Dual GSK-3 β /AChE Inhibitors as a New Strategy for Multitargeting Anti-Alzheimer's Disease Drug DiscoveryXue-Yang Jiang,[†] Ting-Kai Chen,[†] Jun-Ting Zhou,[†] Si-Yu He,[‡] Hong-Yu Yang,[‡] Yao Chen,[§] Wei Qu,[†] Feng Feng,^{*,†} and Hao-Peng Sun^{*,‡,§}[†]Department of Natural Medicinal Chemistry and [‡]Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China[§]School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China

S Supporting Information

ABSTRACT: Designing multitarget-directed ligands (MTDLs) is considered to be a promising approach to address complex and multifactorial maladies such as Alzheimer's disease (AD). The concurrent inhibition of the two crucial AD targets, glycogen synthase kinase-3 β (GSK-3 β) and human acetylcholinesterase (hAChE), might represent a breakthrough in the quest for clinical efficacy. Thus, a novel family of GSK-3 β /AChE dual-target inhibitors was designed and synthesized. Among these hybrids, **2f** showed the most promising profile as a nanomolar inhibitor on both hAChE (IC_{50} = 6.5 nM) and hGSK-3 β kinase activity (IC_{50} = 66 nM). It also showed good inhibitory effect on β -amyloid self-aggregation (inhibitory rate = 46%) at 20 μ M. Western blot analysis revealed that compound **2f** inhibited hyperphosphorylation of tau protein in mouse neuroblastoma N2a-Tau cells. *In vivo* studies confirmed that **2f** significantly ameliorated the cognitive disorders in scopolamine-treated ICR mice and less hepatotoxicity than tacrine. This study provides new leads for assessment of GSK-3 β and AChE pathway dual inhibition as a promising strategy for AD treatment.

KEYWORDS: Dual GSK-3 β /AChE inhibitor, hybrid, multitarget direct ligands (MTDLs)

Among all the neurodegenerative diseases, Alzheimer's disease (AD), an irreversible progressive neurodegenerative disorder of the central nervous system (CNS), is described by a progressive loss of cognitive abilities in old age people.¹ Due to the very limited number of available drugs and their low efficacy, AD remains incurable. Aberrant protein processing is a distinctive feature of AD in which β -amyloid ($A\beta$) and abnormally hyperphosphorylated tau protein misfold and self-accumulate in the brains of affected individuals as aggregates, namely, amyloid senile plaques and neurofibrillary tangles (NFTs), respectively.^{2,3} These assemblies represent the most relevant histopathological hallmarks of AD and have been considered to play crucial roles in its pathogenesis. A series of hypotheses have been proposed, among which tau hypothesis, amyloid cascade hypothesis, and cholinergic hypothesis are of the central importance.⁴

Tau is a microtubule-associated protein mostly found in neuronal axons in CNS.⁵ Under normal physiological conditions, tau is associated with microtubules by preventing their dynamic instability, contributing to morphogenesis of neurons.⁶ Aberrant

hyperphosphorylation of the tau protein loses its ability to stabilize microtubules, which leads to accumulation and formation of NFTs.⁷ As for the tau hypothesis, glycogen synthase kinase-3 β (GSK-3 β), a multitasking serine/threonine kinase largely expressed in CNS, has been proved to play a significant role in regulating tau phosphorylation mainly at Ser396, Ser199, and Ser413, which causes tau to be detached from the microtubules and precipitates as NFTs under both physiological and pathological conditions.⁸ Furthermore, increased GSK-3 β activity also induces $A\beta$ formation in a unique fashion by regulating γ -secretase and results in toxicity to the cultured neurons.⁹ A conditional GSK-3 β overexpressing transgenic mouse exhibited microtubule destabilization, NFTs formation, and cognitive deficits.¹⁰ Both preclinical and early phase clinical studies have validated GSK-3 β as a therapeutic target for AD.

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Figure 1. Design strategy of dual GSK-3 β /AChE inhibitors. (A) Design of a merged GSK-3 β /AChE pharmacophore taking advantage of the solvent exposed sites in GSK-3 β and the probable interactions to be gained by a merged tacrine at the entrance to AChE substrate binding pocket. (B) X-ray cocrystal structure of **1** in the kinase domain of GSK-3 β . Intermolecular interactions are shown as dotted lines with different colors according to the type of the interaction: green, conventional hydrophobic contact; dark pink, π - π stacked; light pink, π -alkyl contact. The thiazolyl methoxy and the primary amide moiety extend into the solvent-exposed region of the protein (red circle).

Recent findings have shown that GSK-3 β inhibitors (GSK-3 β Is) can be a route to shift the equilibrium from neurodegeneration to neurogenesis both *in vitro* and *in vivo*.¹¹ Clearly, hindering generation of A β and hyperphosphorylated tau protein by inhibiting GSK-3 β activity has been envisaged as a suitable strategy for AD treatment, and thus, the development of selective GSK-3 β Is has been popularly pursued (Figure S1).^{12,13} Up until now, a series of new promising high affinity ATP-competitive GSK-3 β I with the basis of a pyridothiazole core has been synthesized.¹⁴

However, the decline of acetylcholine (ACh) levels leads to cognitive and memory deficits; thus, recovering cholinergic function is considered to be clinically beneficial.¹⁵ Several studies have also indicated that AChE seems to be implicated in AD pathogenesis by promoting the formation of both A β fibrils.¹⁶ Fortunately, acetylcholinesterase inhibitors (AChEIs) possibly affect metabolic processing of the APP and thus may influence A β generation.¹⁷ It is worth emphasizing that although many hypotheses and strategies are currently being proposed for the treatment of AD, AChEIs still remain to be a supreme clinical success in AD treatment, which fully demonstrates the value of this target.¹⁸ However, the cholinergic deficit is just one of the hallmarks of the AD pathology. One of the reasons that the single-target directed drugs have failed to reach clinical trials is the pathological complexity found in AD. An open-label study found that the combination of cholinesterase inhibitors and memantine was well tolerated in dementia therapy.¹⁹ As a new paradigm in drug discovery for AD, compared to combination therapies, multifunctional molecules as multitarget direct ligands (MTDL) avoid drug–drug interactions, off-target adverse effects, poor patient compliance, and high development costs.²⁰ Given the fact that AD is a systemic disorder of the central nervous system, multitarget strategies will be more promising. As an AChEI with definite efficacy and binding mode, tacrine is a good scaffold for the design of MTDLs due to its simple structure but high ligand efficiency.^{21,22} Moreover, tacrine has a good endurance against substantial structural modification while retaining the target-based activity and further provides a sound basis for the design of MTDLs.^{23,24} In addition, the more

potent AChEI 6-Cl-tacrine and the less hepatotoxic 7-MeO-tacrine have provided useful scaffolds to generate new hybrids.²⁵

Given the aforementioned evidence, GSK-3 β and AChE, the two main pathways of AD, are ideal candidates for such multitarget approach. Their activities are deeply involved in AD pathogenesis and progression: the cholinergic deficit and NFTs, the two main AD pathological hallmarks. Therefore, the simultaneous modulation of both GSK-3 β and AChE, by blocking hyperphosphorylated tau protein and aggregation of A β plaques, as well as improving cognition, might serve as a promising strategy for AD treatment. Herein, we designed the first GSK-3 β /AChE bifunctional inhibitors by hybridizing the pharmacophores of GSK-3 β I with AChEI. The rationale for the MTDL design originated from the cocrystal structure of the selective GSK-3 β I **1** with the ATP-binding site of GSK-3 β kinase domain. The pyridine carboxamide of **1** acted as the hinge binding head forming hydrogen bonds with the V135 backbone amide and thiazole ring as hinge group, and the carbonyl oxygen of the thiazolyl primary amide formed a critical hydrogen bond with the Lys85 (Figure 1B).¹⁴ It should be noted that the thiazolyl methoxy moiety and the primary amide are located in the solvent exposed sites, to be the best position to attach tacrine to compound **1** by alkylendiamine tethers so that critical binding interactions were not disrupted. It is important that the designed hybrids have no significant effect on the binding of tacrine to the catalytic active site (CAS) of AChE and that the pyridothiazole fragment acts as a binder for the peripheral anionic site (PAS) and thus may enhance AChE inhibition. Therefore, we incorporated tacrine at the thiazolyl ring of **1** with a proper linker to design a new MTDL (Figure 1A).

The route employed to synthesize target compounds **2a–2k** is outlined in Scheme 1. Pyridothiazole ester **3** was obtained from 2-amino-4-cyanopyridine by acylation, addition, and cyclization,²⁶ which was then used in Mitsunobu reaction with *N*-ethanol tacrine,²⁷ giving compound **2a**. Hydrolysis of the ethyl ester in **2a** to afford **2b**, followed by coupling with NH₄Cl afforded compound **2c** or methylation with MeI gave out compound **2d**. Amination of **4** with the corresponding diamines afforded intermediates **5a–5g**. Mitsunobu reaction of **3** with

Scheme 1. Preparation of Compounds 2a–2k^a

^aReagents and conditions: (a) *N*-ethanol tacrine, PPh₃, DIAD, THF, rt, overnight, 52%; (b) 1.5 N LiOH, MeOH/THF/H₂O, rt, 5 h, 87%; (c) NH₄Cl, HATU, DIPEA, DMF, rt, overnight, 38%; (d) CH₃I, K₂CO₃, DMF, rt, 5 h, 43%; (e) i: anhydrous MeOH, PPh₃, DIAD, THF, rt, 18 h; ii: 1 N LiOH, MeOH/THF, rt, 3 h, 80%; (f) HATU, DIPEA, DMF, rt, 10 h, 31–48%.

MeOH, followed by hydrolysis to yield carboxyl 4.¹⁴ Finally, the carboxylic acid 4 was coupled with tacrine intermediates 5a–5g to produce the target compounds 2e–2k, respectively.

The GSK-3 β enzymatic inhibition of all the target compounds was analyzed using human recombinant GSK-3 β using a luminescence method.²⁸ Compounds 2a–2k displayed potent GSK-3 β kinase inhibitory activities. The IC₅₀ values ranged from 18 to 270 nM, similar to compound 1 (Table 1). These results suggested that the GSK-3 β inhibitory effect was durable by introducing tacrine moiety at the solvent exposure zone of 1. The inhibitory potency was significantly reduced when the amino group (1) was replaced by ethoxy group (2a), carboxyl group (2b) and methoxy group (2d). This indicated that the amino group was crucial for the enzymatic inhibition of 1, probably because the NH of the amide bond forms an intramolecular hydrogen bond with the oxygen atom of the thiazole ring to stabilize the orientation of the amide bond. However, 2c did not show potency against hAChE activity. Given the importance of the amino group on GSK-3 β inhibitory activity, tacrine was introduced in the position of the amide group by alkyl chain, resulting in compounds 2e–2k. As shown in Table 1, compound 2e showed potent inhibitory activity against GSK-3 β , with IC₅₀ value of 68 nM, while 6-chlorotacrine and 7-methoxytacrine hybrids (2h–2k) showed similar GSK-3 β inhibitory effects to 2e, with activities ranging from 63 to 98 nM. These results suggested that GSK-3 β inhibition could rarely be affected by changing the length of the side chain, or by addition of the tacrine moiety.

However, 2h–2k exhibited potent antiproliferative activities against human neuroblastoma SH-SY5Y cell lines (IC₅₀ values ranging from 3.1 to 8.9 μ M), the improved cytotoxicity might limit the further cell-based evaluation. It is noteworthy that 2f and 2g, with an *n*-propane or an *n*-hexane linker, displayed potent GSK-3 β inhibition with IC₅₀ of 66 and 18 nM, respectively. In addition, 2g exhibited an IC₅₀ of 30 μ M against SH-SY5Y cells while 2f exhibited a slightly lower IC₅₀ value of 18 μ M. According to their single- or double-digit nanomolar activities on ChEs and GSK-3 β , these two compounds were thought to own the best safety among all the target molecules to exert their biological effects as dual GSK-3 β /AChE inhibitors. Here we chose 2f as the representative for further investigations.

The inhibitory potency of compounds 2a–2k on hAChE and BuChE from human serum (hBuChE) was tested by Ellman's method (Table 1).²⁹ All tested derivatives showed an IC₅₀ against hAChE in nanomolar range, which were consistent with the data on the inhibition of cholinesterase from the electric eel (see Table S1). We retained the amino group of the GSK-3 β 1 or introduced other groups, including carboxylic acid, methyl ester and ethyl ester, at the formyl side chain. Hybrids exhibited slight hAChE inhibitory activities from 130 to 580 nM. Concerning the effect of the spacer chain length on the anti-AChE activity, we introduced various lengths of alkyl chains as linker to the side chain based on the GSK-3 β 1. As shown in Table 1, the compounds with various linkers exhibited potent the anti-AChE activities (from 2.1 to 280 nM), which verified the flexibility of the side chain in AChE inhibitory activity. It should be noted that 2h–2k, bearing 6-Cl-tacrine or 7-MeO-tacrine fragments, were superior AChEIs to tacrine. Besides, these four compounds exhibited much higher inhibitory potency on AChE than on BuChE, indicating that such modifications could help to design highly selective hAChE inhibitors. The most potent hAChE inhibitor was 2h (IC₅₀ = 2.1 nM), which also had the best selectivity toward AChE with 190-fold better than BuChE. Unfortunately, these hybrids possessed a strong antiproliferative activity against SH-SY5Y cell lines, giving IC₅₀ values spanning from 2.3 to 8.9 μ M. The optimal tethers were two–three carbon chains (2e, 2f) for the thiazolylpyridine hybrids bearing tacrine unit, showing IC₅₀ values of 6.4 and 6.5 nM, respectively.

Considering that roles of AChE and BuChE in the modulation of the central cholinergic tone vary with the progression of the disease, it is conceivable that selective AChEIs are more effective

Table 1. Inhibition of hGSK-3 β , hAChE and hBuChE, A β _{1–42} Self-Aggregation, and Anti-Proliferative Activities^a

compd	R	R'	n	hGSK-3 β (IC ₅₀ nM) ^a	hAChE (IC ₅₀ nM) ^a	hBuChE (IC ₅₀ nM) ^a	SI ^c	A β _{1–42} IR (%) ^d	SH-SY5Y (IC ₅₀ μ M) ^e
2a	OEt			180 $\pm$ 20	310 $\pm$ 20	610 $\pm$ 50	2	30 $\pm$ 2.9	7.1 $\pm$ 0.6
2b	OH			270 $\pm$ 30	580 $\pm$ 50	450 $\pm$ 50	0.8	25 $\pm$ 2.1	21 $\pm$ 1.8
2c	NH ₂			34 $\pm$ 3.1	300 $\pm$ 10	150 $\pm$ 10	0.5	15 $\pm$ 1.8	19 $\pm$ 2.5
2d	OMe			99 $\pm$ 10	130 $\pm$ 7.1	28 $\pm$ 2.3	0.2	47 $\pm$ 5.1	13 $\pm$ 0.9
2e		H	1	68 $\pm$ 5.3	6.3 $\pm$ 0.2	51 $\pm$ 6.1	9	40 $\pm$ 4.3	6.3 $\pm$ 0.5
2f		H	2	66 $\pm$ 6.2	6.4 $\pm$ 0.3	260 $\pm$ 32	43	46 $\pm$ 4.1	18 $\pm$ 1.2
2g		H	5	18 $\pm$ 1.4	22 $\pm$ 2.1	100 $\pm$ 7.9	5	39 $\pm$ 4.1	30 $\pm$ 2.8
2h	6-Cl		1	63 $\pm$ 4.9	2.1 $\pm$ 0.9	410 $\pm$ 39 ^b	190	40 $\pm$ 3.7	2.3 $\pm$ 0.3
2i	6-Cl		2	98 $\pm$ 9.3	3.6 $\pm$ 0.3	290 $\pm$ 23 ^b	80	42 $\pm$ 4.1	3.1 $\pm$ 0.2
2j	7-OMe		1	83 $\pm$ 6.5	23 $\pm$ 1.7	2100 $\pm$ 180 ^b	91	47 $\pm$ 4.5	8.9 $\pm$ 0.9
2k	7-OMe		2	65 $\pm$ 4.7	38 $\pm$ 4.2	2300 $\pm$ 170 ^b	60	45 $\pm$ 4.2	2.9 $\pm$ 0.2
Tac.					230 $\pm$ 31	40 $\pm$ 3.7	0.17	<5	120 $\pm$ 15

^aRecombinant human AChE, BuChE, and GSK-3 β were used. Data are the mean of at least three independent determinations. ^bBuChE (EC 3.1.1.8) from horse serum. ^cSelectivity index (SI) = IC₅₀ hBuChE/IC₅₀ hAChE. ^dInhibition of A β _{1–42} self-aggregation investigated by the thioflavin-T fluorescence assay. Assays were carried out in the presence of 20 μ M inhibitor and 50 μ M A β _{1–42}. ^eSH-SY5Y cells exposing for 48 h to compound.

in the early stages of AD. The best selectivity for *hAChE* was demonstrated by **2h**, which was 190-fold more active toward *hAChE* versus *BuChE*. Targeting *BuChE* has become a viable alternative for the treatment of patients with mild-to-late stages of AD. *BuChE* is primarily expressed and secreted by glial cells, and it is thought to play a compensatory role in response to the decrease of *AChE* activity as AD progresses. Selective inhibition of *BuChE* thus represents a useful strategy to ameliorate the cholinergic deficit and improve the cognitive performance of patients in the late stages of AD. Hybrid **2d** was the most potent and selective *hBuChEI* ($IC_{50} = 28$ nM) of this series, with a 4.6-fold better inhibition. The accumulation of oligomeric aggregates of $A\beta$ is considered to play an important role in the pathogenesis of AD. Thus, the inhibition of $A\beta$ oligomerization has been investigated as an attractive therapeutic strategy for AD treatment. All compounds were evaluated for their inhibitory capacity on self-induced $A\beta_{1-42}$ aggregation based on a thioflavin T-based fluorometric assay. Most of the hybrids showed moderate inhibition on $A\beta_{1-42}$ self-aggregation (ranging from 15 to 47%, Table 1), while tacrine was not able to inhibit $A\beta_{1-42}$ self-aggregation. Considering its better inhibition activity of *AChE*, $A\beta_{1-42}$ self-aggregation, and smaller molecular weight compared to **2g**, we assumed that the most promising achievement of the present study is **2f**, which showed a dual low-nanomolar inhibition profile in *in vitro* *GSK-3 β /AChE* (IC_{50} values of 66 and 6.5 nM, respectively).

To investigate the binding pattern of **2f** with *hAChE*, molecular docking studies were performed using Discovery Studio (DS). As shown in Figure 2, **2f** bound to *AChE* in a dual-

Figure 2. Binding mode prediction of **2f** with *hAChE* (PDB id: 4EY7). (A) Compound **2f** was shown in stick mode colored in green. Key residues were labeled as thin stick mode colored in orange. Intermolecular interactions were shown with different colored dotted lines: light green, hydrophobic contact; yellow, π -cation; purple, π -alkyl contact. (B) Lineweaver–Burk plots resulting from subvelocity curves of *AChE* activity with different substrate concentrations (25–450 μ M) in the absence and presence of 1, 5, 10, and 20 nM **2f**.

site manner by occupying both CAS and PAS. The location and orientation of **2f** in *AChE* active-site gorge was consistent with the critical role of tacrine fragments, which binds at the anionic site by stacking against the aromatic rings of Trp86 and Tyr124. The endocyclic nitrogen of **2f** was hydrogen bonded to the main-chain carbonyl oxygen of the catalytic residue His447, indicating that the catalytic triad was disrupted. It was noticeable that pyridothiazole moiety was located at the PAS of *hAChE* binding groove, which formed π – π stacking contacts with the side chain of Trp286. The Lineweaver–Burk plots for *AChE* showed the point of intersection was in the third quadrant, but very close to the *X* axis. It is a mixed but noncompetitive combination, suggesting that **2f** should combine the PAS site as a noncompetitive inhibitor, similarly to donepezil.

It is widely believed that elevated levels of hyperphosphorylated tau (p-tau) protein are causal in the process of forming the neurofibrillary tangles, which are one of the pathologic features in the brains of AD patients.³⁰ Encouraged by the potent inhibitory activity of **2f** against *GSK-3 β* , Western blot assays were conducted to further confirm the effect of the dual-action targeting of *GSK-3 β* and *AChE* on p-tau levels. We treated mouse neuroblastoma N2a-Tau cells with increasing concentrations of **2f** for 6 h, and the lysates were subjected to Western blot analyses. The results in Figure 3B showed that the

Figure 3. (A) Binding mode prediction of **2f** with *hGSK-3 β* (PDB id: 4PTC). Compound **2f** was shown in stick mode colored in green. (B) Compound **2f** effectively reduce tau protein hyperphosphorylation in mouse neuroblastoma N2a-Tau cells. Protein expression was detected by immunoblot analysis with a specific antibody. The effect of **2f** on phosphorylation status of tau.

phosphorylation of tau protein at Ser396 site decreased after treatment with 10 μ M **2f**. Meanwhile, the total tau protein level was not changed resulting in an attenuation of p-tau/total tau. These data suggested that **2f** could significantly inhibit tau protein hyperphosphorylation, thus offering promises for reducing NFTs in the development of thiazolypyridine-based multitarget drug candidates for AD treatment.

Cognition-improving potency is the most important profile of anti-AD agents. Given the interesting multipotent activity profile of **2d** and **2f**, their ability to ameliorate scopolamine-induced cognition impairment in ICR mice was investigated in a behavioral study by a Morris water maze test. Scopolamine-induced cognition-impaired adult mice were used as an animal model to measure the cognitive improvement effects of **2d** and **2f**, compared with tacrine (20 μ mol/kg body weight). Five days of memory training and learning and a probe trial on the sixth day were performed in the test. The mean escape latency values and distance to target of all the groups on the sixth day are shown in Figure 4 (the mean escape latency values for each training day are provided in Table S2 and the trajectories of the mice in each group are shown in Figure S5). Compared to the control group, scopolamine-induced mice exhibited a prominent delay of mean

Figure 4. Effects of oral administration of **2d** (15 mg/kg), **2g** (15 mg/kg), **2f** (15 mg/kg), and tacrine (15 mg/kg) on scopolamine-induced cognitive impairment in ICR mice determined by the Morris water maze test. (A) Latency to target. (B) Distance to target. Data are presented as the mean $\pm$ SEM ($n = 6$; *** $p < 0.001$, **** $p < 0.0001$ vs scopolamine group).

escape latency compared to the control group (41 s vs 8.6 s). Meanwhile, the **2f**-treated group had better performance than the tacrine-treated group, displaying the superior latency to target (17 s) to the tacrine group (26 s). Furthermore, **2d** significantly reduced the latency to target (11 s), closed to the control group. The trend of distance to target was basically consistent with the latency to target (Figure 5B). The results

Figure 5. Histomorphological appearance of livers of male mice after treatment with the solvent only (control, A) or 22 h after administration of tacrine (B), **2d** (C), or **2f** (D). HE staining, original magnification $\times 200$.

suggested **2d** and **2f** considerably ameliorated the cognitive impairment of the treated mice and was much better than tacrine, and also suggested that **2d** and **2f** may penetrate the blood–brain barrier and target the central nervous system.

Given the serious hepatotoxicity of tacrine for its long-term use, the possible drug-induced hepatotoxicity associated with the presence of the tetrahydroacridine nucleus in **2d** and **2f** was investigated by comparing to tacrine. Heparinized serum was collected 8, 22, and 36 h after the administration of tacrine, **2d**, and **2f**, and the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated (Table 2). ALT is primarily distributed in liver, and damaged hepatocytes release ALT and AST into the extracellular space. Thus, the serum levels of AST and ALT are directly related to the extent of the liver damage. Eight hours after tacrine administration, the levels of ALT and AST were slightly induced. However, **2d** and **2f** did not show any hepatotoxicity at all time-points, and the level of ALT and AST even slightly reduced at 8 h, compared to those of the tacrine group. To confirm the hepatotoxicity of **2d** and **2f** ultimately, morphologic studies by immunohistochemical staining were performed. Treatment of tacrine (Figure 5B), **2d** (Figure 5C), or **2f** (Figure 5D) did not result in remarkable

morphologic changes, such as hepatic cell gap and number in liver compared to the control group (Figure 5A). Cytotoxicity profile was also demonstrated in human hepatocellular HepG2 cells, where **2f** showed low impairment of the cell viability ($IC_{50} = 35 \mu M$) (see Figure S4). In general, **2f** showed hepatic safety profile, ensuring its further development.

In conclusion, dual GSK-3 β /AChE inhibitors were successfully designed, which showed nanomolar potency on both targets. To the best of our knowledge, it is the first attempt to design GSK-3 β /AChE dual inhibitor as anti-AD agents. The representative compound **2f** significantly inhibited tau protein hyperphosphorylation, while it also reduced the self-aggregation of A β_{1-42} . Furthermore, ICR male mice treated with scopolamine exhibited significantly ameliorated memory performance in a Morris water maze test when **2f** was administrated. Additionally, **2f** exhibited preliminary safety in hepatotoxicity studies, without improving the level of ALT and AST. Such biological properties highlight **2f** as a very interesting prototype in the search for new disease-modifying drugs in the treatment of AD.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00463.

Synthetic procedures, characterization data, binding mode prediction of **2f** with *ee*AChE and *h*GSK-3 β , and biological assay procedures (PDF)

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Notes

The authors declare no competing financial interest.

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Table 2. ALT and ASAT Activity after the Administration of **2d**, **2f**, and Reference Compound Tacrine (30 mg/kg)^a

group	ALT (U/L)			AST (U/L)		
	8 h	22 h	36 h	8 h	22 h	36 h
control	35 $\pm$ 8.4	39 $\pm$ 9.4	30 $\pm$ 8	120 $\pm$ 20	120 $\pm$ 20	120 $\pm$ 13
tacrine	32 $\pm$ 4.9	25 $\pm$ 7	25 $\pm$ 7	130 $\pm$ 22	120 $\pm$ 30	110 $\pm$ 28
2d	25 $\pm$ 7.9	31 $\pm$ 12	30 $\pm$ 4	110 $\pm$ 24	120 $\pm$ 19	130 $\pm$ 32
2f	28 $\pm$ 7.6	29 $\pm$ 7.9	29 $\pm$ 7	110 $\pm$ 24	120 $\pm$ 24	120 $\pm$ 21

^aValues were expressed as the mean $\pm$ SD ($n = 6$).

Dr. Haopeng Sun graduated in Pharmacy from the China Pharmaceutical University in 2006. He received his Ph.D. in 2011 in Medicinal Chemistry, with a thesis on the structural optimization and mechanism study of the natural product. In 2014, he was promoted to Associate Professor of Medicinal Chemistry at China Pharmaceutical University. So far, he has published more than 70 papers in peer-reviewed journals indexed by Science Citation Index. His major research interests include the design and development of small molecule bioactive compounds targeting neurodegenerative diseases, inflammation modulation, and cancer prevention.

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■ ABBREVIATIONS

MTDLs, multitarget-directed ligands; AD, Alzheimer's disease; GSK-3 β , glycogen synthase kinase-3 β ; h-AChE, human acetylcholinesterase; CAS, catalytic active site; PAS, peripheral anionic site; A β , β -amyloid; NFTs, neurofibrillary tangles; CNS, central nervous system; BuChE, butyrylcholinesterase; PAS, peripheral anionic site; CPCCI, cyclopropanecarbonyl chloride; TFA, trifluoroacetic acid; DIAD, diisopropyl azodiformate; DIPEA, *N,N*-diisopropylethylamine; ALT, alanine aminotransferase; AST, aspartate aminotransferase

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