Effects of α -Mangostin Derivatives on the Alzheimer's Disease Model of Rats and Their Mechanism: A Combination of Experimental Study and Computational Systems Pharmacology Analysis

Yan Chen,[#] Yuemin Bian,[#] Jian-Wei Wang, Ting-Ting Gong, You-Min Ying, Lie-Feng Ma, Wei-Guang Shan, Xiang-Qun Xie, and Zha-Jun Zhan*

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ABSTRACT: α -Mangostin (α -M) is a natural xanthone from the pericarp of fruit *Garcinia mangostana* and possesses versatile biological activities. α -M has a therapeutic potential to treat Alzheimer's disease (AD) because of its anti-inflammatory, antioxidative, and neuroprotective activities. However, the use of α -M for AD treatment is limited due to its cytotoxic activities and relatively low potency. Modifications of its chemical structure were needed to reduce its cytotoxicity and improve its therapeutic potential against AD. For this purpose, 16 α -M carbamate derivatives were synthesized. An animal model of AD was established, and the effects of AMG-1 on the spatial learning ability and memory ability were evaluated using behavioral tests. The effect on neuropathology was tested by histopathological evaluation, Nissl staining, and silver staining. Computational systems pharmacology analysis using the chemogenomics knowledgebase was applied for network studies. Compound-target, target-pathway, and target-disease networks were constructed, integrating both *in silico* analysis and reported experimental data. The results show that AMG-1 can demonstrate its therapeutic effects in a one-molecule, multiple-targets manner to remarkably ameliorate neurological changes and reverse behavioral deficits in AD model rats. The improved cognitive function and alleviated neuronal injury can be observed. The ability of AMG-1 to scavenge β -amyloid in the hippocampus was validated in AD model rats.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative and psychiatric disorder. AD has become the most devastating disease worldwide, and its prevalence is expected to greatly increase over the next few decades as the population ages.¹ The hallmarks of AD are cognitive deficit and memory impairments, which are caused by the massive loss of neurons and synapses.² Accumulating evidence suggests that abnormally folded β -amyloid ($A\beta$) protein can be one of the important pathological contributors to neuronal loss in AD.^{3–5} $A\beta$ plaques can result in oxidative stress, inflammation, and neurotoxicity, all of which can initiate the pathogenic cascade and ultimately lead to cognitive dysfunction.^{6–8} Thus, the strategy to increase the $A\beta$ clearance is being actively pursued as a promising disease-modifying therapy. Currently, five drugs have been approved for the treatment of AD. Four of them are

acetylcholinesterase inhibitors (donepezil, tacrine, rivastigmine, and galantamine) and one *N*-methyl-D-aspartate (NMDA) receptor antagonist (memantine).⁹ Donepezil is usually considered as the first choice for AD patients' treatment.¹⁰ However, donepezil can only improve the cognition and behavior of the patients with AD but does not have any effect on slowing the progression or curing the disease.¹¹ Besides, donepezil has many side effects, including

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Scheme 1. Reagents and Conditions: (a) CH_3I or $\text{C}_2\text{H}_5\text{Br}$, K_2CO_3 , Reflux, and 12 h; (b) DDQ, Toluene, 40 °C, and 5 h; (c) BTC, Et_3N , DMAP, DCM, rt, and 10 h

Table 1. 50% Inhibitory Concentration (IC_{50}) of Derivatives against AChE and 50% Effective Concentration EC_{50} on PC12 Cells^a

compound	IC_{50} (μM) ^a	EC_{50} (μM) ^b	compound	IC_{50} (μM) ^a	EC_{50} (μM) ^b
galantamine	4.24 ± 1.65	ND ^c	AMG-7	43.45 ± 5.27	>100
$\alpha\text{-M}$	34.28 ± 6.06	>100	AMG-8	>100	>100
AMG	41.21 ± 6.28	>100	AMG-9	45.97 ± 15.32	>100
edaravone	ND ^c	69.98 ± 5.73	AMG-10	47.78 ± 16.78	13.07 ± 2.25
AMG-1	52.55 ± 11.27	1.61 ± 0.35	AMG-11	43.60 ± 12.33	12.33 ± 4.65
AMG-2	35.14 ± 9.38	12.77 ± 4.23	AMG-12	19.20 ± 6.87	>100
AMG-3	32.89 ± 7.78	13.36 ± 5.17	AMG-13	15.07 ± 3.56	>100
AMG-4	>100	8.27 ± 2.01	AMG-14	40.64 ± 13.78	>100
AMG-5	>100	>100	AMG-15	34.46 ± 9.38	>100
AMG-6	>100	>100	AMG-16	37.49 ± 11.24	>100

^aMean $\pm$ standard error of the mean (SEM) of three experiments for 50% inhibition of AChE. ^bMean $\pm$ SEM of three independent experiments for 50% antioxidant neuroprotective effect. ^cNot determined. ^dInhibitory activity (IC_{50} , μM) of AChE and neuroprotective effective concentration (EC_{50} , μM) for the studied compounds, galantamine and edaravone.

loss of appetite, diarrhea, gastrointestinal upset, difficulty sleeping, vomiting, and muscle cramping, which further limits its application.¹²

Recently, the studies on active ingredients from natural products and medicinal herbs for treating AD have attracted substantial attention.^{13–15} $\alpha\text{-Mangostin}$ ($\alpha\text{-M}$, Scheme 1) is a natural xanthone separated from the mangosteen pericarp.¹⁶ $\alpha\text{-M}$ has been reported to possess a wide spectrum of biological activities such as anti-inflammatory, cardioprotective, anti-tumor, antidiabetic, antibacterial, antifungal, antioxidant, antiparasitic, and antiobesity activities.^{17–26} Recent evidence further suggests that $\alpha\text{-M}$ can inhibit amyloid aggregation.²⁷ However, the major obstacles facing the application of $\alpha\text{-M}$ are due to its cytotoxic activities, hydrophobic nature, and thus low bioavailability and accumulation in the target organs. To decrease its cytotoxicity and improve the neuroprotective effect, we modified the structure of $\alpha\text{-M}$ through the medicinal chemistry approach. The initial substrate $\alpha\text{-M}$ was converted into the cyclized compound AMG (Scheme 1). Then, the hydroxyl at C-2 in AMG was reacted with different isocyanates to afford a series of $\alpha\text{-M}$ carbamate derivatives.

Hence, one of the aims of our present work is to elucidate AChE inhibitory and neuroprotective effects of AMG derivatives *in vitro*. As a result, **AMG-1**, after structural

optimization, has improved neuroprotective effects. To further investigate whether **AMG-1** can improve the learning and memory of AD's model of rats, we also induced an AD-like rat model by injecting $\text{A}\beta_{25-35}$ into the bilateral hippocampus of rats. At the same time, the molecular mechanism that underlies the experimental observations was further analyzed by computational systems pharmacology.²⁸ Our study showed that **AMG-1** not only protected rat cerebral hippocampal neurons against $\text{A}\beta_{25-35}$ oligomer-induced $\beta\text{-amyloid}$ deposition but also improved the learning and memory ability and alleviated neuronal injury by scavenging $\beta\text{-amyloid}$ in the hippocampus of AD model rats.

RESULTS

Synthesis of AMG Derivatives. The preparation of the target compounds started from $\alpha\text{-M}$ through four steps as described in Scheme 1. The prenyl group is an important factor for the retention of cytotoxicity in prenylated xanthones,²⁹ so $\alpha\text{-M}$ was cyclized to yield AMG (4) to reduce cytotoxicity. The methylation of substituted aniline (**1a–1r**) resulted in substituted *N*-methylaniline (**2a–2r**) and *N*-ethylaniline (**3**). The carbamoyl group has been proven to be an important pharmacophore of acetylcholinesterase inhibitory activity.^{30,31} Furthermore, carbamates could also

affect the expression and processing of the A β -precursor protein, one of the most important therapeutic goals in Alzheimer's disease.³² To improve the bioactivity of α -M, the carbamoyl groups were then introduced into AMG (4) by reacting with chlorides (3a–3r) to afford α -M carbamate derivatives AMG-1–AMG-16.

In Vitro Experiments. Sixteen derivatives of AMG were evaluated for AChE inhibition by an *in vitro* assay based on a microplate. The IC₅₀ values for AChE inhibition are summarized in Table 1. A majority of the derivatives showed potent capacity to inhibit AChE activity, with IC₅₀ values lying in the micromolar range, which demonstrates an important role of the α -M moiety in the AChE inhibition of the developed α -M carbamate group. The neuroprotective activity of target compounds was evaluated on the neuronal-like PC12 cells damaged by H₂O₂. The results expressed as EC₅₀ are shown in Table 1, and most of the α -M derivatives showed better protective effects than the positive control drug edaravone (EC₅₀ = 69.98 $\pm$ 5.73 μ M) on injured PC12 cells. The EC₅₀ of α -M was tested to be more than 100 μ M. Among the AMG derivatives, AMG-1 exhibited the most potent neuroprotective activity, with EC₅₀ values of 1.61 $\pm$ 0.35 μ M. The cytotoxicity was compared between α -M and derivatives in PC12 cells. The results showed that the inhibition ratio of cell proliferation decreased from 73.1 to 45.3% after the cyclization of α -M to AMG (Table 2). Notably, AMG-1, which was then selected for the *in vivo* studies on the rat model of AD, has an inhibition ratio of 7.2%.

Table 2. Cytotoxic Effects of α -M and Derivatives in PC12 Cells

compounds (10 μ M)	inhibition ratio (%)	compounds (10 μ M)	inhibition ratio (%)
edaravone	0	AMG-8	0
α -M	73.1	AMG-9	0
AMG	45.3	AMG-10	0
AMG-1	7.2	AMG-11	0
AMG-2	6.7	AMG-12	0
AMG-3	5.9	AMG-13	3.1
AMG-4	0	AMG-14	5.3
AMG-5	7.8	AMG-15	5.2
AMG-6	7.4	AMG-16	0
AMG-7	0		

In Vivo Experiments. Acute Toxicity. During the acute oral toxicity experiment period, the mice were found to have messy furs and loose gray stool around the anus. Except for the 1310 mg/kg group, death could be found in other groups (Table 3). Particularly, the mice in the highest dosed group (4000 mg/kg) were found dead within 16–48 h after

Table 3. Results of the Acute Oral Toxicity Test of AMG-1 in Mice

group	number of rats	dosage (mg/kg)	deaths	death rate (%)
control	10		0	0
1	10	4000	9	90
2	10	3200	7	70
3	10	2560	6	60
4	10	2048	4	40
5	10	1638	1	10
6	10	1310	0	0

administration. For the 14 day observation, most mice had a recovery. LD₅₀ values and their 95% confidence intervals of AMG-1 in mice were calculated according to the improved Bliss method. The results indicated that the LD₅₀ values of AMG-1 in mice were 2.47 g/kg and the 95% confidence intervals were between 2.13 and 2.89 g/kg. Results showed that AMG-1 had no obvious toxicity at the level of 20 mg/kg or less as the dose for administration in the following studies.

Animal Behavior Tests in the Open Field and Morris Water Maze. The one-way ANOVA analysis of locomotor activity and distance traveled in the open field showed the treatment effects of AMG-1 on AD rats. The total distance moved by the A β model group was the shortest among the six groups (Figure 1A), and the A β model group spent the least time in the central zone (Figure 1B), indicating the low ability of space exploration in the A β model group on average. However, both the AMG-1 (20 mg/kg)-treated group and the donepezil group showed a significant increase compared to the A β model group ($p < 0.05$, $p < 0.01$) in the total distance moved (28.55 $\pm$ 6.87, 31.37 $\pm$ 7.14, vs 17.34 $\pm$ 3.88, respectively). AMG-1-treated groups showed a significant ($p < 0.05$ –0.01) and dose-dependent increase in the time spent in the central zone compared to the A β model group ($p < 0.01$).

To examine whether the ability of spatial learning and memory was significantly improved by administering AMG-1 in AD rats, we adopted a commonly utilized Morris water maze test for evaluation. The results exhibited that in the training trials on the first day, all rats explored the objects equally (Figure 1A, $p > 0.05$). During the retention phase, the escape latency (Figure 1C) and the total distance traveled (Figure 1D) of the rats in the A β -induced model group were significantly increased compared to the rats in the sham control group ($p < 0.01$), while AMG-1-treated rats showed significant improvements compared to the model rats ($p < 0.05$). In the probe trial (Figure 1E), the platform was removed. AMG-1 treatment not only promoted learning during the training trials but also improved memory retention during the probe trials. Rats in AMG-1 treatment groups spent significantly less time to reach the place of the removed platform and spent significantly more time in the target quadrant, compared to the model rats ($p < 0.05$). Thus, these results demonstrate that AMG-1 treatment can improve the memory loss in A β -induced rats.

Neuroprotective Effects of AMG-1 Revealed by Brain Tissue Staining. Hematoxylin and Eosin (HE) staining was performed to detect the pyramidal cells of the hippocampus CA3 region and the neurohistopathological changes in the brain sections in rats in different groups under a light microscope (200 $\times$). The representative morphologic pictures of neuronal cells of the rats from HE staining are illustrated in Figure 2. Figure 2A shows no remarkable neuronal abnormalities in the hippocampus CA3 region of rats in the sham control group. The pyramidal cells of the CA3 region are arranged neatly and tightly, and no cell loss can be found. Cells are round and intact with nuclei stained clearly in dark blue (red arrows in Figure 2A). However, obvious hippocampal histopathological damage is observed in the model group. The pyramidal layered structure is disintegrated, and neuronal loss can be found in the CA3 region. Pyramidal neurons with pyknotic nuclei and a shrunken or irregular shape can also be observed (Figure 2B). These abnormalities were attenuated by AMG-1 treatment. The pyramidal cells in the AMG-1 (5, 10,

Figure 1. Effect of AMG-1 administration on behaviors related to locomotor activity and the improvement of spatial cognition in AD rats. (A) Total distance moved and (B) time spent in the central zone in the OFT. (C) Effect of AMG-1 on escape latency, (D) total distance moved, and (E) number of crossings in the MWM. (Values are expressed as mean $\pm$ SEM and were analyzed using the *t*-test. #*P* < 0.05, ##*P* < 0.01 compared with the sham group and **P* < 0.05 ***P* < 0.01 compared with the $A\beta$ model group in each figure).

and 20 mg/kg) groups have better cell morphology and density than those in the model group (Figure 2D–F).

In the hippocampal CA1 and CA3 areas, representative Nissl-stained neurons can be observed in Figure 3. Intact cells can be found in sham-operated animals as well as all unaffected regions following AD. The results from the Nissl staining showed that in the hippocampal CA1 area of AD rats, the cells were sparsely distributed and the number of cells with eumorphism was significantly reduced. Based on the results, $A\beta$ significantly increased necrotic death in the hippocampal CA1 region of the rats in the $A\beta$ model group, compared with the rats in the sham group (Figure 3A,B). However, with the AMG-1 treatment, neuronal damage was significantly attenuated in rats in 10 and 20 mg/kg groups, which suggested that AMG-1 can significantly prevent the neuronal loss in $A\beta$ -induced AD rats (Figure 3E–G).

To evaluate the effects of AMG-1 on the AD rat model, neurofibrillary tangle pathology was observed by modified Bielschowsky silver staining. The notable dark neurofibrillary

tangles (red arrows in Figure 4) that characterize the neuronal damage in the hippocampus can be found for rats in the $A\beta_{25-35}$ -treated group (Figure 4B–F). The damaged neurons can rarely be found in the hippocampus for rats in the sham group (Figure 4A). A large amount of dark staining of neurons and senile plaques can be frequently observed in the hippocampus for rats in the AD model group (Figure 4B). Meanwhile, the number of senile plaques for rats in the AMG-1 treatment (10, 20 mg/kg) groups was less (Figure 4E,F). Our data demonstrate that amyloid exposure can induce senile plaque deposition. Furthermore, the results suggest that AMG-1 can reduce the senile plaques and repair the neuron injury for the AD model rats.

AMG-1 Treatment Decreases $A\beta_{1-42}$ in the Hippocampus of the AD Rat Brain. Quantification of immunohistochemical staining for $A\beta_{1-42}$ showed that there are significantly more positive-stained neurons for rats in the model group (*p* < 0.01) than in the sham group (Figure 5A,B). Compared with the model group, the AMG-1 treatment groups (10, 20 mg/kg)

Figure 2. HE staining of the neuronal cells of the hippocampus CA3 region. Hematoxylin and eosin staining of the hippocampus CA3 region. The red arrows indicate the histologic changes ($\times 200$). (A) Sham group, (B) $A\beta$ model group, and (C) donepezil group. (D–F) AMG-1 (5, 10, and 20 mg/kg) + the model group, respectively.

Figure 3. Nissl staining of the hippocampal CA1 region after induction of $A\beta$. Damaged cells ($n = 5$ rats per group, three sections per rat) were sparsely arranged, and their shapes were fuzzy (the red arrows indicate the necrotic cells). (A) Sham group, (B) $A\beta$ model group, (C) donepezil group, and (D–F) AMG-1 (5, 10, and 20 mg/kg) + model group, respectively. The results are shown as mean $\pm$ SED. (G) Quantitative analysis of the necrotic cell count.

showed a significantly reduced expression of $A\beta_{1-42}$ ($p < 0.05$) (Figure 5E–G). Red arrows show areas of $A\beta_{1-42}$ -positive amyloid plaques.

Chemogenomics Knowledgebase Network Analysis. In the observation of experimental evidence, AMG-1 remarkably reversed behavioral deficits and significantly improved cognitive functions in AD model rats. The results suggest that the neuroprotective effects of AMG-1 are realized through scavenging β -amyloid in the hippocampus. To (1) better understand the complex interaction of the compound and the corresponding targets and (2) establish a comprehensive

understanding of the therapeutic mechanisms, the chemogenomics knowledgebase was adapted for network analysis.

Chemogenomics databases for Alzheimer's disease, drug abuse research, and cardiovascular disease, as well as two chemogenomics tools, TargetHunter and HTDocking, were combined for retrieving the information for protein targets as detailed in the Materials and Methods section. The compound-target network was first constructed (Figure 6). In total, 55 nodes of protein targets were connected with α -mangostin and AMG through 68 edges (Table 4). A total of 33 protein targets were predicted to have an association with α -mangostin, while 35 protein targets were predicted to interact with AMG-1.

Figure 4. Silver staining. Administration of AMG-1 to animals for 30 days (D–F) in comparison to (C) donepezil, (B) model, and (A) sham rats.

Figure 5. Effect of AMG-1 on Aβ₁₋₄₂ expression in the hippocampal CA1 region. (A) Sham rat group, (B) Aβ model group, (C) donepezil treatment group, and (D–F) AMG-1 treatment rat groups (5, 10, 20 mg/kg/day) ($n = 4$, four sections per rat). (G) Quantification of the immunohistochemistry of Aβ plaque numbers in the hippocampus ($n = 4$, four sections per rat).

Thirteen nodes were shared between the two compounds. The annulation and the substitution of two hydroxyl groups changed the size and the physical–chemical properties of α -mangostin. As a result, distinctive targets can be observed between these two compounds. The reported targets for α -mangostin were recurred and marked in green. The effect of AMG-1 on preventing Tau hyperphosphorylation was proposed through the experiments and marked in yellow.

The analysis of signaling pathways (Figure 7) was followed to connect the protein targets with both physiological and pathological processes. Targets were identified to be involved in a total of 37 nodes of pathways through 94 edges. The

complexity of the network reflects the polypharmacology feature of α -mangostin and its derivative, AMG-1, which is consistent with the broad therapeutic spectrum of these compounds. Pathways connected with more than three targets were placed in the center and colored in orange for emphasis. Among the highly shared pathways, signaling in cholinergic neurons, cholesterol and sphingolipid transport and biosynthesis, and tau pathology can have a direct or indirect association with AD. Oxidative phosphorylation, neuro-protective action of lithium, etc. can also have influences on the pathology of AD. The effect of AMG-1 on treating diseases can be in a one-molecule, multiple-targets manner.

Figure 6. Chemogenomics-based target mapping for α -mangostin and AMG-1. Pink nodes represent the purely predicted targets. Yellow nodes represent the experimentally validated targets. Green nodes represent the known targets. Known or experimentally validated targets are connected with the corresponding target through solid edges. Predicted targets are connected through dashed edges.

The target-pathway network bridged the gap between the targets and the specific diseases. The target-disease network (Figure 8) was constructed afterward. A total of 25 nodes of diseases were connected with targets through 125 edges. Most diseases can be categorized into four groups: neurological, cancer-related, cardiovascular, and inflammatory diseases. The reported effects of α -mangostin on treating cancers, benefiting cardiovascular disorders, and improving cognitive functions can be recurred or verified in the target-disease network. Limited reports can be found to associate α -mangostin and neurological disorders, except AD. However, the potential of α -mangostin and AMG-1 for treating other neurological disorders, including depression, Parkinson's disease, and Huntington's disease, can be investigated. One explanation that the identified diseases are mainly neurological and cardiovascular is that the chemogenomics knowledgebase we

used focuses on AD, drug abuse, and the cardiovascular system. It is a shortcoming that using focused knowledgebases can have a limited target coverage. However, on the other hand, using focused knowledgebases according to reported compound activities can better develop a systematic understanding of the specific areas, especially for the AD research in this case.

For the node of AD, 18 edges were connected to a variety of protein targets. An AD-specific target-disease network of α -mangostin and **AMG-1** was then constructed (Figure 9). Seven nodes of targets were connected with α -mangostin, and 15 nodes of targets were connected with **AMG-1**, while four targets were shared between these two compounds. Seventeen nodes of targets (except TPH1) were categorized into five groups: β -amyloid ($A\beta$) deposit, deficits of acetylcholine, tau hyperphosphorylation, oxidative stress, and lipid metabolism,

Table 4. Protein Annotation for the Predicted Targets^a

protein	docking score	gene name	compound
cytochrome P450 1B1 (CP1B1_HUMAN)	11.7	CP1B1	α -mangostin
pyruvate kinase isozymes M1/M2 (KPYM_HUMAN)	11.6	KPYM	α -mangostin
amine oxidase [flavin-containing] B, Monoamine oxidase type B, MAO-B	11.0	MAO-B	α -mangostin
lanosterol synthase (ERG7_HUMAN)	10.9	ERG7	α -mangostin
acetylcholinesterase, AChE	10.9	ACHE	α -mangostin
cAMP-specific 3',5'-cyclic phosphodiesterase 4B (PDE4B_HUMAN)	10.8	PDE4B	α -mangostin
cAMP-specific 3',5'-cyclic phosphodiesterase 4D (PDE4D_HUMAN)	10.7	PDE4D	α -mangostin
prostaglandin G/H synthase 2, Cyclooxygenase-2, COX-2	10.7	COX-2	α -mangostin
tryptophan 5-hydroxylase 1 (TPH1_HUMAN)	10.6	TPH1	α -mangostin
aldo-keto reductase family 1 member C2 (AK1C2_HUMAN)	10.5	AK1C2	α -mangostin
cGMP-specific 3',5'-cyclic phosphodiesterase (PDE5A_HUMAN)	10.5	PDE5A	α -mangostin
medium-chain specific acyl-CoA dehydrogenase, mitochondrial (ACADM_HUMAN)	10.3	ACADM	α -mangostin
proto-oncogene tyrosine-protein kinase Src (SRC_HUMAN)	10.3	SRC	α -mangostin
nitric oxide synthase, inducible; NOS type II; NOS2	10.3	NOS2	α -mangostin
cyclooxygenase	10.3	COX-1	α -mangostin
T-cell surface glycoprotein CD1d (CD1D_HUMAN)	10.2	CD1D	α -mangostin
dual specificity protein kinase CLK1 (CLK1_HUMAN)	10.2	CLK1	α -mangostin
serine/threonine-protein kinase haspin (HASP_HUMAN)	10.2	HASP	α -mangostin
catalase (CATA_HUMAN)	10.1	CATA	α -mangostin
catalase (CATA_HUMAN)	10.1	CATA	α -mangostin
carnitine O-acetyltransferase (CACP_HUMAN)	10.1	CRAT	α -mangostin
cAMP and cAMP-inhibited cGMP 3',5'-cyclic phosphodiesterase 10A (PDE10_HUMAN)	10.1	PDE10	α -mangostin
pirin (PIR_HUMAN)	10.1	PIR	α -mangostin
liver carboxylesterase 1; Brain carboxylesterase hBr1; CES1	9.9	CES1	α -mangostin
cyclooxygenase	9.7	COX-2	α -mangostin
cyclin-dependent kinase 5; Cell division protein kinase 5; CDK5	9.6	CDK5	α -mangostin
sphingomyelin phosphodiesterase 2; SMPD2	9.4	SMPD2	α -mangostin
matrix metalloproteinase-16 (MMP16_HUMAN)	9.3	MMP16	α -mangostin
matrix metalloproteinase-9 (MMP9_HUMAN)	9.3	MMP9	α -mangostin
E3 ubiquitin-protein ligase Mdm2 (MDM2_HUMAN)	7.9	MDM2	α -mangostin
apoptosis regulator Bcl-2 (BCL2_HUMAN)	7.6	BCL2	α -mangostin
G1/S-specific cyclin-D3@Cell division protein kinase 4(CCND3_HUMAN/CDK4_HUMAN)	7.0	CDK4	α -mangostin
fatty acid synthase (FAS_HUMAN)	6.7	FAS	α -mangostin
induced myeloid leukemia cell differentiation protein Mcl-1 (MCL1_HUMAN)	5.7	MCL1	α -mangostin
acetylcholinesterase, AChE	12.3	ACHE	AMG-1
cholinesterase, Butyrylcholine esterase, BCHE	12.3	BCHE	AMG-1
nitric oxide synthase, brain; Neuronal NOS; NOS type I; NOS1	12.2	NOS1	AMG-1
peptidyl-prolyl cis-trans isomerase FKBP1A (FKBP1A_HUMAN)	12.1	FKBP1A	AMG-1
aldo-keto reductase family 1 member C3 (AK1C3_HUMAN)	12.0	AK1C3	AMG-1
T-cell surface glycoprotein CD1d (CD1D_HUMAN)	11.9	CD1D	AMG-1
T-cell surface glycoprotein CD1a (CD1A_HUMAN)	11.8	CD1A	AMG-1
NAD(P)H dehydrogenase [quinone] 1 (NQO1_HUMAN)	11.8	NQO1	AMG-1
collagen type IV α -3-binding protein (C43BP_HUMAN)	11.7	C43BP	AMG-1
prostaglandin reductase 2 (PTGR2_HUMAN)	11.7	PTGR2	AMG-1
cytochrome P450 1B1 (CP1B1_HUMAN)	11.5	CP1B1	AMG-1
advanced glycosylation end product-specific receptor (RAGE_HUMAN)	11.4	RAGE	AMG-1
calcitonin gene-related peptide type 1 receptor, Receptor activity-modifying protein 1(CALRL_HUMAN/RAMP1_HUMAN)	11.3	CALRL	AMG-1
serine/threonine-protein kinase Pim-2 (PIM2_HUMAN)	11.2	PIM2	AMG-1
cholesteryl ester transfer protein (CETP_HUMAN)	11.2	CETP	AMG-1
cyclin-dependent kinase 5; Cell division protein kinase 5; CDK5	11.1	CDK5	AMG-1
glycogen synthase kinase-3 β , GSK3 β , GSK3B	10.6	GSK3 β	AMG-1
amine oxidase [flavin-containing] B, Monoamine oxidase type B, MAO-B	10.6	MAO-B	AMG-1
β -secretase 1, Aspartyl protease 2, BACE1	10.5	BACE1	AMG-1
high-affinity cGMP-specific 3',5'-cyclic phosphodiesterase 9A, PDE9A	10.3	PDE9A	AMG-1
3-hydroxy-3-methylglutaryl-coenzyme A reductase; HMG-CoA reductase; HMGCR	10.2	HMGCR	AMG-1
α -synuclein (Tau)	10.2	SNCA	AMG-1
steroid 5 α -reductase 1	10.1	SRD5A1	AMG-1
prostaglandin G/H synthase 2, Cyclooxygenase-2, COX-2	10.0	COX-2	AMG-1
adenosine A2a receptor	10.0	A2a	AMG-1

protein	docking score	gene name	compound
cyclooxygenase	9.9	COX-1	AMG-1
amyloid β A4 protein	9.7	A β A4	AMG-1
collagenase 3 (MMP13_HUMAN)	9.5	MMP13	AMG-1
neutrophil collagenase (MMP8_HUMAN)	9.3	MMP8	AMG-1
E3 ubiquitin-protein ligase Mdm2 (MDM2_HUMAN)	8.9	MDM2	AMG-1
G1/S-specific cyclin-D3@Cell division protein kinase 4 (CCND3_HUMAN/CDK4_HUMAN)	8.3	CDK4	AMG-1
apoptosis regulator Bcl-2 (BCL2_HUMAN)	8.1	BCL2	AMG-1
fatty acid synthase (FAS_HUMAN)	8.0	FAS	AMG-1
arachidonate 12-lipoxygenase	7.8	ALOX12	AMG-1
induced myeloid leukemia cell differentiation protein Mcl-1 (MCL1_HUMAN)	5.7	MCL1	AMG-1

^aThe docking score represents the predicted $-\log K_d$.

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Figure 8. Target-disease network. Blue nodes represent the targets for α -mangostin and AMG-1 identified through the target mapping. Salmon nodes represent different diseases. Diseases are categorized into four categories, neurological, cancer-related, cardiovascular, and inflammatory. Diseases are connected with categories with edges in the corresponding color.

according to their association in pathology hypothesis regarding AD. Among these five groups, lipid metabolism may attract relatively less attention. Besides having been reported to be associated with $A\beta$ deposit and tau hyperphosphorylation, lipid metabolism can also have a direct or unclear effect on AD pathology, including (1) biosynthesis of neuroprotective allopregnenolone, (2) regulation of cholesteryl ester transformation, and (3) interference in the metabolism of sphingomyelin. The distinctions in predicted targets for α -mangostin and AMG-1 reflected the alteration in physical-chemical properties caused by the annulation and the substitution on two hydroxyl groups of α -mangostin. The experimentally observed inhibition of tau hyperphosphorylation from straining can be caused directly by interacting SNCA and indirectly by interfering GSK3 β . In addition, the prevention of $A\beta$ deposit can be the result of the interaction

of BACE1, CDK5, and HMGR. Also, AMG-1 can inherit the ability of α -mangostin to inhibit cholinesterases, which further benefits the AD treatment. Further investigation can be conducted toward NQO1 and CETP. In response to the increased oxidatives, NADPH quinone oxidoreductase (NQO1) can be activated. The reduced HDL level can be observed in AD, which can be a result of regulating the cholesteryl ester transfer protein (CETP). The regulation in lipid metabolism and oxidative stress can contribute to the therapeutic potential of AMG-1. The finding supports the hypothesis that AMG-1 demonstrates its therapeutic effects in a one-molecule, multiple-targets manner.

In Silico Property Prediction. The experimental results clearly show the neuroprotective effects of AMG-1. Given that the targeting site for treating AD is in the central nervous system, it is critical to evaluate the ability of a compound to

Figure 9. AD-specific target-disease network. Pink nodes represent the purely predicted targets. Yellow nodes represent the experimentally validated targets. Green nodes represent the known targets. Targets are categorized into five AD pathology hypothesis groups, lipid metabolism, tau hyperphosphorylation, β -amyloid deposit, deficits of acetylcholine, and oxidative stress, with edges in the corresponding color.

pass the blood–brain barrier (BBB). Eight combinations of fingerprints and algorithms were calculated (Table 5) for both

Table 5. BBB Permeability Prediction

algorithms and fingerprints	threshold	α -mangostin	AMG-1
SVM and MACCS	0.02	−0.046	−0.094
SVM and Openbabel (FP2)	0	0.146	0.155
SVM and Molprint 2D	0	0.341	0.681
SVM and PubChem	0	−0.070	−0.135
AdaBoost and MACCS	0	−4.006	−0.070
AdaBoost and Openbabel (FP2)	0	8.623	9.675
AdaBoost and Molprint 2D	0	−1.614	3.049
AdaBoost and PubChem	0	0.089	1.226

α -mangostin and AMG-1 as specified in Materials and Methods. The lipophilicity was increased for AMG-1 ($c \log P = 7.83$) after the annulation and the substitution of two hydroxyl groups of α -mangostin ($c \log P = 6.07$). Although the combination of SVM with different fingerprint types can result in contradictory predictions, the algorithm AdaBoost provided consistent results that AMG-1 can have a better chance to

cross the BBB. The differences among the abilities for these two compounds to pass the BBB help explaining the distinctive neuroprotective effects to some extent. Both the compounds passed the pan-assay interference compounds (PAINS)-Remover, indicating that there are no undesired substructural features. The toxic hazard estimation using Toxtree suggested no improved toxicity profile of AMG-1. Both compounds were estimated to have their toxic hazard high. The chemical modification should be considered to decrease the toxicity while maintaining or increasing the activity to move further.

DISCUSSION

For this work, the study began with the natural product, α -mangostin. AMG-1 was designed and synthesized as a derivative for α -M in an effort to reduce the toxicity and increase the lipophilicity. Notably, the cyclization significantly reduced the toxicity of α -M, while the improved neuroprotective activities after the cyclization can also be observed at the cell level. Animal behavior studies using an open-field test and a Morris water maze showed that AMG-1 treatment can improve the memory loss and cognitive function in $A\beta$ -induced rats. After the sacrifice of the animals, multiple

staining methods, including HE staining, Nissl staining, and Bielschowsky silver staining, were applied to brain slices for mechanism studies. The **AMG-1** treatment can (1) have neuron cells with better morphology and density compared with the disease model, (2) significantly prevent the neuronal loss in $A\beta$ -induced AD rats, and (3) reduce the senile plaque and repair the neuron injury in the AD model. The quantification of immunohistochemical staining further demonstrated that the **AMG-1** treatment significantly reduced the expression of $A\beta_{1-42}$. A computational systems pharmacology analysis using the chemogenomics knowledgebase was applied for network studies. Compound-target, target-pathway, and target-disease networks were constructed, integrating both *in silico* analysis and reported experimental data. The systematic network analysis (1) confirmed the broad therapeutic spectrum of α -mangostin, (2) bridged the corresponding targets with specific pathways and diseases, and (3) speculated that **AMG-1** can demonstrate its therapeutic effects in a one-molecule, multiple-targets manner. In a nutshell, **AMG-1** is reported here to be a promising compound with the potential for future modification and development for the study of Alzheimer's disease.

MATERIALS AND METHODS

All chemicals, reagents, and solvents were purchased from commercial suppliers and used without further purification unless otherwise noted. Isolation and reactions were monitored by analytical thin-layer chromatography (TLC) on silica gel 60 F254 percolated plates with a fluorescent indicator (purchased from Qingdao Haiyang Inc.). Visualization on TLC was achieved by UV light (254 or 365 nm) and a typical TLC indicator solution (10% sulfuric acid/ethanol solution). Column chromatography was performed on silica gel (200–300 mesh) for the purification of intermediates and final compounds. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance (500 MHz) spectrometer at 300 K in CD_3COCD_3 or CDCl_3 with tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm (δ); coupling constants (J) are given in hertz; and peak multiplicities are reported as singlet (s), doublet (d), triplet (t), and multiplet (m). The electrospray ionization mass spectrometry (ESI-MS) and high-resolution ESI-MS data were recorded on an Agilent-6210-LC/TOF mass spectrometer. All final compounds were more than 95% pure, which was analyzed by a high-performance liquid chromatography (HPLC) system (Waters Corporation, Milford, MA). α -Mangostin was isolated from the pericarp of *G. mangostana*, as reported in the literature.³³

General Procedures for the Synthesis and Spectral Data of the Synthesized Compounds. *General Procedure for the Synthesis of 2a–2r and 3.* To a solution of compound **1** (0.01 mmol) and K_2CO_3 (0.012 mmol) in acetone, CH_3I or $\text{C}_2\text{H}_5\text{Br}$ (0.011 mmol) was added dropwise. The reaction mixture was heated to reflux for 12 h. Then, the mixture was filtrated and the filtrate was concentrated to afford the crude product. The crude product was chromatographed on silica gel (petroleum ether (PE)/EtOAc = 40:1, v/v) to give compounds **2** and **3** as colorless oils (isolated yield 80.0–91.2%).

Synthesis of AMG (4). To a solution of α -M (4.0 mmol) in toluene, **2**, 3-dicyano-5, 6-dichlorobenzoquinone (DDQ) (8.0 mmol) was added at ice bath conditions and stirred at 40 °C for 5 h. The reaction mixture was evaporated to remove

toluene. Then, the crude product was purified by silica gel chromatography (PE/EtOAc = 10:1) to afford **3** (isolated yield 85.0%).

General Procedure for the Synthesis of AMG-1–AMG-16. BTC (0.20 mmol) was added to 10 mL of anhydrous CH_2Cl_2 . A solution of **2** or **3** (0.20 mmol) and Et_3N (0.18 mmol) in 5 mL of anhydrous CH_2Cl_2 was added dropwise to the above mixture at ice bath conditions and stirred at room temperature for another 4 h. Then, a solution of AMG or BMG (0.1 mol) and 4-dimethylaminopyridine (DMAP, catalytic amount) in 5 mL of anhydrous CH_2Cl_2 was added dropwise to the above mixture at ice bath conditions. After a 6 h reaction at room temperature, the reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layers were then combined and washed with brine and dried over anhydrous Na_2SO_4 . The solution was evaporated to afford the crude product. Then, the crude product was purified by silica gel column chromatography (PE/EtOAc = 20:1) to give **AMG-1–AMG-16** as a yellow solid (isolation yield 70.0–85.0%).

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl methyl(phenyl)carbamate (AMG-1). ^1H NMR (CDCl_3 , 500 MHz): δ 13.55 (1H, s, 5-OH), 7.46–7.40 (4H, m, Ar-H), 7.32 (1H, t, 7.8 Hz, Ar-H), 7.28 (1H, s, H-1), 6.74 (1H, d, J = 10.2 Hz, H-9), 6.26 (1H, s, H-8), 5.59 (1H, d, J = 10.1 Hz, H-10), 5.20 (1H, brs, H-15), 4.12 (2H, d, J = 4.0 Hz, H-14), 3.46 (3H, s, OCH_3), 3.39 (3H, s, N- CH_3), 1.83 (3H, s, H_3 -18), 1.68 (3H, s, H_3 -17), 1.48 (6H, s, H_3 -12 and H_3 -13). ^{13}C NMR: δ 182.2, 160.3, 158.0, 156.4, 153.8, 152.4, 149.5, 146.7, 142.5, 138.5, 131.9, 130.9, 129.6, 129.3, 127.2, 123.1, 116.3, 115.6, 110.4, 104.5, 104.0, 94.2, 78.1, 61.6, 38.6, 28.4, 26.3, 25.8, 19.2. ESI-MS m/z (%): 540 [$\text{M} - \text{H}$] $^-$.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl ethyl(phenyl)carbamate (AMG-2). ^1H NMR (CD_3COCD_3 , 500 MHz): δ 13.55 (1H, s, 5-OH), 7.45–7.36 (5H, m, Ar-H), 7.38 (1H, s, H-1), 6.70 (1H, d, J = 10.1 Hz, H-9), 6.29 (1H, s, H-8), 5.74 (1H, d, J = 10.1 Hz, H-10), 5.22 (1H, brs, H-15), 4.10 (2H, d, J = 5.0 Hz, H-14), 3.86 (3H, s, OCH_3), 3.60 (2H, q, J = 8.0 Hz, N- CH_2), 1.81 (3H, s, H_3 -18), 1.65 (3H, s, H_3 -17), 1.48 (6H, s, H_3 -12 and H_3 -13), 1.08 (3H, t, J = 8.0 Hz). ^{13}C NMR: δ 182.3, 160.3, 157.8, 156.1, 153.9, 152.1, 150.1, 146.8, 141.1, 138.4, 130.9, 129.3, 128.3, 127.7, 126.6, 123.2, 115.6, 110.4, 104.6, 104.1, 94.2, 78.0, 61.5, 52.7, 46.2, 28.4, 26.4, 25.6, 18.2, 12.8. ESI-MS m/z (%): 556 [$\text{M} + \text{H}$] $^+$.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl methyl(o-tolyl)carbamate (AMG-3). ^1H NMR (CDCl_3 , 500 MHz): δ 13.55 (1H, s, 5-OH), 7.32–7.27 (4H, m, Ar-H), 7.28 (1H, s, H-1), 6.74 (1H, d, J = 10.0 Hz, H-9), 6.26 (1H, s, H-8), 5.58 (1H, d, J = 10.0 Hz, H-10), 5.17 (1H, t, J = 5.5 Hz, H-15), 4.12 (2H, d, J = 5.5 Hz, H-14), 3.36 (3H, s, OCH_3), 3.35 (3H, s, N- CH_3), 2.11 (3H, s, Ar- CH_3), 1.81 (3H, s, H_3 -18), 1.66 (3H, s, H_3 -17), 1.48 (6H, s, H_3 -12 and H_3 -13). ^{13}C NMR: δ 183.4, 161.5, 159.0, 157.5, 154.7, 152.0, 151.3, 146.6, 139.0, 132.1, 131.1, 129.0, 128.8, 127.4, 127.2, 126.4, 123.1, 121.4, 116.1, 115.6, 110.1, 104.5, 104.0, 94.1, 78.1, 61.3, 38.8, 28.4, 28.4, 27.0, 26.0, 21.1, 18.2. ESI-MS m/z (%): 554 [$\text{M} - \text{H}$] $^-$.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-ylmethyl(m-tolyl)carbamate (AMG-4). ^1H NMR (CD_3COCD_3 , 500 MHz): δ 13.63 (1H, s, 5-OH), 7.47–7.31 (4H, m, Ar-H), 7.38 (1H, s, H-1), 6.70 (1H, d, J = 10.0 Hz, H-9), 6.31 (1H, s,

H-8), 5.73 (1H, d, J = 10.0 Hz, H-10), 5.26 (1H, t, J = 5.5 Hz, H-15), 4.17 (2H, d, J = 5.5 Hz, H-14), 3.16 (3H, s, OCH₃), 2.83 (3H, s, N-CH₃), 2.07 (3H, s, Ar-CH₃), 1.83 (3H, s, H₃-18), 1.67 (3H, s, H₃-17), 1.49 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 183.4, 161.6, 159.0, 157.6, 154.8, 152.4, 148.1, 146.7, 142.4, 139.2, 138.4, 132.1, 129.8, 129.0, 127.1, 123.2, 123.2, 116.2, 115.9, 115.6, 111.9, 105.1, 104.7, 95.0, 79.2, 62.2, 38.6, 28.4, 28.4, 26.3, 25.8, 21.3, 18.2. ESI-MS m/z : 556 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-ylmethyl(*p*-tolyl)carbamate (AMG-5). ¹H NMR (CDCl₃ 500 MHz): δ 13.55 (1H, s, 5-OH), 7.29 (2H, d, J = 8.0 Hz, Ar-H), 7.28 (1H, s, H-1), 7.22 (2H, d, J = 8.0 Hz, Ar-H), 6.74 (1H, d, J = 10.0 Hz, H-9), 6.26 (1H, s, H-8), 5.73 (1H, d, J = 10.0 Hz, H-10), 5.25 (1H, brs, H-15), 4.17 (2H, d, J = 3.5 Hz, H-14), 3.19 (3H, s, OCH₃), 3.11 (3H, s, N-CH₃), 2.38 (3H, s, Ar-CH₃), 1.83 (3H, s, H₃-18), 1.67 (3H, s, H₃-17), 1.48 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 177.4, 157.7, 156.2, 152.8, 152.7, 152.6, 149.6, 148.0, 146.6, 138.4, 131.9, 129.9, 129.8, 126.3, 123.6, 118.7, 116.2, 111.9, 110.9, 110.2, 110.0, 99.6, 77.6, 62.4, 38.6, 28.3, 28.3, 26.1, 25.8, 21.0, 18.1. ESI-MS m/z (%): 556 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(2-methoxyphenyl)(methyl)carbamate (AMG-6). ¹H NMR (CDCl₃ 500 MHz): δ 13.55 (1H, s, 5-OH), 7.34 (2H, m, Ar-H), 7.28 (1H, s, H-1), 7.00 (2H, m, Ar-H), 6.74 (1H, d, J = 10.0 Hz, H-9), 6.26 (1H, s, H-8), 5.68 (1H, d, J = 10.0 Hz, H-10), 5.17 (1H, t, J = 5.5 Hz, H-15), 4.06 (2H, d, J = 5.5 Hz, H-14), 3.92 (3H, s, OCH₃), 3.49 (3H, s, OCH₃), 3.42 (3H, s, N-CH₃), 1.83 (3H, s, H₃-18), 1.66 (3H, s, H₃-17), 1.47 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 182.3, 160.2, 158.0, 156.4, 155.1, 153.2, 153.0, 149.8, 146.6, 138.2, 131.8, 131.0, 129.2, 128.8, 127.1, 123.0, 120.8, 115.9, 115.6, 111.8, 110.1, 104.5, 104.0, 94.1, 78.0, 61.2, 55.6, 37.7, 28.4, 28.4, 26.3, 25.8, 18.1. ESI-MS m/z : 572 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(3-methoxyphenyl)(methyl)carbamate (AMG-7). ¹H NMR (CDCl₃ 500 MHz): δ 13.55 (1H, s, 5-OH), 7.35–7.25 (2H, m, Ar-H), 7.28 (1H, s, H-1), 7.02–6.87 (2H, m, Ar-H), 6.74 (1H, d, J = 10.0 Hz, H-9), 6.26 (1H, s, H-8), 5.68 (1H, d, J = 10.0 Hz, H-10), 5.16 (1H, brs, H-15), 4.11 (2H, d, J = 3.5 Hz, H-14), 3.84 (3H, s, OCH₃), 3.49 (3H, s, OCH₃), 3.45 (3H, s, N-CH₃), 1.83 (3H, s, H₃-18), 1.63 (3H, s, H₃-17), 1.49 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 182.2, 160.3, 160.2, 158.0, 156.4, 153.8, 152.4, 149.5, 146.7, 143.6, 138.5, 131.9, 129.9, 127.3, 127.2, 123.2, 123.1, 116.3, 115.6, 112.6, 110.5, 104.5, 104.0, 94.2, 78.1, 61.2, 55.5, 38.6, 28.4, 28.4, 26.4, 25.8, 18.2. ESI-MS m/z : 572 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(4-methoxyphenyl)(methyl)carbamate (AMG-8). ¹H NMR (CDCl₃ 500 MHz): δ 13.55 (1H, s, 5-OH), 7.32 (2H, d, J = 8.0 Hz, Ar-H), 7.27 (1H, s, H-1), 6.94 (2H, d, J = 8.0 Hz, Ar-H), 6.74 (1H, d, J = 10.0 Hz, H-9), 6.26 (1H, s, H-8), 5.58 (1H, d, J = 10.0 Hz, H-10), 5.18 (1H, brs, H-15), 4.11 (2H, d, J = 3.5 Hz, H-14), 3.83 (3H, s, OCH₃), 3.48 (3H, s, OCH₃), 3.38 (3H, s, N-CH₃), 1.82 (3H, s, H₃-18), 1.67 (3H, s, H₃-17), 1.48 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 182.2, 160.2, 159.7, 158.0, 156.4, 153.8, 152.7, 149.5, 146.7, 138.4, 131.9, 127.7, 127.2, 123.2, 123.1, 116.2, 115.6, 114.5, 110.5, 104.5,

104.0, 94.1, 78.1, 61.6, 55.5, 38.9, 28.4, 28.4, 26.3, 25.8, 18.2. ESI-MS m/z (%): 570 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(3-ethylphenyl)(methyl)carbamate (AMG-9). ¹H NMR (CD₃COCD₃ 500 MHz): δ 13.55 (1H, s, 5-OH), 7.35–7.14 (4H, m, Ar-H), 7.26 (1H, s, H-1), 6.73 (1H, d, J = 10.0 Hz, H-9), 6.25 (1H, s, H-8), 5.58 (1H, d, J = 10.0 Hz, H-10), 5.20 (1H, brs, H-15), 4.11 (2H, d, J = 3.5 Hz, H-14), 3.50 (3H, s, OCH₃), 3.46 (3H, s, N-CH₃), 2.68 (2H, m, Ar-CH₂), 1.82 (3H, s, H₃-18), 1.67 (3H, s, H₃-17), 1.48 (6H, s, H₃-12 and H₃-13), 1.26 (3H, t, J = 7.5 Hz, CH₃). ¹³C NMR: δ 182.3, 160.3, 158.0, 156.4, 153.8, 152.6, 149.6, 146.6, 145.6, 142.5, 138.5, 131.9, 129.1, 127.2, 127.2, 123.0, 123.0, 116.2, 115.6, 115.6, 110.4, 104.5, 104.0, 94.2, 78.1, 61.6, 38.4, 30.9, 28.4, 28.4, 26.4, 25.8, 18.2, 15.4. ESI-MS: 568 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(3-chlorophenyl)(methyl)carbamate (AMG-10). ¹H NMR (CDCl₃ 500 MHz): δ 13.52 (1H, s, 5-OH), 7.47 (1H, d, J = 1.5 Hz, Ar-H), 7.44–7.31 (2H, m, Ar-H), 7.28 (1H, s, H-1), 7.24 (1H, m, Ar-H), 6.73 (1H, d, J = 10.0 Hz, H-9), 6.26 (1H, s, H-8), 5.58 (1H, d, J = 10.0 Hz, H-10), 5.21 (1H, brs, H-15), 4.12 (2H, d, J = 5.5 Hz, H-14), 3.56 (3H, s, OCH₃), 3.46 (3H, s, N-CH₃), 1.83 (3H, s, H₃-18), 1.68 (3H, s, H₃-17), 1.48 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 182.2, 160.3, 158.0, 156.4, 153.8, 152.4, 149.2, 146.7, 143.6, 138.7, 134.7, 134.7, 132.1, 130.2, 127.3, 123.1, 123.0, 116.5, 115.7, 115.6, 110.5, 104.5, 104.0, 94.2, 78.1, 61.7, 38.4, 28.4, 28.4, 26.3, 25.8, 18.2. ESI-MS m/z : 574 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(4-chlorophenyl)(methyl)carbamate (AMG-11). ¹H NMR (CDCl₃ 500 MHz): δ 7.40 (2H, d, J = 8.5 Hz, Ar-H), 7.36 (2H, d, J = 8.5 Hz, Ar-H), 7.28 (1H, s, H-1), 6.73 (1H, d, J = 10.0 Hz, H-9), 6.26 (1H, s, H-8), 5.58 (1H, d, J = 10.0 Hz, H-10), 5.20 (1H, brs, H-15), 4.11 (2H, d, J = 5.5 Hz, H-14), 3.50 (3H, s, OCH₃), 3.43 (3H, s, N-CH₃), 1.83 (3H, s, H₃-18), 1.68 (3H, s, H₃-17), 1.48 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 182.2, 160.2, 158.0, 156.4, 153.7, 152.1, 149.3, 146.6, 141.2, 138.4, 132.0, 129.4, 127.2, 123.1, 123.0, 116.2, 115.6, 115.5, 110.5, 104.8, 104.1, 94.2, 78.1, 61.4, 37.4, 28.4, 28.4, 26.3, 25.8, 18.2. ESI-MS m/z : 576 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(2-bromophenyl)(methyl)carbamate (AMG-12). ¹H NMR (CDCl₃ 500 MHz): δ 13.54 (1H, s, 5-OH), 7.48–7.71 (1H, d, J = 8.0 Hz, Ar-H), 7.47–7.42 (2H, m, Ar-H), 7.32 (1H, s, H-1), 7.24 (1H, d, J = 7.5 Hz, Ar-H), 6.73 (1H, d, J = 10.0 Hz, H-9), 6.25 (1H, s, H-8), 5.58 (1H, d, J = 10.0 Hz, H-10), 5.17 (1H, t, J = 7.5 Hz, H-15), 4.10 (2H, d, J = 7.5 Hz, H-14), 3.50 (3H, s, OCH₃), 3.37 (3H, s, N-CH₃), 1.81 (3H, s, H₃-18), 1.66 (3H, s, H₃-17), 1.48 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 182.2, 160.2, 158.0, 156.4, 153.7, 152.1, 149.3, 146.6, 141.2, 138.4, 133.6, 131.9, 129.7, 129.4, 128.7, 127.2, 123.1, 123.0, 116.3, 115.6, 110.3, 104.5, 104.0, 94.2, 78.1, 61.5, 38.6, 28.4, 28.4, 26.4, 25.8, 18.2. ESI-MS m/z : 620 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(3-bromophenyl)(methyl)carbamate (AMG-13). ¹H NMR (CDCl₃ 500 MHz): δ 13.52 (1H, s, 5-OH), 7.46–7.60 (1H, d, J = 1.5 Hz, Ar-H), 7.45–7.24 (3H, m, Ar-H), 7.28 (1H, s, H-1), 6.73 (1H, d, J = 10.0 Hz, H-9), 6.26 (1H, s, H-8), 5.58

(1H, d, $J = 10.0$ Hz, H-10), 5.20 (1H, brs, H-15), 4.11 (2H, d, $J = 4.0$ Hz, H-14), 3.49 (3H, s, OCH₃), 3.43 (3H, s, N-CH₃), 1.83 (3H, s, H₃-18), 1.68 (3H, s, H₃-17), 1.48 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 182.1, 160.3, 158.0, 156.4, 153.8, 152.2, 149.2, 146.7, 143.7, 138.6, 132.0, 130.5, 127.2, 127.2, 123.0, 123.0, 122.5, 116.3, 115.6, 115.6, 110.5, 104.6, 104.0, 94.2, 78.1, 61.7, 38.4, 28.4, 26.4, 25.8, 18.2. ESI-MS m/z : 620 [M + H]⁺.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(4-bromophenyl)(methyl)carbamate (AMG-14). ¹H NMR (CDCl₃ 500 MHz): δ 13.52 (1H, s, 5-OH), 7.55 (2H, d, $J = 8.5$ Hz, Ar-H), 7.28 (1H, s, H-1), 7.23 (2H, d, $J = 8.5$ Hz, Ar-H), 6.73 (1H, d, $J = 10.0$ Hz, H-9), 6.26 (1H, s, H-8), 5.58 (1H, d, $J = 10.0$ Hz, H-10), 5.19 (1H, brs, H-15), 4.11 (2H, d, $J = 4.0$ Hz, H-14), 3.49 (3H, s, OCH₃), 3.43 (3H, s, N-CH₃), 1.83 (3H, s, H₃-18), 1.68 (3H, s, H₃-17), 1.48 (6H, s, H₃-12 and H₃-13). ¹³C NMR: δ 182.1, 160.3, 158.0, 156.4, 153.8, 152.2, 149.2, 146.7, 141.5, 138.6, 132.3, 132.0, 127.2, 123.0, 123.0, 116.3, 115.6, 115.6, 110.5, 104.5, 104.0, 94.2, 78.1, 61.7, 38.4, 28.4, 26.3, 25.8, 18.2. ESI-MS m/z : 618 [M - H]⁻.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(4-butylphenyl)(methyl)carbamate (AMG-15). ¹H NMR (CDCl₃ 500 MHz): δ 13.55 (1H, s, 5-OH), 7.29 (2H, d, $J = 8.0$ Hz, Ar-H), 7.28 (1H, s, H-1), 7.23 (2H, d, $J = 8.0$ Hz, Ar-H), 6.74 (1H, d, $J = 10.0$ Hz, H-9), 6.26 (1H, s, H-8), 5.58 (1H, d, $J = 10.0$ Hz, H-10), 5.19 (1H, brs, H-15), 4.11 (2H, d, $J = 4.0$ Hz, H-14), 3.45 (3H, s, OCH₃), 3.41 (3H, s, N-CH₃), 2.62 (2H, t, $J = 7.0$ Hz, Ar-CH₂), 1.83 (3H, s, H₃-18), 1.68 (3H, s, H₃-17), 1.60 (2H, m, CH₂), 1.48 (6H, s, H₃-12 and H₃-13), 1.37 (2H, m, CH₂), 0.94 (3H, t, $J = 7.0$ Hz, CH₃). ¹³C NMR: δ 182.3, 160.3, 158.0, 156.4, 153.8, 152.6, 149.6, 146.7, 140.1, 138.4, 131.9, 129.2, 127.2, 126.4, 123.1, 116.1, 115.8, 115.7, 110.4, 104.5, 104.0, 94.2, 78.1, 61.6, 38.6, 35.2, 33.5, 28.4, 28.4, 26.3, 25.8, 22.3, 18.2, 13.9. ESI-MS m/z : 596 [M - H]⁻.

5-Hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2,6-dihydropyrano[3,2-b]xanthen-9-yl(3-ethoxyphenyl)(methyl)carbamate (AMG-16). ¹H NMR (CDCl₃ 500 MHz): δ 13.55 (1H, s, 5-OH), 7.31–7.28 (4H, m, Ar-H), 7.24 (1H, s, H-1), 6.74 (1H, d, $J = 10.0$ Hz, H-9), 6.26 (1H, s, H-8), 5.58 (1H, d, $J = 10.0$ Hz, H-10), 5.19 (1H, brs, H-15), 4.12 (2H, d, $J = 4.0$ Hz, H-14), 4.06 (2H, q, $J = 7.5$ Hz, OCH₂CH₃), 3.49 (3H, s, OCH₃), 3.44 (3H, s, N-CH₃), 1.83 (3H, s, H₃-18), 1.68 (3H, s, H₃-17), 1.48 (6H, s, H₃-12 and H₃-13), 1.26 (3H, t, $J = 7.5$ Hz, OCH₂CH₃). ¹³C NMR: δ 182.3, 160.2, 159.6, 158.0, 156.4, 153.8, 152.4, 149.5, 146.7, 143.8, 138.4, 131.9, 129.8, 127.3, 127.2, 123.1, 123.0, 116.3, 115.6, 113.2, 110.4, 104.5, 104.0, 94.2, 78.1, 61.6, 58.4, 38.6, 28.4, 28.4, 26.3, 25.8, 18.2, 14.7. ESI-MS m/z : 584 [M - H]⁻.

In Vitro Experiments. Neuroprotective Effect Assay. PC12 cells (pheochromocytoma) were obtained from the Chinese Academy of Medical Sciences, Shanghai Institutes for Biological Sciences. Cells were grown in a culture flask at a humidified atmosphere of 5% CO₂ and in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum supplemented with 100 U/mL penicillin/streptomycin at 37 °C. The culture medium was changed every 2 days. Cells were seeded in 96-well plates (3 × 10⁴ cells per well) 48 h prior to treatment. All compounds were dissolved in dimethyl sulfoxide (DMSO), with the final concentration of DMSO less than 0.1%. PC12 cells were pretreated with various concentrations

of all compounds for 24 h and exposed to 300 μM H₂O₂ for an additional 12 h. The control cells were treated with an equal amount of DMEM. Edaravone was set as a positive drug. The cell viability was assessed by the MTT assay.³⁴

AChE Inhibition Assay. AChE inhibitory activity was studied using Ellman's method with slight modifications.³⁵ Briefly, 220 μL of an assay mixture containing 50 μL of the sample solution, 140 μL of 100 mM phosphate buffer, 10 μL of 2.5 mM DTNB, and 20 μL of 1.0 U/mL AChE solution was added to each well of a 96-well plate. The microplate was incubated at 37 °C for 10 min, and then 40 μL of 7.5 mM AtCHI was added and incubated at 37 °C for 10 min. The absorbance of the mixture was determined at 412 nm using a microplate reader instrument (Multiskan MK3, Thermo Fisher).

Cytotoxicity. The *in vitro* cytotoxicity of α-M, AMG, and 16 derivatives of AMG was evaluated by the MTT assay in PC12 cell lines with edaravone as a positive control. All compounds were prepared by dissolving in a certain amount of DMSO and diluted with culture medium to give a single concentration of 10 μM. PC12 cells were seeded in 96-well plates at a density of 10⁵/well and allowed to accommodate for 24 h in normal conditions. The cultures were then exposed to above compounds in concentrations of 10 μM for 24 h. After 24 h, the compound-treated cells were incubated for 2 h with 200 μL of MTT. Formazan was solubilized with 200 μL of DMSO, and the absorbance of each sample was read at 490 nm using a microplate reader instrument.³⁴

In Vivo Experiments. Animals. We used healthy ICR mice (18–22 g, half male and half female) for the acute toxicity assessment and adult Sprague–Dawley (SD) rats (8 weeks old, 280 ± 10 g) for the pharmacodynamic experiment of AMG-1. All animals were purchased from the Zhejiang Academy of Medical Sciences Laboratory Animal Center (license number: SCXK-20140001). All animals were housed and kept on a 12 h/12 h light/dark cycle in standard conditions with temperature and relative humidity set at 23 ± 2 °C and 55 ± 10%, respectively. All animals had free access to diet and water. Animal sacrifice at the end of the study was performed under deep anesthesia with 5% isoflurane, and then decapitation was performed. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals in the Zhejiang University of Technology, Hangzhou, China, and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996).

Acute Oral Toxicity Study. The acute toxicity test of AMG-1 was performed in healthy ICR mice.³⁶ The mice were randomly divided into seven groups (one control group and six treated groups) of 10 animals. AMG-1 was suspended in 0.5% carboxymethyl cellulose solution, and the mice were exposed to a single dose of AMG-1 1310, 1638, 2048, 2560, 3200, and 4000 mg/kg body weight by the oral gavage, which were determined through respective pretesting. Control groups were administered a 0.5% carboxymethyl cellulose solution equally. Then, mice that survived were observed for 120 min and once daily for the next 14 day after the treatment. The general behavior, signs of toxicity, and mortality of the mice were assessed carefully. The number of survivors after the 14 day period was noted. The toxicological effect was evaluated based on the mortality for 14 day, which was expressed by the median lethal dose value (LD₅₀) estimated from the regression of the log-probit mortality rate.

Surgery and Drug Treatment. The experimental procedure for creating AD model rats was slightly modified from that in the previously reported study.³⁷ In brief, $A\beta_{25-35}$ was first dissolved in sterile 0.9% saline at a concentration of 5 $\mu\text{g}/\mu\text{L}$ and incubated at 37°C for 4 days to allow aggregation before the hippocampal injection. Rats were weighed and general anesthetized with 5% isoflurane and fixed into a stereotaxic apparatus. A burr hole was drilled through the skull above the bilateral hippocampal coordinates (anterior–posterior (AP) = −3.5 mm, medial–lateral (ML) = ± 2.5 mm from the bregma, and dorsal–ventral (DV) = 3.0 mm from the skull surface) according to the stereotaxic atlas. $A\beta_{25-35}$ (2 μL containing 10 μg) was subsequently injected over 5 min through a microsyringe into the hole. The needle was left for an additional 5 min before the withdrawal. Sham-vehicle rats underwent the same surgical procedure, except that normal saline (as a vehicle of $A\beta_{25-35}$) was bilaterally injected. After 1 day of $A\beta_{25-35}$ hippocampal injection, all operated rats were randomly divided into six groups ($n = 20$) and administered orally different drug treatments once daily for 30 days (for experimental schedules, see Supporting Figure 1): sham control group (vehicle only), positive group (1 mg/kg donepezil), $A\beta_{25-35}$ -induced model group (vehicle only), $A\beta_{25-35}$ + AMG-1 5 mg/kg group, $A\beta_{25-35}$ + AMG-1 10 mg/kg group, and $A\beta_{25-35}$ + AMG-1 20 mg/kg group.

Open-Field Test for the Locomotor Ability. After AMG-1 administration for 30 days, rats were subjected to an open-field test to evaluate the exploratory activity and locomotor ability.³⁸ The open-field apparatus was a clear cubic box with a Plexiglas floor, was 100 $\times$ 100 $\times$ 50 cm^3 in size (BW-OF362, Shanghai, China), and was dimly illuminated during the test. After 1 h of acclimation in the dim room, each rat was placed in the same place of the open arena and allowed to freely explore for 2 min. The floor and walls were cleaned with 70% ethanol to eliminate the odor between each trial. The rats' behavior was recorded by a video digital recorder suspended 1.8 m above the area. The locomotor activity was analyzed by the total distance traveled (m).

Morris Water Maze. The spatial learning and memory ability of all rats was tested by the Morris water maze (XR-XM101, Shanghai, China).³⁹ Briefly, a circular pool (180 cm in diameter and 60 cm in depth) was filled with water and maintained at 25 ± 1 °C. Animals were trained to seek an escape platform (25 cm^2 Plexiglas square, 2 cm below the surface of the water in one of the four quadrants) with four trials for 2 min per trial with a 30 min interval between the trials, daily, for 5 consecutive days. On locating the platform, the rat was allowed to remain there for 30 s before being returned to its cage. If the rat did not find the platform within 2 min, the time was recorded as 2 min. The rat was then manually guided to the platform where it stayed for 30 s. The escape latency and swim pattern were measured by a video tracking system for the analysis of performance. After completion of the escape latency test, rats were subjected to a probe trial in which the platform was removed from the pool and replaced by a visible platform. Rats were placed in the pool at the same pole and allowed to swim for 2 min. The number of rats crossing the target quadrant was recorded.

Tissue Preparation. On the terminal day, the rats were deeply anesthetized with 5% isoflurane. The whole brains of eight rats were isolated after performing intracardiac perfusion with 100 mmol/L phosphate-buffered saline (PBS). Then, the brains were quickly removed and the brain tissues were fixed in

10% neutral buffered formalin, dehydrated, and embedded in paraffin for coronal microtome sections with hematoxylin staining and Bielschowsky silver staining. Similarly, the whole brains of six rats perfused with PBS, followed by ice-cold 4% paraformaldehyde, were sectioned into 30 μm slices for immunohistochemical staining. The remaining brains of the rats were isolated and postfixed in 50 mmol/L PBS containing 4% paraformaldehyde overnight, immersed in a solution containing 30% sucrose in 50 mmol/L PBS, and stored at 4 °C until sectioning. The frozen brains were sectioned by a freezing microtome (Thermo Fisher) at 25 μm for Nissl staining.

Hematoxylin and Eosin (HE) Staining. After animal behavior test experiments, four rats in each group were chosen randomly to be sacrificed. Their brains were quickly removed and fixed in 4% formalin. The brains were then dehydrated in alcohol and embedded in paraffin for coronal microtome sections (5 μm thick) with HE staining for histomorphological study under the light microscope (200 $\times$, Nikon, Japan).⁴⁰

Nissl Staining. Hippocampi of brain tissues of six rats were cut into 25 μm thick sections by a freezing microtome (Thermo Fisher) and series stained using a Nissl staining solution (Beyotime Institute of Biotechnology, Nantong, China) according to the manufacturer's instructions. In brief, the sections were mounted onto gelatin-coated slides. After being dipped in 98% EtOH for 2 min, slides were immersed in xylene for 2 min and rehydrated in a series of concentrations of EtOH from 98 to 70 to 50 to 20 to 0% (distilled water) for 2 min each. Sections were then stained with 2% cresyl violet for 5 min. After two dips in distilled water, sections were dehydrated with a series of concentrations of EtOH from 50 to 70 to 98% and then were cleaned in xylene and mounted with Permount. At the end of staining, the neuronal survival and loss of Nissl bodies in the hippocampus were observed using light microscopy (Nikon, Japan).⁴¹

Bielschowsky Silver Staining. Bielschowsky silver staining was used to assess the $A\beta$ plaques and neurofibrillary tangles (NFTs), the hallmark lesions of AD. Representative tissue sections were stained by a modified Bielschowsky method.⁴² Briefly, coronal brain sections (40 μm) were cut with the microtome, and the rehydrated sections were covered with 20% AgNO_3 in the dark for 20 min, followed by rinsing in distilled water and incubation in the ammoniacal silver solution for 10 min in the dark. After the incubation, the slides were immersed in ammoniated water and developed. Finally, sections were rinsed, dehydrated, cleared, and coverslipped. Positive silver-stained neurons were counted and photographed under 10 $\times$ 40 magnification with light microscopy (Nikon, Japan).

Immunohistochemistry Analysis for $A\beta_{1-42}$. Immunohistochemistry for $A\beta_{1-42}$ was performed as described.⁴³ Briefly, hippocampal sections of five rats were deparaffinized in xylene and hydrated through graded alcohol. Endogenous peroxidase was blocked with 3% hydroxide in 10% methanol. Then, sections were incubated in blocking serum, 5% normal goat serum in 0.3% Triton with PBS. Subsequently, sections were incubated in a primary antibody anti- $A\beta_{1-42}$ (rabbit anti-rat amyloid β protein 1-42 monoclonal antibody 1:300 dilution, Dakopatts A/C; Bioss China) solution overnight at 4 °C. Sections were washed in PBS with 0.3% Triton X-100 and incubated in a secondary antibody (biotinylated goat anti-rabbit antibody, 1:500) solution for 1 h at room temperature, which was followed by washing and staining with DAB

(1:1000) for another 10 min at room temperature. Stained sections were mounted on resin-coated slides and dried. Photomicrographs of brain sections were taken using a digital camera (Nikon, Japan), and at least 20 fields from each brain section were analyzed using the Image J software.

Chemogenomics Databases and Tools. Chemogenomics Database for Alzheimer's Disease (<http://www.cbligand.org/AD/>),⁴⁴ Chemogenomics Database for Drug Abuse Research (<http://www.cbligand.org/CDAR/>),⁴⁵ and Chemogenomics Database for Cardiovascular Disease (<http://www.cbligand.org/CVD/>)⁴⁶ were combined for the target prediction on α -mangostin and AMG-1. Two chemogenomics tools, TargetHunter⁴⁷ and HTDocking,⁴⁸ were used specifically for the identification of both therapeutic targets and off-target interactions. The inquiry compounds were α -mangostin (1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methylbut-2-en-1-yl)-9H-xanthen-9-one) and AMG-1 (5-hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-en-1-yl)-6-oxo-2H,6H-pyrano[3,2-b]xanthen-9-yl methyl(phenyl)carbamate). For TargetHunter, the fingerprint type was set to be extended-connectivity fingerprints 6 (ECFP6).^{49,50} The threshold for 2D similarity was set to be 60%. For HTDocking, high-throughput molecular docking studies were performed between the inquired compounds and protein targets in each database. Networks were constructed using Cytoscape.⁵¹ Both the *in silico* prediction and the reported experimental validations were combined. The layout format was set to the degree-sorted circle.

Blood–Brain Barrier (BBB) Prediction, PAINS-Remover, and Toxtree. The BBB Prediction Server (<http://www.cbligand.org/BBB/>)^{44,52} was used for the *in silico* evaluation of whether the inquired compounds can pass the BBB. For each compound, four fingerprint types, MACCS, Openbabel (FP2), Molprint 2D, and PubChem, were applied. For each fingerprint type, two algorithms, AdaBoost and SVM, were adapted. A total of eight combinations of fingerprints and algorithms were calculated for both α -mangostin and AMG-1. PAINS-Remover (<http://www.cbligand.org/PAINS/>)⁵³ was used to detect and remove the pan-assay interference compounds (PAINS). Both α -mangostin and AMG-1 were analyzed to identify the existence of the undesired substructural features.

Toxtree (<http://toxtree.sourceforge.net/index.html>)⁵⁴ was used to estimate any toxic hazard by applying a tree approach. The SMILES formats of α -mangostin and AMG-1 functioned as the input. Three levels, low (Class I), intermediate (Class II), and high (Class III), of estimated toxic hazards were reported.

Statistical Analysis. Data were analyzed using one-way ANOVA, followed by the least significant difference post-hoc tests or the independent samples *t*-tests. The results are expressed as mean $\pm$ SEM. Differences between groups were considered statistically significant when $P < 0.05$. All statistical analyses were performed using the SPSS 19.0 software (SPSS Inc; 19.0).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c00057>.

Animal study timeline (Figure S1); ¹H and ¹³C NMR spectra of all of the reported synthesized compounds (Figures S2–S33) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Zha-Jun Zhan – College of Pharmacology Sciences Zhejiang University of Technology, Hangzhou 310014, P. R. China; orcid.org/0000-0001-7865-5435; Phone: 86(571)-88871075; Email: zjnpr@zjut.edu.cn

Authors

Yan Chen – College of Pharmacology Sciences Zhejiang University of Technology, Hangzhou 310014, P. R. China; Department of Pharmaceutical Sciences and Computational Chemical Genomics Screening Center, School of Pharmacy and NIH National Center of Excellence for Computational Drug Abuse Research, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, United States

Yue-min Bian – Department of Pharmaceutical Sciences and Computational Chemical Genomics Screening Center, School of Pharmacy and NIH National Center of Excellence for Computational Drug Abuse Research, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, United States

Jian-Wei Wang – College of Pharmacology Sciences Zhejiang University of Technology, Hangzhou 310014, P. R. China

Ting-Ting Gong – College of Pharmacology Sciences Zhejiang University of Technology, Hangzhou 310014, P. R. China

You-Min Ying – College of Pharmacology Sciences Zhejiang University of Technology, Hangzhou 310014, P. R. China; orcid.org/0000-0002-2231-5068

Lie-Feng Ma – College of Pharmacology Sciences Zhejiang University of Technology, Hangzhou 310014, P. R. China; orcid.org/0000-0001-7711-0767

Wei-Guang Shan – College of Pharmacology Sciences Zhejiang University of Technology, Hangzhou 310014, P. R. China

Xiang-Qun Xie – Department of Pharmaceutical Sciences and Computational Chemical Genomics Screening Center, School of Pharmacy, NIH National Center of Excellence for Computational Drug Abuse Research, Drug Discovery Institute, and Departments of Computational Biology and Structural Biology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, United States; orcid.org/0000-0002-6881-6175

Complete contact information is available at:
<https://pubs.acs.org/doi/10.1021/acsomega.0c00057>

Author Contributions

*Y.C. and Y.B. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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