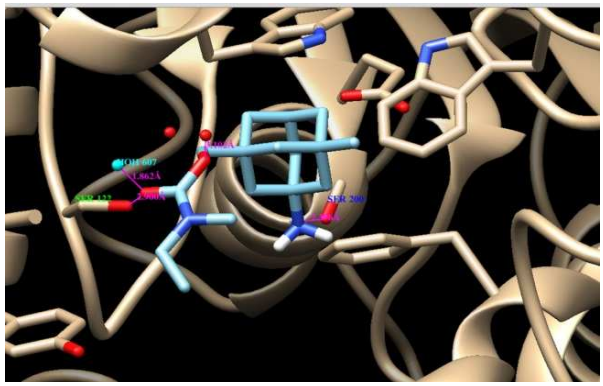


Figure 8. The top-scored docking poses of tacrine (red), 22 (yellow) and 31 (blue).



(A)



(B)

Figure 9. Hydrogen bond interactions of 22 and 31 with AChE. (A), Compound **22** forms four Hydrogen bonds with the residuals Try 121 (3.165 Å), Ser 122 (2.702 Å), Glu 199 (3.357 Å) and a water molecule HOH 634 (2.068 Å). **(B),** Compound **31** forms four Hydrogen bonds interacting with main chain of Ser 122 (2.900 Å), Ser 200 (3.267 Å) and tow solvent water molecule HOH 607 (1.862 Å), HOH 643 (3.102 Å).

CONCLUSION

We designed and synthesized four new compounds which were memantine derivatives with carbamate moiety, and hoped they had the function of NMDAR and AChE inhibition. Of them only compound **31** showed NMDAR antagonism at a high concentration (10-100 μM), the other three compounds **22, 23, 24** has no NMDAR inhibition. On the other hand, all four new compounds showed a moderate AChE inhibition, and compound **31** didn't show superiority than the other three compounds. According to the results, lengthening the carbon chain can reduce the steric interaction of the two moieties, and increase the NMDAR antagonism, but it can't increase the AChE inhibition. The molecular docking revealed that the structure which can form π-π interactions with AChE is important for AChE inhibition. So, linked the carbamate group and the memantine moiety with an electron-riched plane-conjugated structure maybe achieve the bifunctional compounds with NMDA antagonism and AChE inhibition.

EXPERIMENTAL SECTION

Unless otherwise noted, all chemicals and solvents were purchased as reagent grade from commercial

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suppliers and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 F254 pre-coated glass plates. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker Avance 300 spectrometer. Chemical shifts are reported as δ value in parts per million (ppm) relative to tetramethylsilan (TMS) as internal standard. Coupling constants are reported in units of Hertz [Hz]. Low-resolution mass spectra (MS) were recorded with a Waters Quattro premier XE mass spectrometer.

Compounds **4-6** were synthesized using a procedure similar to that described below for the synthesis of 4.Compound **4-6** have been previously described in literature[34].

1-Bromo-3-methyladamantane (4).
1-methyladamantane (1.64 g, 10 mmol)and Br2(20 mL, 40 mmol) were added in 100 mL round-bottom flask. After refluxed for 4 h under N_2 atmosphere, the mixture cooled to room temperature and stirred overnight. 40 mL of CCl_4 was added and the mixture was poured into 100 mL of ice-water. With vigorous stirring, sufficient Na_2SO_3 was added to decolorize the product. The CCl_4 layer was separated and the aqueous layer was extracted with 3 × 30 mL CCl_4. The extractions were combined and washed with 50 mL 5% $NaHCO_3$ and dried over Na_2SO_4. Evaporation of solvent to yield yellow liquid. Separated with Silica gel column with petroleum ether as the eluent to give Compounds4 as colorless oil, yield 87%.

1-Bromo-3, 5-dimethyladamantane(5). Colorless oil, yield 85%.

1-Bromo-3-ethyladamantane(6). Colorless oil, yield 87%.

The general synthesis of compounds **7-9** were according to a procedure similar to that described below for the synthesis of **7**.[47] Compound **7** and **9** have been previously described in literature[34], compound **8** has been described in literature[48].

N-Acetyl-3-methyl-1-aminoadamantane(7).
Compound **4**(6.9 g, 30 mmol) and CH_3CN (26 mL, 50 mmol) was added and mixed in 250 mL two-neck flask. Concentrated H_2SO_4 (47 mL, 86 mmol) was added dropwise into the mixture. The dropping speed was about 1 drop per second. The reaction liquid stirred for 12 h in room temperature after addition of the H_2SO_4 was completed. Poured the yellow viscous liquid into 300 mL ice-water and vigorous stirred, and then stationed for 12 h. Separated the precipitate with vacuum suction filtration and washed the white solid product with water to afford compound 7 as a White solid, yield 90%.

N-acetyl-3, 5-dimethyl-1-aminoadamantane(8). White solid, yield 88%.

N-Acetyl-3-ethyl-1-aminoadamantane(9). White solid, yield 83%.

Compounds **10-12** were synthesized using a procedure similar to that described below for the synthesis of **10**.[39] Compound **7** and **9** have been previously described in literature[34], compound **8** has been described in literature[48].

3-Methyl-1-aminoadamantane(10). Compound 7(620 mg, 3 mmol) and NaOH (1.5 g, 37.5 mmol) were added in 10 mL diethylene glycol and heated to 175 °C for 15 h. After cooled to room temperature, 20 mL of ice-water was added and the mixture was extracted with 4 × 20 mL ethyl acetate. The combined organic layer was washed with 20 mL brine and water in turn. After dried over Na_2SO_4 and evaporate the solution, yellow crude oil was obtained. 5 mL of ethyl acetate dissolved the oil and HCl saturated ethyl acetate was added in the solution. Vacuum suction filtration to separate the precipitates and washed with ethyl acetate to get pure product. Dried the white solid to get compounds 10 as a white solid, yield 67%.

3,5-Dimethyl-1-aminoadamantane hydrochloride (11). White solid, yield 63%.

3-Ethyl-1-aminoadamantane hydrochloride(12). White solid, yield64%.

Compounds **13-15** were synthesized using a procedure similar to that described below for the synthesis of **13**.[36]

1-Amino-3-methyl-5-hydroxyadamatane hydrocloride(13).HNO_3 (1 mL) and H2SO4 (9.4 mL) were added and mixed in a 50 mL flask in 0 °C. Compound 10 (8.0 g, 40 mmol) was added in small batches and stirred for 2 h in ice bath. The mixture was reacted in room temperature for another 30 h. The pale yellow liquid was poured into 10 g ice-water and stirred for 30 min in ice bath. After removed the bath, NaOH (18 g, 450 mmol) was added into the yellow-bull liquid in batches. Keep the temperature below 80 °C. Ethyl acetate 100 mL was added into the solid and stirred for 1 h. Vacuum filtration and 3 × 20 mL ethyl acetate washed the residue. The combined filtrate was washed with 50 mL brine and water and dried over Na_2SO_4. Evaporated the solution to get the white solid crude product. Recrystallization with ethyl acetate to afforded compound 13 as a white solid.

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Yield 65%. ESI-MS: *m/z* 182.2 ([M+H]$^+$). ^{1}H-NMR (DMSO-*d*6, ppm): 0.86 (s, 3 H, CH_3), 1.18-1.32 (m, 4 H, CH_2), 1.45 (s, 4 H, CH_2), 1.58-1.72 (m, 4 H, CH_2), 2.20 (s, 1 H, CH), 4.80 (s, 1 H, OH), 8.39 (s, 3 H, NH_3Cl), ^{13}C-NMR (DMSO-*d*6, ppm): 29.67, 30.10, 33.81, 38.58, 41.70, 43.12, 46.07, 47.52, 50.97, 53.86, 68.07.

1-Amino-3,5-dimethyl-7-hydroxyadamatane hydrocloride (14). Yield 65%. The MS, ^{1}H-NMR and ^{13}C-NMR were identical with the previous paper[35].

1-Amino-3-ethyl-5-hydroxyadamatane(15). Yield 67%. ESI-MS: *m/z* 196.1 ([M + H]$^+$). ^{1}H-NMR (DMSO-*d*6, ppm): 0.76 (t, 3 H, J = 7.6 Hz, CH_3), 1.11-1.22 (m, 6 H, CH_2), 1.39-1.51 (m, 4 H, CH_2), 1.57-1.69 (m, 4 H, CH_2), 2.11 (m, 1 H, CH), 4.40 (s, 1 H, OH), 5.42 (s, 1 H, NH_2), ^{13}C-NMR (DMSO-*d*6, ppm): 29.92, 34.80, 36.61, 39.00, 43.45, 43.75, 47.90, 48.33, 53.91, 68.17.

The method described in a previous studies was used to synthesis compound **16-18**.[35] The typical synthesis method was described below for prepare compound **16**.

1-Tert-butoxycarbonylamino-3-methyl-5-hydroxyadamamtane (16). Compound **12**(540 mg, 3 mmol) was suspended in 10 mL tetrahydrofuran. Triethylamine (600 mg, 6 mmol), di-tert-butyl dicarbonate (980 mg, 4.5 mmol) and 4-dimethylaminopyridine 10 mg were added sequentially. The mixture was reacted for 5 h at room temperature. The completion of the reaction was monitored using conventional TLC analysis. 10 mL of saturated NH_4Cl solution was added and the solvent was removed in vacuo. The aqueous residue was extracted with 4 × 20 mL ethyl acetate. The organic solution was washed with 30 mL brine and water and dried over Na_2SO_4. Evaporated ethyl actetate to get colorless oily product. Separated with Silica gel column (petroleum ether: ethyl acetate = 3:1) to afford compound 16as a white solid, yield 87%. ESI-MS: *m/z* 282.3 ([M+H]$^+$). ^{1}H-NMR (DMSO-*d*6, ppm): 0.82 (s, 3 H, CH_3), 1.17-1.27 (m, 4 H, CH_2), 1.36 (s, 9 H, 3×CH_3), 1.39-1.50 (m, 4 H, CH_2), 1.63-1.66 (m, 4 H, CH_2), 2.10 (s, 1 H, CH), 4.04 (s, 1 H, OH), 8.39 (s, ^{1}H, NH), ^{13}C-NMR(DMSO-*d*6, ppm): 28.76, 30.19, 30.48, 33.73, 42.48, 43.89, 47.47, 48.91, 51.72, 53.07, 68.37, 77.54, 153.97.

1-Tert-butoxycarbonylamino-3, 5-dimethyl-7-hydroxyadamamtane(17). White solid, yield 87%. The compound 17 has been described in the previous paper[35].

1-Tert-butoxycarbonylamino-3-ethyl-5-hydroxyadamamtane(18). White solid, yield 84%. ESI-MS: *m/z* 295.4 ([M + H]$^+$). ^{1}H-NMR (DMSO-*d*6, ppm): 0.75 (t, 3 H, J = 7.6 Hz, CH_3), 1.11-1.22 (m, 6 H, CH_2), 1.36 (s, 9 H, 3×CH_3), 1.44 (d, 4 H, CH_2), 1.66 (d, 4 H, CH_2), 2.12 (m, 1 H, CH), 4.43 (s, 1 H, OH), 6.45 (s, 1 H, NH), ^{13}C-NMR(DMSO-*d*6, ppm): 7.64, 28.77, 30.31, 35.27, 36.44, 39.88, 44.27, 45.03, 49.02, 49.31, 53.07, 68.41, 77.51, 154.37.

Compounds **19-21** were synthesized using a procedure similar to that described below for the synthesis of **19**.

3-Tert-butoxycarbonylamino-5-methyladamantan-1-yl-ethyl(methyl)carbamate(19). Compound **16**(560 mg, 2 mmol) were dissoloved in 30 mL anhydrous THF. 60% NaH (96 mg, 2.4 mmol) was added to the solution. The suspension solution was refluxed for 2-3 h under the N_2 atmosphere. N-ethyl-N-methyl carbamoylchloride (480 mg, 4 mmol) was syringed in and refluxed for another 2 h. After the reaction completed, 2 mL H2O was added dropwise and 20 mL more H_2O was added. Evaporated the THF and the residual solvent was extracted with ethyl acetate (40 mL×4). Combined the ethyl acetate and washed with 30 mL brine and water and dried over Na_2SO_4. Evaporated ethyl actetate to get colorless oily product. Separated with Silica gel column (petroleum ether: ethyl acetate = 3:1) to afford compound 19 as a colorless oil. Yield 63%. ESI-MS: *m/z* 367.6 ([M+H]$^+$). ^{1}H-NMR (DMSO-*d*6, ppm): 0.85 (s, 3 H, CH_3), 1.00 (t, 3 H, *J* = 7.0 Hz, CH_3), 1.26 (d, 2 H, CH_2), 1.36 (s, 9 H, 3×CH_3), 1.57-1.77 (m, 6 H, CH_2), 1.82-19.2 (m, 2 H, CH_2), 2.12 (s, 2 H, CH_2), 2.17 (s, 1 H, CH), 2.74 (s, 3 H, CH_3), 3.15 (q, 2 H, J = 7.0 Hz, CH_2), 6.56 (s, 1 H, NH), ^{13}C-NMR (DMSO-*d*6, ppm): 28.76, 29.65, 33.66, 33.90, 42.29, 44.09, 46.70, 46.86, 49.65, 53.47, 77.65, 79.95, 154.28, 197.13.

3-Tert-Butoxycarbonylamino-5,7-dimethyladamantan-1-yl-ethyl(methyl)-carbamate (20). Yield 45%. ESI-MS: *m/z* 398.6 ([M+H2O]$^+$). ^{1}H-NMR (DMSO-*d*6, ppm): 0.87 (s, 6 H, 2×CH_3), 1.00 (t, 3 H, *J* = 6.6 Hz, CH_3), 1.05 (s, 2 H, CH_2), 1.36 (m, 11 H, 3×CH_3, CH_2), 1.61 (m, 6 H, CH_2), 2.07 (s, 2 H, CH_2), 2.74 (s, 3 H, CH_3), 3.16 (q, 2 H, *J* = 6.6 Hz, CH_2), 6.57 (s, 1 H, NH),^{13}C-NMR(DMSO-*d*6, ppm): 28.76, 29.34, 30.63, 31.86, 36.23, 37.69, 43.02, 45.38, 51.25, 73.64, 77.64, 154.42, 155.80.

3-Tert-Butoxycarbonylamino-5-ethyladamantan-1-yl-ethyl(methyl)carbamate (21). Yield 56%. ESI-MS: *m/z* 381.4 ([M + H]$^+$). ^{1}H-NMR (DMSO-*d*6, ppm): 0.76 (t, 3 H, *J* = 7.6 Hz, CH_3), 1.01 (t, 3 H, J = 7.1 Hz, CH_3), 1.18 (q, 2 H, *J* = 7.6 Hz, CH_2), 1.26 (s, 2 H, CH_2), 1.36 (s, 9 H, 3×CH_3), 1.43 (d, 1 H, J = 12.2 Hz, CH2), 1.64 (dd, 4 H, J = 19.8 Hz, CH_2), 1,76 (d, 1 H, *J* = 12.2 Hz, CH_2), 1.89 (s, 2 H, CH_2), 2.15 (s, 2 H, CH_2), 2.20 (m, 1 H, CH), 2.74 (s, 3

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H, CH_3), 3.16 (t, 2 H, *J* = 7.1 Hz, CH_2), 6.56 (s, 1 H, NH), ^{13}C-NMR(DMSO-*d*6, ppm):7.64, 28.76, 30.16, 33.63, 35.14, 36.58, 44.98, 45.11, 53.04, 77.69, 79.60, 154.50.

Compounds **22-24** were synthesized using a procedure similar to that described below for the synthesis of **22**.

3-Amino-5-methyladamantane-1-yl-ethyl(methyl)carbamate hydrochloride(22). Compound 19 2 mmol was dissolved in 1 mL ether and 5 mL HCl saturated ether was added and stirred. TLC analysis monitored the completion of the reaction. The precipitate was filtered and washed with ether. Compound **22-24** was afforded as a white solid. Yield 68%. ESI-MS: *m/z* 267.1 ([M+H]$^+$). ^{1}H-NMR (DMSO-*d*6, ppm): 0.91 (s, 3 H, CH_3), 1.01 (t, 3 H, *J* = 7.1 Hz, CH_3), 1.25-1.38 (m, 2 H, CH_2), 1.47-1.56 (t, 2 H, CH_2), 1.62-1.72 (m, 4 H, CH_2), 1.82-1.97 (m, 2 H, CH_2), 2.09-2.20 (m, 2 H, CH_2), 2.29 (s, 1 H, CH), 2.75 (s, 3 H, CH_3), 3.17 (q, 2 H, *J* = 7.1 Hz, CH_2), 8.31 (s, 3 H, NH_3Cl), ^{13}C-NMR(DMSO-*d*6, ppm): 29.43, 29.89, 33.70, 33.97, 38.51, 43.62, 45.94, 47.01, 53.77, 78.48, 154.59.

3-Amino-5, 7-dimethyladamantane-1-yl-ethyl(methyl)carbamate hydrochloride (23). Yield 82%. ESI-MS: *m/z* 281.1 ([M+1]$^+$). ^{1}H-NMR (DMSO-*d*6, ppm): 0.92 (s, 6 H, 2×CH_3), 1.01 (t, 3 H, *J* = 7.2 Hz, CH_3), 1.12 (m, 2 H, CH_2), 1.45 (q, 4 H, *J* = 11.7 Hz, CH_2), 1.67 (dd,4 H, *J* = 21.9 Hz, 11.7 Hz, CH_2), 2.10 (s, 2 H, CH_2), 2.70 (s, 3 H, CH_3), 3.17 (q, 2 H, *J* = 7.2 Hz, CH_2), 8.29 (s,3 H, NH_3Cl).

3-amino-5-ethyladamantane-1-yl-ethyl(methyl)carbamate hydrochloride (24). Yield 85%. ESI-MS: *m/z* 280.9 ([M+H]+). ^{1}H-NMR (DMSO-*d*6, ppm): 0.77 (t, 3 H, J = 7.5 Hz, CH_3), 1.00 (t, 3 H, *J* = 6.9 Hz, CH_3), 1.12-1.30 (m, 6 H, CH_2), 1.39 (q, 2 H, *J* = 11.7 Hz, CH_2), 1.65 (q, 2 H, *J* = 11.7 Hz, CH_2), 1.73-1.83 (m, 2 H, CH_2), 1.87 (m, 2 H, CH_2), 2.15-2.23 (m, 1 H, CH), 2.74 (s, 3 H, CH_3), 3.16 (q, 2 H, *J* = 6.9 Hz, CH_2).

3-Methyl-1-adamantanecarboxylic acid (25).[37] Concentrated H_2SO_4 (20 mL), n-hexane (2 mL) and compound 4 (920 mg, 4 mmol) were sequentially added in a 50 mL flask and cooled to 0-10 °C. Formic acid (1.8 mL, 48 mmol) was added dropwise. The mixture was stirred for 3 h in ice bath. Poured the reaction mixture into 30 mL ice-water and stirred. The precipitate was filtered and washed with water. The solid was dissolve in ethyl acetate. Aqueous NaOH solution was added to alkalize the organic layer to pH=9-10. Separated the aqueous layers and acidify with HCl solution to the pH was about 3. Filtered the precipitate and washed with water and dried to afford compound 25 as a white solid (675 mg, 85% yield). The compound was without further purification. Compound 27has been described in previous paper[49].

3-Acetamido-5-methyladamantane-1-carboxylic acid (26).[38] To a 50 mL reactor was added compound 4 (1.16 g, 6 mmol) and HNO_3 (1.1 mL). The suspension was cooled at 0 °C with a condenser. Concentrated H_2SO_4 (7 mL) was added slowly to the suspension liquid. Once the addition completed, CH_3CN (5 mL, 9.6 mmol) was added at such a rate that the temperature was kept below 10 °C. After the addition of CH_3CN was completed, the mixture was stirred for another 1 h at 0 °C. The yellow viscous solution was poured into 20 mL ice-water and stirred vigorously. And then stationed overnight and the solid was precipitated. The precipitation was filtrated, washed with water and dried to afforded compound **26** as a white solid (1.05 g, 70% yield). The product was without further purification.The MS, ^{1}H NMR were identical with those reported in literature[50].

1-Acetamido-3-methyl-5-hydroxymethyladamantane(27).[35]Triethylamine (2 mL, 14.5 mmol) and ethyl chloroformate (2 mL, 21 mmol) were added sequentially into a suspension of compound 25 (2.71 g, 10.8 mmol) in THF at 0 °C. The mixture was stirred at room temperature for 4 h. The suspension was filtrated and the solid was washed with THF. $NaBH_4$(5 g, 13.2 mmol) was added into the combined THF. Water (2 mL) was added dropwise over 1 h and then 50 mL water was added. The organic solvent was removed in vacuo and the aqueous solution was extracted with 4 × 30 mL ethyl acetate. The extraction was washed with 0.5 N HCl, water and brine and then dried over Na_2SO_4. Evaporated the solvent to afford oily product. Separated with Silica gel column (petroleum ether: ethyl acetate = 1:1) to afford compound 26 as a white solid, yield 65.2%, mp 143.7-144.3 oC.ESI-MS: *m/z* 238.4 [M+H]$^+$. ^{1}H-NMR (DMSO-*d*6, ppm): 0.81 (s, 3H,CH_3), 1.09 (s, 2H), 1.28 (q, 4H, *J* = 12 Hz), 1.57 (m, 4H), 1.73 (s, 3H, $COCH_3$), 1.75 (s, 2H), 2.07 (s, 1H), 3.02 (d, 2H, *J* = 5.5 Hz, CH_2O), 4.36 (t, 1H, *J* = 5.5 Hz, OH), 7.31 (s, 1H, NH), ^{13}C-NMR(DMSO-*d*6, ppm): 24.22, 29.58, 30.75, 31.90, 37.43, 37.85, 42.66, 43.34, 45.64, 48.03, 52.47, 71.39 169.05.

The synthesis of compound **28-31** was similar to the synthesis of compound **13, 16, 19** and **21** respectively.

1-Amino-3-methyl-5-hydroxymethyladamantane hydrochlorid (28). White solid, yield 73.7%. ESI-MS: *m/z* 196.2 ([M +

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H]+). ^{1H}-NMR (DMSO-*d*6, ppm): 0.86 (s, 3 H, CH_3), 1.11 (s, 2 H, CH_2), 1.28 (m, 4 H, CH_2), 1.47 (m, 4 H, CH_2), 1.66 (s, 2 H, CH_2), 2.17 (s, 1 H, CH), 3.05 (d, 2 H, J = 5.5 Hz, CH_2), 4.58 (t, 1 H, *J* = 5.5 Hz, OH), 8.15 (s, 3 H, NH_3Cl), ^{13}C-NMR(DMSO-*d*6, ppm): 29.15, 30.20, 32.03, 37.08, 37.62, 41.63, 42.50, 44.90, 46.79, 52.80, 70.65.

1-Tert-butoxycarbonylamino-3-methyl-5-hydroxymethyladamatane (29). White solid, yield 64%. The MS, ^{1}H-NMR and ^{13}C-NMR were identical with the previous studies[51].

3-Tert-butoxycarbonylamino-5-methyladamantan-1-yl-methylethyl(methyl)carba-mate (30). Colorless oil, yield 73%. ESI-MS: *m/z* 381.6 ([M + H]+). ^{1}H-NMR (DMSO-*d*6, ppm): 0.82 (s, 3 H, CH3), 1.04 (s, 3 H, CH_3), 1.14 (s, 2 H, CH_2), 1.28 (m, 4 H, CH_2), 1.36 (s, 9 H, 3×CH_3), 1.55 (m, 4 H, CH_2), 1.70 (s, 2 H, CH_2), 2.09 (s, 1 H, CH), 2.81 (s, 3 H, CH_3), 3.23 (q, 2 H, CH_2), 3.63 (s, 2 H, CH_2), 6.43 (s, 1 H, NH).

3-Amino-5-methyladamantane-1-yl-methyl-ethyl(methyl)carbamate hydrochloride(31). White solid, yield 71%. ESI-MS: *m/z* 281.6 ([M + H]+). ^{1}H-NMR (DMSO-*d*6, ppm): 0.88 (s, 3 H, CH_3), 1.05 (s, 3 H, CH_3), 1.22 (m, 2 H, CH_2), 1.36 (m, 4 H, CH_2), 1.49 (m, 4 H, CH_2), 1.66 (s, 2 H, CH_2), 2.21 (s, 1 H, CH), 2.81 (s, 3 H, CH_3), 3.24 (q, 2 H, CH_2), 3.71 (s, 2 H, CH_2), 7.96 (s, 3 H, NH_3Cl), ^{13}C-NMR(DMSO-*d*6, ppm):28.91, 30.06, 31.92, 36.36, 36.88, 38.78, 39.52, 41.94, 43.92, 49.76, 70.15, 153.24.

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