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PII: S0960-894X(17)30966-6
DOI: https://doi.org/10.1016/j.bmcl.2017.09.057
Reference: BMCL 25321

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 29 July 2017
Revised Date: 22 September 2017
Accepted Date: 27 September 2017

Please cite this article as: Sang, Z., Wang, K., Wang, H., Wang, H., Ma, Q., Han, X., Ye, M., Yu, L., Liu, W., Design, synthesis and biological evaluation of 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives as multifunctional agents for the treatment of Alzheimer's disease, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: https://doi.org/10.1016/j.bmcl.2017.09.057

Design, synthesis and biological evaluation of 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives as multifunctional agents for the treatment of Alzheimer's disease

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Abstract

A series of 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives was designed, synthesized and evaluated as multi-function inhibitors for the treatment of Alzheimer's disease (AD). The results revealed that compound **TM-3** indicated selective AChE inhibitory potency (*ee*AChE, IC_{50} = 0.69 μM, selective index (SI) = 32.7). Both kinetic analysis of AChE inhibition and molecular modeling study suggested that **TM-3** could simultaneously bind to the catalytic active site and peripheral anionic site of AChE. And **TM-3** was also a highly selective MAO-B inhibitor (IC_{50} = 6.8 μM). Moreover, **TM-3** could act as antioxidant (ORAC value was1.5*eq*) and neuroprotectant, as well as a selective metal chelating agent. More interestingly, compound **TM-3** could cross the blood-brain barrier (BBB) *in vitro* and abided by Lipinski's rule of five. Therefore, compound **TM-3**, a promising multi-targeted active molecule, offers an attractive

starting point for further lead optimization in the drug-discovery process against AD.

Keywords: Alzheimer's disease; 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives; designed; synthesized; multi-target inhibitor; blood–brain barrier.

Abbreviations: AD, Alzheimer's disease; AChEIs, acetylcholinesterase inhibitors; NMDA, *N*-methyl-*D*-aspartate; Aβ, β-amyloid; τ, tau protein; ACh, acetylcholine; MTDLs, multi-target-directed ligands; MAO-B, monoamine oxidase B; MAO-A, monoamine oxidase A; BuChE, butyrylcholinesterase; *Ee*AChE, *Electrophorus electricus* AChE; eqBuChE, *equine serum* BuChE; PAS, peripheral anionic site; CAS, catalytic active site; ORAC-FL, oxygen radical absorbance capacity by fluorescein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; PAMPA-BBB, parallel artificial membrane assay for the blood- brain barrier; SD, standard deviation; MW, molecular weight; TPSA, topological polar surface area; ADME, absorption, distribution, metabolism, excretion.

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by deterioration of memory, language skills, and other cognitive impairments in elder people. This situation intensely influences the patient's social life and activity. It is evaluated by World Alzheimer's Report that more than 35 million people suffered from AD in 2015, and this number will be double by 2030 and approximately triple to 131 million by 2050.[1] Current clinical treatment of AD is mainly focused on controlling symptoms by providing temporary improvement. To date, there are four acetylcholinesterase inhibitors (AChEIs), named donepezil, tacrine, rivastigmine, galantamine and one *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine have been approved for the treatment of AD. Unfortunately, these drugs are just palliative treatment and do not address the molecular mechanisms that underlie the pathogenic processes due to the multifactorial nature of AD.[2]

The etiology of AD is not completely known, but several characteristic pathological features such as β-amyloid (Aβ) deposits, tau protein (τ) aggregation, oxidative stress, decreased level of acetylcholine (ACh), neuroinflammation, and dyshomeostasis of biometals have been thought to play significant roles in the pathogenesis of AD.[3] In this regard, one single drug that acts on a specific target to produce the desired clinical effects might not be suitable for the complex nature of AD, the development of multitarget-directed ligands (MTDLs), i.e., single chemical entities able to hit different targets involved in the cascade of AD pathological events, to act as multifunctional agents to treat this disease has been applied by many research groups, and the results obtained have been encouraging and convince researchers that MTDLs might present the best pharmacological option for tackling the multifactorial nature of AD and for halting the progression of the disease.[4-6] Several MTDLs candidate drugs with disease modifying potential are now in the pipeline and have reached testing stage in clinical trials.[7] Therefore, the discovery of a lead compound that can modulate multi-factors simultaneously is a crucial step in the search for a candidate for the clinical treatment of AD.

2-Acetyl-5-alkoxyphenol analog **DDDT-2d**, is a potent and selective MAO-B inhibitor, the IC_{50} values of MAO-B was 2.9 nM, as well as 17000-flod selective for

MAO-B over the MAO-A.[8] Recent studies shows that the selective MAO-B inhibitor has been shown to significantly improve learning and memory deficits in animal models associated with AD and to slow the disease progression in AD patients,[9] and seem to be an important treatment of AD.[10] So, it is appropriate for the design of therapies for neurodegenerative disorders such as Alzheimer's disease. Recently, our group has reported the synthesis of scutellarein-*O*-alkylamine derivatives and discovered representative **EJMC-16d** as a potential multifunctional agent for the treatment, and the alkylamine side chain is required pharmacophore for the AChE inhibition.[11] Therefore, in this paper, 2-acetylphenol was selected to combine with different length alkylamine fragment to design a series of novel 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives, to evaluate whether these novel molecules might possess more potency in various multifunctional activities.

In this study, we report the study of the design, synthesis and evaluation of a series of 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives based on MTDLs (**Figure 1**). They were found to show potentially applicable biological activities, including inhibition of ChEs (AChE and BuChE) and MAOs (MAO-A and MAO-B), antioxidant properties, neuroprotective effects, and metal chelation.

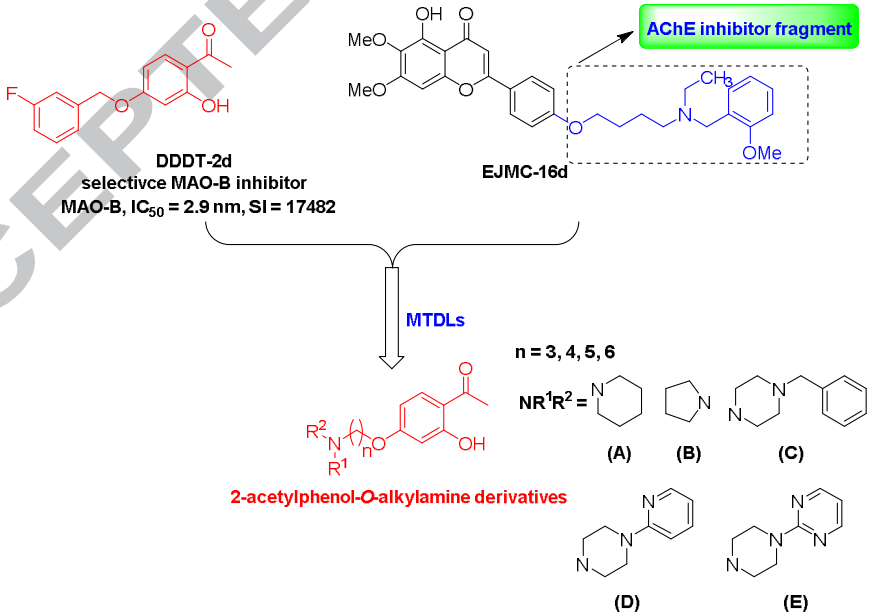
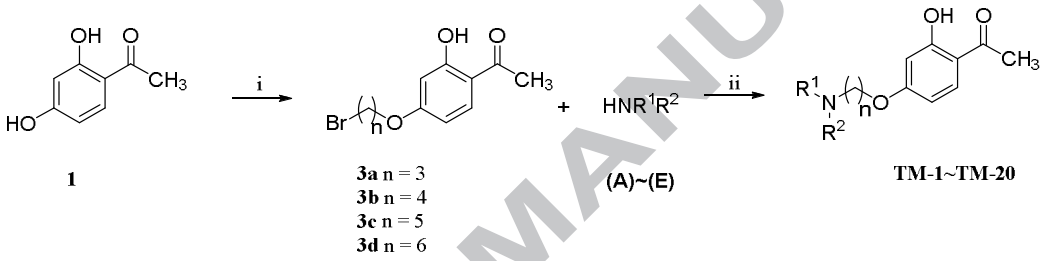


Figure 1. Design strategy for the 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives.

Total 20 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives were synthesized in good yields. The synthetic pathway of these target compounds was outlined in **Scheme 1**. Briefly, the starting material **1** was treated with excessive amounts of 1,3-dibromopropane (**2a**), 1,4-dibromobutane (**2b**), 1,5 –dibromopentane (**2c**) or 1,6-dibromohexane (**2d**) in the presence of K_2CO_3 in CH_3CN at 60–65°C to obtain the intermediates **3a~3d**. Subsequently, the target compounds **TM-1~TM-20** were got by the reaction of intermediates **3a~3d** with secondary amines (**A~E**) in the presence of K_2CO_3 in anhydrous CH_3CN at 60–65°C. All target compounds were purified by chromatography, and the analytical and spectroscopic data confirmed their structures, as detailed in the experimental section.



Scheme 1. Synthesis of target compounds **TM**. *Reagents and conditions:* (i) $Br(CH_2)_nBr$ (**2a~2d**), K_2CO_3, acetone, reflux, 6-8 h; (ii) K_2CO_3, NR^1R^2 (**A~E**) (Structures of NR^1R^2 are shown in **Figure 1**) anhydrous CH_3CN, 65°C, 6-10h.

The 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives **TM-1~TM-20** were tested *in vitro* for their cholinesterase inhibitory activities were determined by the modified Ellman method using *ee*AChE (from *electric eel*) and *eq*BuChE (from *equine serum*).[12] Donepezil, a well-known cholinesterase inhibitor approved by FDA, was used as the positive control, and the precursor compound 2,4-dihydroxyacetophenone (**1**) was also tested, the results are listed in **Table 1**. Compared with compound **1** and donepezil, all the target compounds were selective AChE inhibitors, and showed moderate to good *Ee*AChE inhibition potent with IC_{50} values ranging from 0.96 to 57.0 μM. It revealed that the introduction of *O*-alkylamines increased ChEs inhibitory activity and improved the selectivity for *ee*AChE over *eq*BuChE, which was consistent with our previous work.[11,13,14] The selectivity may be helpful to diminish

peripheral cholinergic side effects. The results data also displayed that both the structure of terminal groups NR^1R^2 of side chain and the methylene chain length significantly affected the AChE inhibitory potencies. When the terminal groups NR^1R^2 was piperidine and pyrrolidine, the AChE inhibitory activity remarkably enhance as the methylene chain increases, for example, **TM-1 < TM-6 < TM-11 < TM-16**; **TM-2 < TM-7 < TM-12 < TM-17**, while the compounds containing two basic centers (benzylpiperazine, 1-(2-pyridyl)piperazine and 2-(1-piperazinyl)pyrimidine group) demonstrated the opposite result, that is, the inhibitory activity decrease gradually with the increase of methylene chain, such as **TM-3 > TM-8 > TM-13 > TM-18**; **TM-4 > TM-9 > TM-14 > TM-19**; **TM-5 > TM-10 > TM-15 > TM-20**. This phenomenon revealed that the alkylamine side chain can interact with catalytic active site (PAS) of AChE in different ways. In addition, in the same methylene chain length situation, and for the compounds possessing piperazine groups, benzylpiperazine indicated better inhibitory potency than the other groups. From the screening data, all the target compounds showed weak BuChE inhibitory potency, and were significantly selective AChE inhibitors. Especially, compound **TM-3** was the best AChE inhibitor with IC_{50} value was 0.96μM, and the selective index was 32.7.

Table 1 *ee*AChE and *eq*BuChE inhibitory activities, MAO-A and MAO-B inhibitory potencies and oxygen radical absorbance capacity (ORAC, Trolox equivalent) of target compounds and control compounds.

Comp.	n	NR^1R^2	IC_{50} (μM)[a]		SI[f]	IC_{50} (μM)[a]		ORAC[g]
			*ee*AChE[b]	*eq*BuChE[c]		MAO-A[d]	MAO-B[e]	
TM-1	3	**(A)**	29.2±0.66	n.a.[h]	—	n.a.[j]	2.8±0.05	1.1±0.04
TM-2	3	**(B)**	41.3±0.78	n.a.[h]	—	n.a.[j]	0.62±0.01	1.0±0.03
TM-3	3	**(C)**	0.96±0.01	31.4±0.89	32.7	n.a.[j]	6.8±0.31	1.5±0.01
TM-4	3	**(D)**	15.2±0.23	8.8±0.66%[i]	—	n.a.[j]	11.7±0.20	0.98±0.02
TM-5	3	**(E)**	26.3±0.96	n.a.[h]	—	n.a.[j]	15.1±0.36	0.95±0.04
TM-6	4	**(A)**	8.8±0.15	21.4±0.38%[i]	—	n.a.[j]	2.5±0.08	1.0±0.03
TM-7	4	**(B)**	16.7±0.58	18.4±0.52%[i]	—	n.a.[j]	1.8±0.04	1.0±0.02
TM-8	4	**(C)**	2.2±0.01	11.5±0.31%[i]	—	n.a.[j]	15.9±0.11	1.4±0.05
TM-9	4	**(D)**	18.4±0.23	n.a.[h]	—	n.a.[j]	12.0±0.33	1.1±0.02
TM-10	4	**(E)**	27.5±0.58	n.a.[h]	—	n.a.[j]	15.2±0.77	0.98±0.03
TM-11	5	**(A)**	5.5±0.11	27.9±0.65%[i]	—	n.a.[j]	6.1±0.14	1.1±0.02

TM-12	5	**(B)**	6.3±0.22	37.2±0.81	5.9	n.a.[j]	3.5±0.13	1.0±0.04
TM-13	5	**(C)**	8.5±0.13	46.1±1.5	5.4	n.a.[j]	26.8±0.47	1.3±0.02
TM-14	5	**(D)**	21.9±0.62	n.a.[h]	—	n.a.[j]	16.7±0.39	0.99±0.03
TM-15	5	**(E)**	51.8±0.16	137±1.2	2.6	n.a.[j]	15.5±0.76	1.0±0.02
TM-16	6	**(A)**	2.9±0.08	45.7±0.79	15.8	n.a.[j]	26.6±0.43	0.98±0.04
TM-17	6	**(B)**	3.9±0.09	46.5±0.64	11.9	n.a.[j]	12.2±0.26	1.0±0.02
TM-18	6	**(C)**	16.8±0.67	75.4±0.83	4.5	n.a.[j]	30.8±0.27	1.3±0.03
TM-19	6	**(D)**	25.8±1.1	n.a.[h]	—	n.a.[j]	20.7±0.69	1.1±0.04
TM-20	6	**(E)**	57.0±1.8	n.a.[h]	—	n.a.[j]	34.6±0.88	0.98±0.05
1			n.a.[h]	n.a.[h]		n.t.[k]	n.t.[k]	2.5±0.03
donepezil			0.019±0.0003	4.76±0.02	251	n.t.[k]	n.t.[k]	—
clorgyline			n.t.[k]	n.t.[k]	—	**0.0034±0.0001**	5.02±0.02	—
rasagiline			n.t.[k]	n.t.[k]	—	2.13±0.01	**0.086±0.003**	—
iproniazid			n.t.[k]	n.t.[k]	—	3.18±0.03	1.78±0.01	—

Structures (n and NR^1R^2) are shown in **Figure 1** and **Scheme 1**. [a] IC_{50} values represent the concentration of inhibitor required to decrease enzyme activity by 50% and are the mean of three independent experiments, each performed in triplicate (SD = standard deviation). [b] From electrophorus electricusc. [c] From equine serum. [d] From recombinant human MAO-A. [e] From recombinant human MAO-B. [f] SI (selectivity index) = IC_{50} (*Ee*AChE)/IC_{50} (*eq*BuChE). [g] Results are expressed as μM of Trolox equivalent/μM of tested compounds. [h] n.a. = no active. Compounds defined "no active" means percent inhibition less than 5.0% at a concentration of 50 μM in the assay conditions. [i] the inhibition percent ratio of compounds for eqBuChE at a concentration of 50 μM in the assay conditions. [j] n.a. = no active. Compounds defined "no active" means percent inhibition less than 5.0% at a concentration of 10 μM in the assay conditions. [k] n.t. = no test.

The Lineweaver–Burk plots (**Figure 2**) showed that both inhibitions had rising slopes and increasing intercepts at higher concentration, which indicated a mixed-type inhibition.[13] The result indicated that compound **TM-3** could bind to both CAS and PAS of AChE.

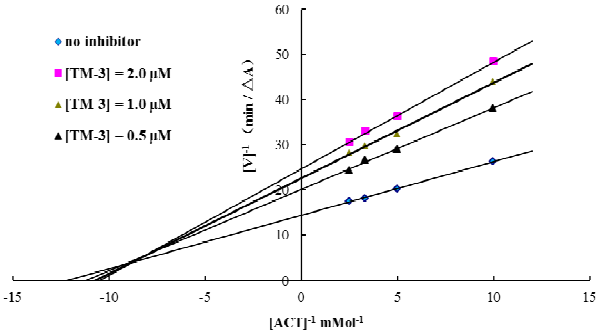


Figure 2. Steady state inhibition by compound **TM-3** of the AChE hydrolysis of ACh. The plots show mixed-type AChE inhibition for compound **TM-3.**

In order to explore possible interactions of compounds with the enzyme *Ee*AChE, compounds **TM-3** and **TM-16** were selected to do molecular modeling study using the docking program, AutoDock 4.2 package with Discovery Studio 2.5, based on the X-ray crystal structure of *Tc*AChE (PDB code: 1EVE).[12] **TM-3** was the most AChE inhibitor with three methylene chain length, As shown in **Figure 3**, the results revealed that **TM-3** occupied the entire enzymatic catalytic site (CAS), the mid-gorge sites and the peripheral site (PAS), and could simultaneously bind to both the CAS and the PAS thus providing a reasonable explanation for its highly potent inhibitory potency against AChE. In the **TM-3**-*Tc*AChE complex, the 2-hydroxy and carbonyl group at the 2,4-dihydroxyacetophenone nucleus could simultaneously bind to ARG289 *via* two intermolecular hydrogen bonds, and the 2-hydroxy additionally bind to PHE288 via a intermolecular hydrogen bond. In addition, the chain of methylene and piperazine ring could fold into a conformation in the gorge that allowed them to interact with TYR121, SER122, PHE330, PHE331 and TYR334 through hydrophobic interaction. Meanwhile, the benzene ring of benzylpiperazine was observed to bind to the CAS *via* potential hydrophobic interactions with residues TYR70, ASP72, TRP84 and ASN85. Therefore, both the kinetic study of AChE inhibition and the molecular docking showed that **TM-3** was a highly potent AChE inhibitor and was reasonably selected to further study. As shown in **Table 1**, **TM-16** was also a potent AChE inhibitor, but with six methylene chain length. The docking result was shown in **Figure S1**, **TM-16** also has one intramolecular hydrogen bond between the 2-hydroxy and carbonyl group of the 2,4-dihydroxyacetophenone nucleus, the oxygen atom of 2-hydroxy group could bind to ARG289 and PHE288 *via* two intermolecular hydrogen bonds, respectively, and the hydrogen atom of 2-hydroxy group could also bind to ARG289 *via* a intermolecular hydrogen bond, moreover, **TM-16** was observed to bind to the PAS and CAS *via* potential hydrophobic interactions with residues TYR121, SER122, PHE330, PHE331, TYR334, TYR70, ASP72 and TRP84, these phenomenon accord with the docking of **TM-3**. However, lack of the intermolecular hydrogen bond between carbonyl group of the 2,4-dihydroxyacetophenone nucleus and ARG289 presented compared with **TM-3**. That's maybe the reason why **TM-16**

showed the weaker AChE inhibitory activity than that of **TM-3**.

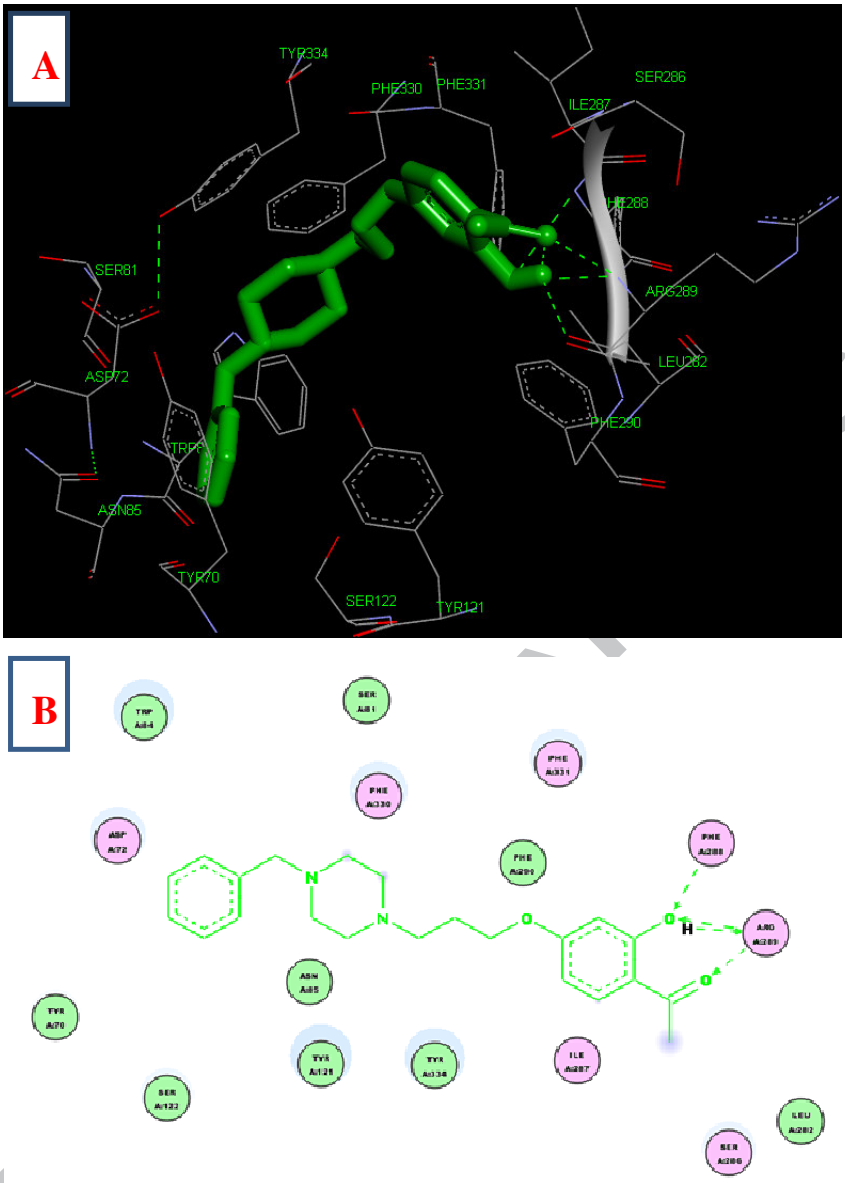


Figure 3 (A) 3D docking model of compound **TM-3** (green stick) interacting with residues in the binding site of *Tc*AChE (PDB code: 1EVE). (B) 2D schematic diagram of docking model of compound **TM-3** with *Tc*AChE.

The target compounds were also investigated for their inhibitory activity against hMAOs using clorgyline, rasagiline and iproniazid as reference drugs. Recombinant human MAO-A and –B were employed as enzyme sources, and their activities were determined by a fluorimetric method with kynuramine as a common substrate.[15,16] As shown in **Table 1**, all the synthesized target compounds were significantly selective

hMAO-B inhibitors, and displayed moderate to good MAO-B inhibition (IC_{50} values range from 0.62 to 34.6 μM). Among of which, compound **TM-2**, with pyrrolidine, exhibited the most potent MAO-B inhibitory potency with IC_{50} values of 0.62 μM. The screening data revealed that MAO-B inhibitory potency were susceptible to the substituent groups NR^1R^2 and the length of methylene. In most situations, the inhibitory activity sharply decreased with the increase of the mehtylene chain, for example, **TM-1** > **TM-6** < **TM-11** < **TM-16**; **TM-2** < **TM-7** < **TM-12** < **TM-17**; **TM-3** < **TM-8** < **TM-13** < **TM-18**; **TM-4** < **TM-9** < **TM-14** < **TM-19**; **TM-5** < **TM-10** < **TM-15** < **TM-20**. Generally speaking, expect compound **TM-1**, the optimal length of linker was three. In addition, the substituent groups NR^1R^2 was also a key role, under the same methylene chain condition, the potency to inhibit MAO-B were in the order pyrrolidine > piperidine > benzylpiperazine > 1-(2-pyridyl)piperazine > 2-(1-piperazinyl)pyrimidine. The promising compound **TM-3** was a potent selective MAO-B inhibitor (IC_{50} = 6.8 μM), which was also beneficial for AD.

The antioxidant activities of all synthesized target compounds were determined by following the well-established ORAC-FL method (oxygen radical absorbance capacity by fluorescein)[17,18] and the results were shown in **Table 1**. Trolox, a vitamin E analogue, was used as the standard, and the results were expressed as Trolox equivalents. 2,4-dihydroxyacetophenone was also tested, which has an ORAC-FL value of 2.5 Trolox equivalents. All the tested compounds exhibited favorable antioxidant capacities. As expected, the introduction of *O*-alkylamine group side chain in the 2,4-dihydroxyacetophenone nucleus decreased the radical capture capacity but the different tertiary amine fragment on the side chain has little influence on the radical capture capacity. Generally speaking, the compounds possessing benzylpiperazine group (**TM-3, TM-8, TM-13** and **TM-18**) displayed higher radical capture capacity than that of other substituent groups (pyrrolidine, piperidinem, 1-(2-pyridyl)piperazine and 2-(1-piperazinyl)pyrimidine). Compound **TM-3** exhibited the highest antioxidant potency, with an ORAC value of 1.5 Trolox equivalents.

The protective effect of **TM-3** against free radicals damage was evaluated by testing the ability of the compound to protect against H_2O_2 injury based on the

reported protocol.[18] After 100 μM H_2O_2 exposure, cell viability as determined by MTT reduction was markedly decreased to 46.5 % ($P < 0.01$ *vs* control), suggesting high sensitivity to H_2O_2-induced injury. However, compound **TM-3** showed protective effect in a dose-dependent manner against H_2O_2-induced PC12 cell injury (**Figure 4**). At concentrations of 10.0 μM, compound **TM-3** showed remarkably neuroprotective effect and the cell viability was 73.3%. When the concentration was reduced to 1.0 μM, the cell viability decreased to 59.6%. It revealed that the compound **TM -3** could capture the hydroxyl radical, generated by H_2O_2.

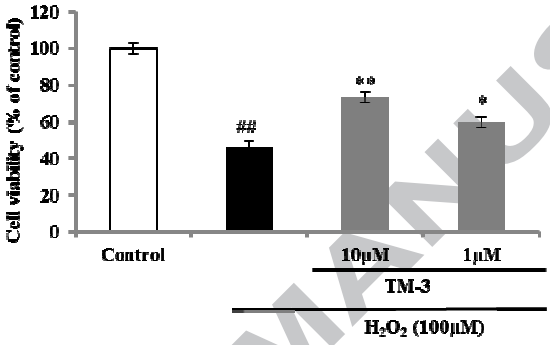


Figure 4. Protective effects of **TM -3** on cell injury induced by H_2O_2 (100 μM) in PC12 cells. $^{\#\#}P < 0.01$ *vs* control; $^{*}P < 0.05$ *vs* H_2O_2 group and $^{**}P < 0.01$ *vs* H_2O_2 group.

To further study the complexing ability of the promising compound **TM-3** toward biometals such as Cu^{2+}, Fe^{2+}, Zn^{2+} and Al^{3+}, UV-visual spectrometry test was performed.[19,20] The results were shown in **Figure 5**. The electronic spectra of **TM-3** exhibited a red shift (the peak at 314 nm shifted to 356 nm) after the addition of $CuCl_2$. But, when $FeSO_4$, $ZnCl_2$ and $AlCl_3$ were added, the electronic spectra of **TM-3** displayed no obvious change. Therefore, **TM-3** was a selective metal chelator.

The stoichiometry of the **TM-3**-Cu^{2+} complex was determined using the molar ratio method, by preparing the solution of compound **TM-3** with ascending amounts of $CuCl_2$. The UV spectra were recorded and treated by numerical subtraction of $CuCl_2$ and **TM-3** at corresponding concentrations at 356nm. As shown in **Figure 6**, the absorbance linearly increased initially and then plateaued. The two straight lines intersected at a mole fraction of 0.99, indicating a 1:1 stoichiometry for the complex **TM-3**-Cu^{2+}.

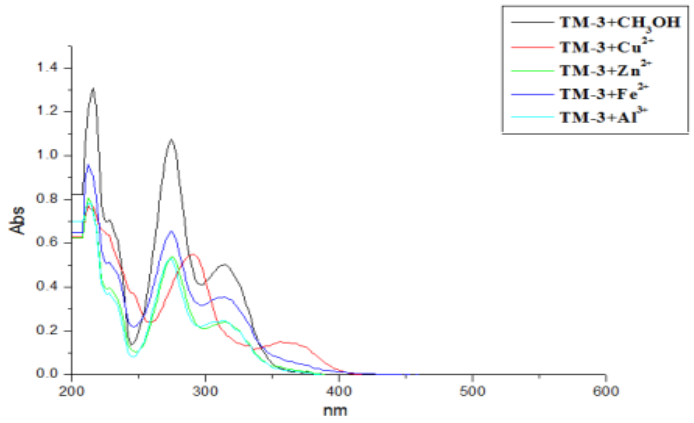


Figure 5. The UV spectrum of compound **TM-3** (37.5 μM, in methanol) alone or in the presence of $CuCl_2$, $FeSO_4$, $ZnCl_2$ and $AlCl_3$ (37.5 μM, in methanol).

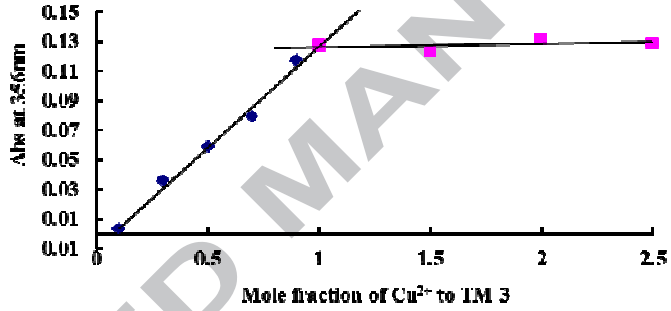


Figure 6. Determination of the stoichiometry of complex-Cu^{2+} by using molar ratio method through titrating the methanol solution of compound **TM-3** with ascending amounts of $CuCl_2$. The final concentration of tested compound was 37.5 μM, and the final concentration of Cu^{2+} ranged from 3.75 to 93.75 μM.

Brain penetration is a necessary requirement for successful anti-AD drugs. To access the brain penetration of potential compound **TM-3**, a parallel artificial membrane assay for the blood- brain barrier (PAMPA-BBB) described by Di et al was performed.[12,21] We compared the permeability of 11 commercial drugs with reported values to validate the assay (**Table 2**). A plot of the experimental data versus the reported values produced a good linear correlation, P_e (exp) = 0.9163P_e (bibl.) –0.2247 (r^2 = 0.9558) (**Figure 7**). From this equation, and considering the limit established by Di *et al.* for blood–brain barrier permeation, we confirmed that compound with permeability above 3.44×10^{-6} cm/s could cross the blood–brain barrier (**Table 3**). Based on the measured permeability (**Table 4**), **TM-3** could cross

the BBB and reach the biological targets located in the CNS.

Table 2. Permeability ($P_e \times 10^{-6}$ cm/s) in the PAMPA-BBB assay for 11 commercial drugs used in the experiment validation.

Commercial drugs	Bibl[a]	PBS:EtOH(70:30) [b]
Verapamil	16	16.90
Oxazepam	10	9.60
Diazepam	16	11.86
Clonidine	5.3	5.10
Imipramine	13	10.10
Testosterone	17	16.30
Caffeine	1.3	1.28
Enoxacine	0.9	0.47
Piroxicam	2.5	0.72
Norfloxacin	0.1	0.42
Theophylline	0.12	0.10

[a] Taken from ref. [21]

[b] Data are the mean ± SD of three independent experiments

Figure 7. Linear correlation between experimental and reported permeability of commercial drugs using the PAMPA-BBB assay. P_e(exp) = 0.9163, P_e(bibl.) −0.2247 (r^2 = 0.9558).

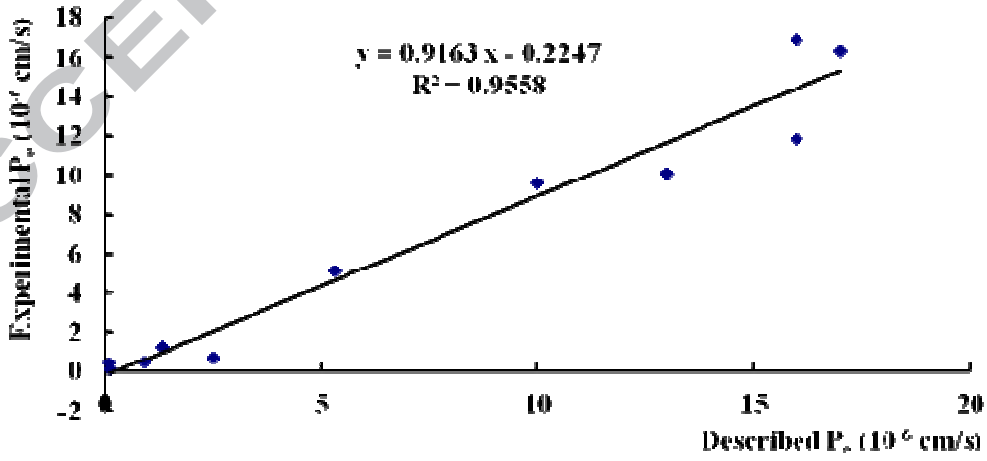


Table 3. Ranges of permeability of PAMPA-BBB assays ($P_e \times 10^{-6}$ cm/s)

Compounds of high BBB permeation (CNS +)	P_e>3.44
Compounds of uncertain BBB permeation (CNS +/-)	3.44>P_e> 1.61
Compounds of low BBB permeation (CNS -)	P_e<1.61

Table 4. Permeability P_e ($\times 10^{-6}$ cm/s) in the PAMPA-BBB assay of the selected compound **TM-3** and the predictive penetration in the CNS.

Compound[a]	P_e ($\times 10^{-6}$ cm/s)[b]	Prediction
TM-3	13.31 ± 0.12	CNS +

[a]Compound **TM-3** was dissolved in DMSO at 5 mg/mL and diluted with PBS/EtOH (70:30). The final concentration of the compound was 100 μg/mL.

[b] Values are expressed as the mean ± SD of three independent experiments.

To further evaluate the drug-likeness property of compound **TM-3**, we applied the Molinspiration property program to calculate the items including log *P*, molecular weight (MW), topological polar surface area (TPSA), the number of hydrogen-bond acceptors, and the number of hydrogen-bond donors.[22] The theoretical prediction properties of compound **TM-3** was listed in **Table 5**. The screening data illustrated showed that compound **TM-3** did not break any point of the Lipinski's rule of five, and TPSA and log P values were compatible with those described as a predictive indicator of a drug's capacity for membrane penetration. Therefore, compound **TM-3** might be a promising drug candidate and was selected to further investigate for the treatment of AD.

Table 5. Theoretical prediction of the ADME properties of compound **TM-3**

Comp.	Log *P*	MW	TPSA (Å^2)	n-ON	n-OHNH	volume (Å^3)
TM-3	3.4	368.48	53.01	5	1	357.14

[a] MW, Molecular weight; TPSA, topological polar surface area; n-OH, number of hydrogen acceptors; n-OHNH, number of hydrogen bond donors. The data was determined with the Molinspiration calculation software.

In brief, a series of 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives was designed, synthesized and evaluated as multi-target-directed ligands for the treatment of Alzheimer's disease. Among the target compounds, compound **TM-3** indicated highly selective AChE inhibitory potency with IC_{50} values of 0.96 μM, both the kinetic

analysis and molecular modeling study suggested that **TM-3** showed mixed-type inhibition, and could bind to both CAS and PAS of AChE. In addition, compound **TM-3** was a favourable selective MAO-B inhibitor with IC_{50} value of 6.8 μM. More interestingly, our study displayed that **TM-3** was an antioxidant and neuroprotectant, as well as a selective metal chelating agent. Further, **TM-3** could cross the blood-brain barrier (BBB) *in vitro* and complied with Lipinski's rule of five of the drug-likeness. Therefore, the present study revealed that **TM-3** could be considered as a promising multi-targets lead compound for the treatment of AD. Further investigations to evaluate compound **TM-3** *in vivo* and to develop structural refinements are in progress and will be reported in due course.

Notes

The authors declare no competing financial interest.

Acknowledgments

This work was supported in part by the Scientific and Technological Project of Henan Provincial (162300410103), the Key Scientific Research Project of Colleges and Universities in Henan Province (17B350002), the Special Project of Nanyang Normal University (ZX2016017).

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Design, synthesis and biological evaluation of 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives as multifunctional agents for the treatment of Alzheimer's disease

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