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Highly selective carbamate-based butyrylcholinesterase inhibitors derived from a naturally occurring pyranoisoflavone

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ABSTRACT

This current study described the design and synthesis of a series of derivatives based on a natural pyranoisaflavone, which was obtained from the seeds of *Millettia pachycarpa* and displayed attractive BChE inhibition and high selectivity in our previous study. The inhibitory potential of all derivatives against two cholinesterases was evaluated. Only a few compounds demonstrated AChE inhibitory activity at the tested concentrations, while 26 compounds showed significant inhibition on BChE (the IC_{50} values varied from 9.34 μM to 0.093 μM), most of them presented promising selectivity to ward BChE. Prediction of ADME properties for 7 most active compounds was performed. Among them, **9g** (IC_{50} = 222 nM) and **9h** (IC_{50} = 93 nM) were found to be the most potent BChE inhibitors with excellent selectivity over AChE (SI ratio = 1339 and 836, respectively). The kinetic analysis demonstrated both of them acted as mixed-type BChE inhibitors, while the molecular docking results indicated that they interacted with both residues in the catalytic active site. A cytotoxicity test on PC12 cells showed that both **9g** and **9h** had a therapeutic safety range similar to tacrine. Overall, the results indicate that **9h** could be a good candidate of BChE inhibitors.

1. Introduction

Alzheimer's disease (AD) is a serious neurodegenerative disorder characterized by progressive memory loss and central cognitive dysfunction. And the deficit of cholinergic neurons is one of the key pathological hallmarks of AD [1]. According to cholinergic dysfunction hypothesis, inadequate levels of the neurotransmitter acetylcholine is the main cause of cognitive decline [2]. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), two metabolic serine hydrolases, can catalyze the hydrolysis of acetylcholine [3]. AChE, as the predominant cholinesterase in the brain, can improve cognitive function by enhancing cholinergic neurotransmission. Thus, the AChE inhibitors have been employed to treat AD. Moreover, most of current AChE inhibitors to treat AD available in the clinic are related to natural products, such as rivastigmine, galantamine, and huperzine A [4].

Compared with AChE, BChE plays a supportive role in the cholinergic neurotransmission. However, during the progression of AD, level of AChE in the patient brain decreases while the level and activity of BChE significantly increases, which suggests that the compensation of BChE in the late AD stage is of great importance [5,6]. In addition, selective BChE inhibitors do not display the adverse cholinergic effect of AChE inhibitors [7]. Therefore, highly selective inhibition of BChE presents a promising therapeutic strategy in promoting acetylcholine level and improving cognition for the treatment of advanced AD [8–11]. Furthermore, the key role of BChE is also emphasized in detoxication and scavenging of polyproline-rich peptides [12], regulation of the serum metabolism associated with obesity [13,14], diabetes mellitus and insulin resistance [13,15], and cardiovascular risk factors [16,17]. Thus, the development of selective BChE inhibitors becomes an attractive topic for researchers in medicinal chemistry currently.

The seeds of *Millettia pachycarpa* Benth are widely used as anthelminthic drugs in China [18]. In our recent study, the 95% ethanol extract of these seeds demonstrated interesting BChE inhibitory activity *in vitro* [19]. A potential natural BChE inhibitor **8** with significant inhibition (IC_{50} = 2.34 μM) and good selectivity (SI ratio = 56.1), which possesses a 6″, 6″-dimethylpyranoisoflavone scaffold and demonstrates apoptosis-inducing effects [20] and antiinflammatory activities previously [21], was obtained by subsequent bioassay-guided isolation [19] (Fig. 1). More importantly, its occurrence in plant kingdom is relatively rare, as this type of scaffold was detected only in *M. pachycarpa* and *Spiranthes sinensis*.

Previously, only a few isoflavones were reported as cholinesterase inhibitors with weak inhibition and poor selectivity towards BChE [22–26] (Fig. 2). Additionally, structural modification of pyranoisoflavone core as potential site to improve cholinesterase inhibition were not reported. Considering the importance of natural products as AD

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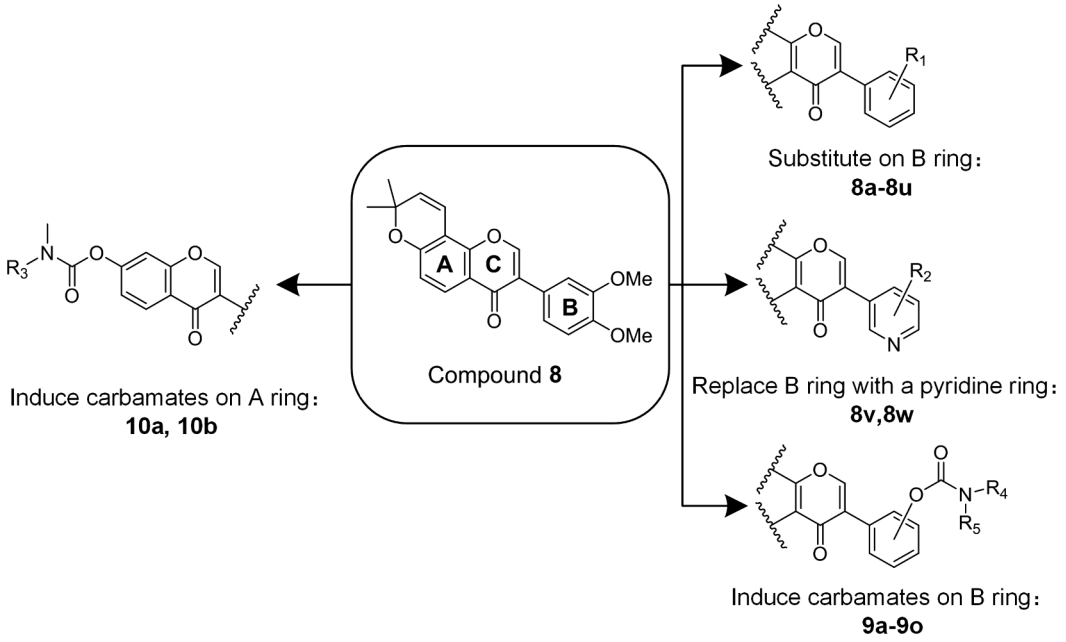


Fig. 1. Design strategy carried out starting from compound **8**.

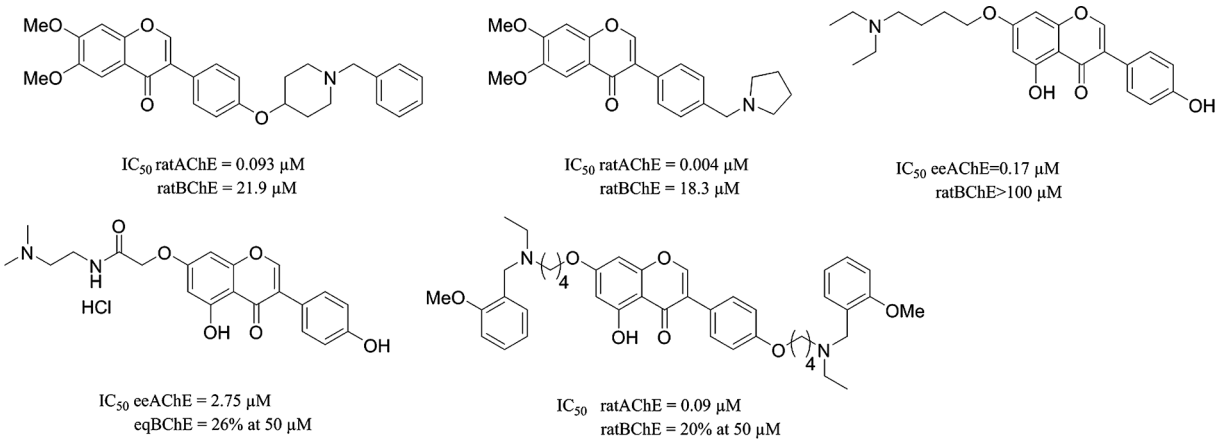


Fig. 2. Isoflavone-based structures which have been investigated as ChE inhibitors.

drugs, a series of structurally modified derivatives based on 6″,6″-dimethylpyranoisoflavone were designed and synthesized in current study. The strategy for the design of the inhibitors, including the introduction of a series of substituents on B ring, replacing the B ring with a pyridine ring, and the introduction of various carbamates on A ring and B ring, is illustrated in Fig. 1. Some of derivatives presented more potential (IC_{50} values varied from 0.093 μM to 1.47 μM) against BChE than parent compound **8**. Preliminary kinetic and modeling data are also presented to investigate their inhibition mechanism.

2. Results and discussion

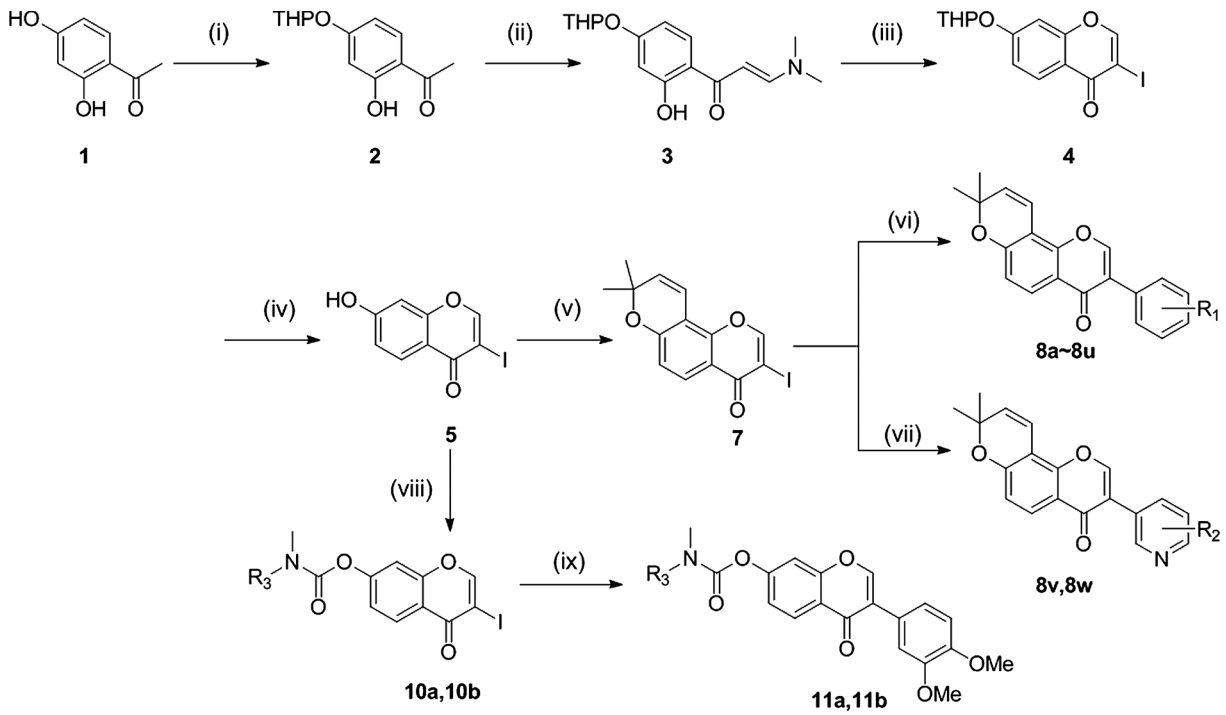
2.1. Chemistry

The synthesis of **8a–8w**, **11a** and **11b** are outlined in Scheme 1. Compounds **2–5** were prepared using the methods reported previously [27]. 1,1-Diethoxy-3-methyl-2-butene was obtained from 3-methylbut-2-enal by following method [28]. Treatment of **5** with 3-picoline and the crude 1,1-diethoxy-3-methyl-2-butene resulted in **7** [29], which was treated with different boronic acids to give **8a–8w** [27,30]. In addition, the reaction of **5** and acyl chlorides give **10a** and **10b**, which coupled with 3,4-dimethoxyphenylboronic acid to afford **11a** and **11b**, respectively [27]. The synthetic route of **9a-9o** is presented in Scheme 2. Treatment of **8f-8h** with isocyanates and various types of acyl chlorides yield **9a-9n** [31–33], while **8t** was converted into **9o** by reacting with triethylamine and *n*-heptylisocyanate. The structure features of the derivatives are presented in Tables 1 and 2. Twenty-nine compounds were synthesized for the first time.

2.2. Inhibitory studies of AChE and BChE

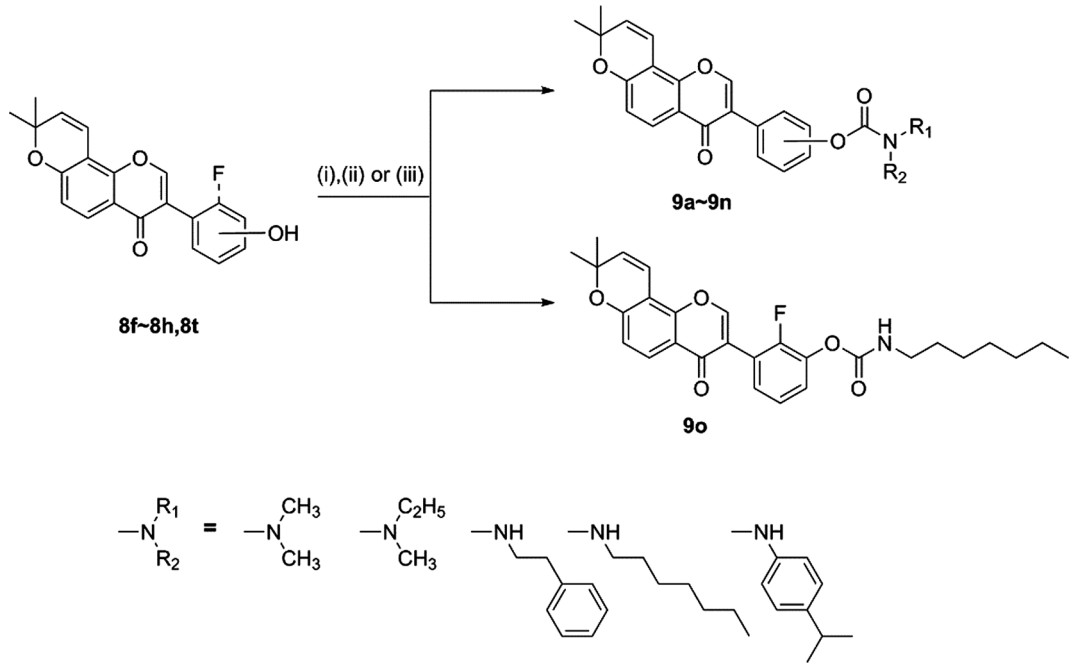
The ChEs inhibitory capacity of all 41 derivatives was evaluated by Ellman's method using tacrine and galantamine as positive controls [34]. Further IC_{50} values determination were performed for compounds with higher inhibition rate than 50% at 10 μM (final concentration in the reaction system). Their inhibitory activity and selectivity results are listed in Tables 1 and 2. Most of the synthesized derivatives did not present good inhibition toward AChE, while their inhibition potency on BChE depended on structure features.

Firstly, a series of substituents were introduced on the B ring to provide derivatives **8a-8u**. Introduction of three small substituents (hydroxyl, methoxy, and fluorine) in different positions (*ortho*, *meta* and *para*) of the B ring resulted in the decline of inhibitory activity (**8a-8l**). On the whole, introduction these substituents on *para* position of the B



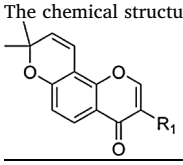
8a R_1 = 2-fluoro	**8h** R_1 = 4-hydroxy	**8o** R_1 = 4-acetylphenyl	**8v** R_2 = 5-fluoropyridin-3-yl
8b R_1 = 3-fluoro	**8i** R_1 = 2-methoxy	**8p** R_1 = 4-cyanophenyl	**8w** R_2 = 5-methoxypyridin-3-yl
8c R_1 = 4-fluoro	**8j** R_1 = 3-methoxy	**8q** R_1 = 2,3-difluoro	**10a** R_3 = methyl
8d R_1 = 3,4-(methylenedioxy)	**8k** R_1 = 4-methoxy	**8r** R_1 = 2,5-difluoro	**10b** R_3 = ethyl
8e R_1 = 3,4-(ethylenedioxy)	**8l** R_1 = 3-(methoxycarbonyl)	**8s** R_1 = 2-fluoro-5-methoxy	**11a** R_3 = methyl
8f R_1 = 2-hydroxy	**8m** R_1 = 2,3-dimethoxy	**8t** R_1 = 2-fluoro-3-methoxy	**11b** R_3 = ethyl
8g R_1 = 3-hydroxy	**8n** R_1 = 4-(trifluoromethyl)	**8u** R_1 = 2-fluoro-3-hydroxy	

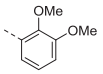
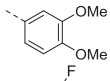
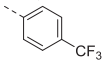
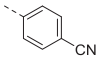
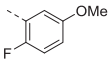
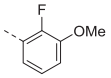
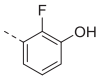
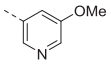
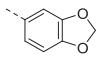
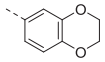
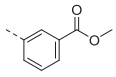
Scheme 1. Synthesis of compounds **8a-8w** and **11a-11b. Reagents and conditions:** (i) DHP, PPTS, CH_2Cl_2, rt, 4 h; (ii) DMF-DMA, 95 °C, 3 h; (iii) I_2, pyridine, $CHCl_3$, rt, 12 h (74.7% for three steps); (iv) pTsOH, CH_3OH, THF, 60 °C, 1 h; (v) 1,1-diethoxy-3-methyl-2-butene, 3-picoline, xylene, reflux, 24 h; (vi) $ArB(OH)_2$, 10% Pd/C, Na_2CO_3, H_2O, DME, 45 °C, 1 h; (vii) heteroaryl boronic acids, $Pd[P(C_6H_5)_3]_4$, Na_2CO_3, THF, H_2O, 60 °C, 6 h; (viii) acyl chloride, K_2CO_3, DMF, CH_3CN, 95 °C, 3 h; (ix) $ArB(OH)_2$, 10% Pd/C, Na_2CO_3, H_2O, DME, 45 °C, 1 h;



Scheme 2. Synthesis of compounds **9a-9o. Reagents and conditions:** (i) acyl chloride, K_2CO_3, DMF, CH_3CN, 95 °C, 3 h; (ii) isocyanate, NEt_3, CH_2Cl_2, rt, 6 h; (iii) acyl chloride, K_2CO_3, CH_3CN, rt, 24 h.

Table 1
The chemical structure of the tested compounds and their Inhibitory activities against eeAChE and eqBChE.



Compd.	R_1[a]	Chain Position	IC_{50} (μM) ± SEM[b] AChE	BChE	SI[c]	Compd.	R_1[a]	Chain Position	IC_{50} (μM) ± SEM[b] AChE	BChE	SI[c]
7			24.47%	5.27 ± 0.13	> 1.9	**8m**			19.17%	21.24%	ND[d]
8			131.17 ± 13.77	2.34 ± 0.15	56.1	**8n**			5.60%	17.04%	ND[d]
8a		ortho	79.60 ± 9.81	2.94 ± 0.26	27.1	**8o**			2.71 ± 0.11	9.34 ± 0.49	0.3
8b		meta	18.18%	4.27 ± 0.12	> 2.3	**8p**			15.45%	13.10%	ND[d]
8c		para	8.26%	13.65%	ND[d]	**8q**			13.28%	4.59 ± 0.25	> 2.2
8d		ortho	7.19%	6.04 ± 0.31	> 1.7	**8r**			10.77%	2.92 ± 0.13	> 3.4
8e		meta	13.87%	8.20 ± 0.16	> 1.2	**8s**			14.50%	2.34 ± 0.06	> 4.3
8f		para	15.05%	6.47 ± 0.23	> 1.6	**8t**			349.36 ± 16.56	1.13 ± 0.09	309
8g		ortho	8.27%	5.09 ± 0.14	> 2.0	**8u**			8.36%	11.46 ± 0.31	> 0.9
8h		meta	130.27 ± 17.90	2.36 ± 0.19	55.2	**8v**			16.74 ± 0.23	8.26 ± 0.10	2.0
8i		para	17.73%	8.32 ± 0.34	> 1.2	**8w**			8.67 ± 0.18	3.39 ± 0.13	2.6
8j			15.72%	3.11 ± 0.12	> 3.2	Galantamine			3.75 ± 0.04	44.07 ± 0.91	0.09
8k			23.57%	4.96 ± 0.13	> 2.0	Tacrine			0.0438 ± 0.006	0.0101 ± 0.0005	4.34
8l			4.81%	9.34 ± 0.51	> 1.1						

[a] The dash line represents connecting bond.
[b] The inhibition activities are expressed as IC_{50} (μM) or as percentage of inhibition at 10 μM, the IC_{50} values are the mean of three independent experiments ± SEM.
[c] SI represents selectivity index which is determined as ratio AChE IC_{50}/BChE IC_{50}.
[d] Not determined.

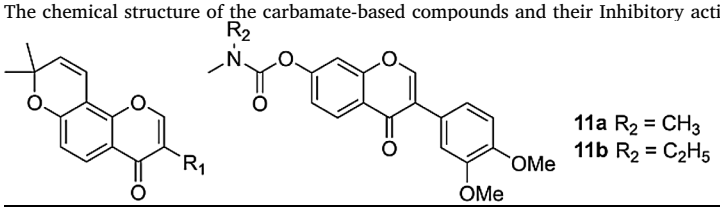
ring have a higher negative influence on BChE inhibition than on *meta* and *ortho* position. Among them, **8h** and **8a** demonstrated similar potency against BChE (IC_{50} = 2.36 μM and 2.94 μM, respectively)

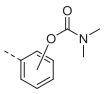
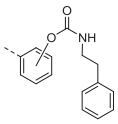
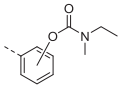
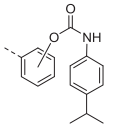
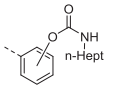
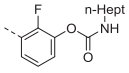
No obvious improvements in BChE inhibition activity were observed when a methylenedioxy or ethylenedioxy group was introduced in *ortho* positions of the B ring (**8j** and **8k**). Also, **8n**-**8p**, obtained by the introduction of trifluoromethyl acetyl and cyano in the *para* position of B ring respectively, presented poor BChE inhibition. Considering the slightly potency difference of **8a** and **8h**, **8q**-**8t,** bearing two methoxy and/or fluorine groups in *meta* and *ortho* position of the B ring, were further synthesized, only **8t** displayed a noticeable activity and significant selectivity toward BChE (IC_{50} = 1.13 μM, SI = 309).

Previously study revealed that selective BChE inhibitors can be developed with a scaffold bearing carbamate to fit into the correct position in the gorge of BChE [35]. Therefore, fifteen carbamate-based derivatives (**9a**-**9o**) were synthesized. In general, introduction of carbamates in the B ring result in the significantly increase of the BChE inhibition activity. Four derivatives (**9b**, **9g**, **9h**, and **9k**) showed submicromolar potency, while most of them demonstrated low micromolar activities. The BChE inhibition potency varied in the following sequence: heptyl > phenethyl > dimethyl > ethyl (methyl) > 4-isopropylphenyl. Notably, their BChE inhibitory activities were closely associated with the substituted position in following pattern *meta* > *ortho* > *para* (i.e., **9a**-**9n**). This trend can be attributed to a more advantageous position in the gorge of BChE in the case of the *meta*-substituted compounds, which was partly confirmed by the docking results of two most potent compounds among this series, **9g** (IC_{50} = 93 nM, SI = 836) and **9h** (IC_{50} = 222 nM, SI = 1339). However, further introduction of an *ortho*-fluoride in **9h** significantly and unexpectedly decreased the BChE inhibitory capacity (**9o**, IC_{50} = 4.84 μM).

In order to investigate the contribution of pyran ring in the scaffold, **11a** and **11b** were synthesized and showed poor activity toward BChE,

Table 2
The chemical structure of the carbamate-based compounds and their Inhibitory activities against eeAChE and eqBChE.



Compd.	R_1[a]	Chain Position	IC_{50} (μM) ± SEM[b] AChE	BChE	SI[c]	Compd.	R_1[a]	Chain Position	IC_{50} (μM) ± SEM[b] AChE	BChE	SI[c]
9a		ortho	26.78%	8.72 ± 0.46	> 1.1	**9j**		ortho	49.71 ± 7.26	1.09 ± 0.09	45.6
9b		meta	33.55 ± 1.71	0.681 ± 0.019	49.3	**9k**		meta	164.80 ± 17.97	0.367 ± 0.009	449
9c		para	27.60%	11.79 ± 0.52	> 0.8	**9l**		para	5.54%	4.67%	ND[d]
9d		ortho	6.27%	7.85 ± 0.33	> 1.3	**9m**		meta	14.21%	13.44%	ND[d]
9e		meta	31.51 ± 1.52	1.47 ± 0.08	21.4	**9n**		para	6.69%	11.47%	ND[d]
9f		para	10.12 ± 0.22	28.65%	< 1.0	**9o**			20.72%	4.84 ± 0.13	> 2.1
9g		ortho	297.28 ± 11.28	0.222 ± 0.014	1339	**11a**			10.44%	5.68%	ND[d]
9h		meta	77.79 ± 6.74	0.093 ± 0.001	836	**11b**			6.33%	6.42%	ND[d]
9i		para	12.18%	32.07%	ND[d]						

[a] The dash line represents connecting bond.
[b] The inhibition activities are expressed as IC_{50} (μM) or as percentage of inhibition at 10 μM, the IC_{50} values are the mean of three independent experiments ± SEM.
[c] SI represents selectivity index which is determined as ratio AChE IC_{50}/BChE IC_{50}.
[d] Not determined.

which indicated that the pyran ring in the flavonoid nucleus played a prominent role to keep BChE inhibition, It is also evident from the moderate inhibition and selectivity of **7** (IC_{50} = 5.27 μM, 24.47% of AChE inhibition at 10 μM).

Considering that nitrogen of the pyridine ring may interact with other residues as a result of slight changes in the scaffold. Replacement of B ring of **8** with a pyridine ring bearing methoxy and fluoro increased the AChE over BuChE inhibitory potency (**8v** and **8w**), which suggested the importance of pyridine ring for AChE inhibition. All other compounds demonstrated weak to moderate AChE inhibition.

Due to their most potent activities and good selectivity toward BChE, **9g** and **9h** were selected for following studies.

2.3. ADME prediction

The ADME (absorption, distribution, metabolism and excretion) properties are considered as critical parameters for drug candidates [36]. Hence, the ADME properties of seven potent inhibitors (IC_{50} < 2 μM) were predicted *in silico* using the QikProp v. 5.5 (Schrodinger). Compared to the characteristics of galantamine and tacrine, the data (Table S1, see Supporting Information) indicated that all of seven compounds possessed adequate clog P, good serum albumin binding and human absorption, low levels of primary metabolites and good Caco-2 permeability. Among the tested compounds, the solubility of three compounds (**8t**, **9b** and **9e**) were within the limits. As an important parameter, LogBB is frequently used to assess the permeability of compounds through the blood-brain barrier. The calculated logBB of all these compounds ranged from −0.880 to 0.231, which were also within the reported limitation (−3 < logBB < 1.2), indicating that seven investigated inhibitors could be administered orally as CNS-active compounds.

2.4. Kinetic characterization of BChE inhibition

Two potent BChE inhibitors (**9g** and **9h**) were selected for further kinetic analysis to gain the information on their mechanisms of inhibition. The experiments were performed using the combination of six concentrations of the substrate (from 0.05 to 0.8 mM) and four concentrations of the inhibitors. Double-reciprocal Lineweaver-Burk plots were used to evaluate the type of inhibition. As shown in Fig. 3, increasing the concentrations of **9g** (Fig. 3A) and **9h** (Fig. 3B) decreased the V_{max} and increased the K_m, suggesting a mixed-type inhibition. The dissociation constants K_i for **9g** and **9h** were estimated to be 0.158 μM and 0.045 μM, respectively.

2.5. Molecular docking

To help explain the higher potential of **9g** and **9h** than **8** and illustrate their binding mode in the active sites of BChE enzyme, molecular docking simulations were performed using the molecular docking program GOLD 5.0 (The Cambridge Crystallographic Data Centre). Hydrogen bonds were defined by Gold, and other interactions were described by Discovery Studio 2016 (BIOVIA) based on the docking data. The calculated GoldScores were 60.5219, 53.6077 and 40.6528 for the **9h**-, **9g**- and **8**-*h*BChE complexes, respectively, which were highly correlated with their inhibitory activity *in vitro*.

In the **8**-*h*BChE complex (Fig. 4A and B), two hydrogen bonds were observed between the oxygen atom of the pyran ring and Ser287 (2.6 Å), the oxygen atom of the carbonyl group and Gly117 (3.1 Å) in the oxyanion cavity of BChE. Additionally, three carbon-hydrogen bonds were formed between 4-OMe group and Tyr128 (2.7 Å), among 3-OMe group, Tyr128 (3.4 Å) and Gly115 (3.2 Å). Moreover, the B ring of **8** was also involved in a *T*-shaped interaction with Trp82.

A further comparison between the **9g**- and **9h**-*h*BChE complexes

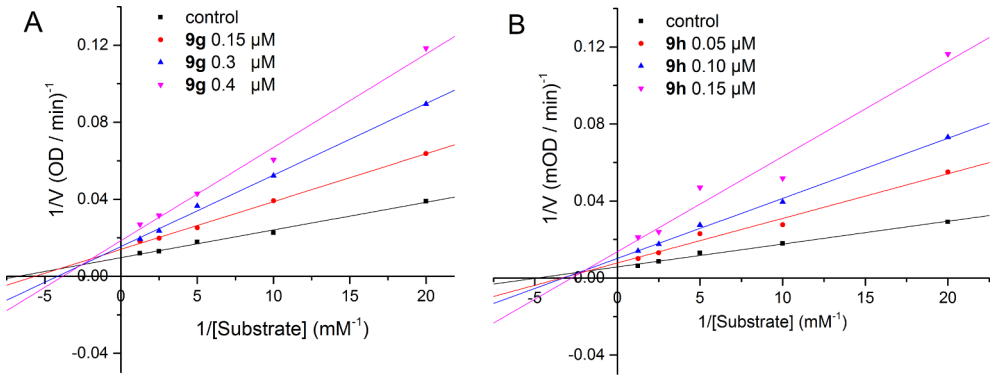


Fig. 3. Lineweaver-Burk plots of BChE inhibition of **9g** (A) and **9h** (B).

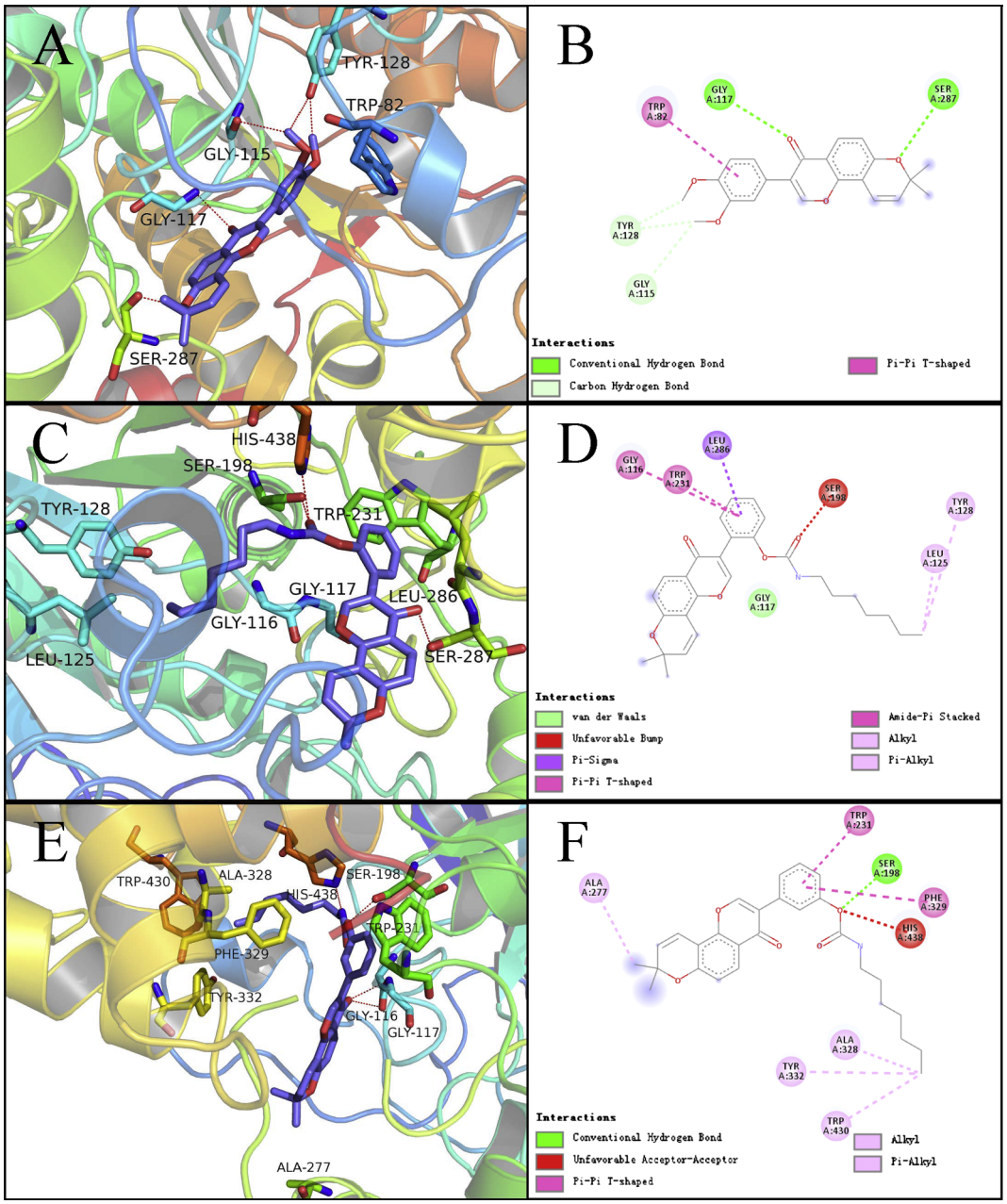


Fig. 4. Binding mode of **8** (A and B), **9g** (C and D) and **9h** (E and F) in the BChE active site. Figures (B, D and F) were made by Discovery Studio 2016 Client software; Figures (A, C and E) were created with PyMol 1.8.2.0.

revealed additional interesting interaction modes. In the **9g**-*h*BChE complex (Fig. 4C and D), the oxygen atom of the carbamate moiety contributed to the hydrogen bonds with Ser198 (2.1 Å) and His438 (2.9 Å), two key residues in the catalytic triad, and the carbonyl group provided another hydrogen bond with Ser287. This complex was further stabilized by the π-sigma interaction with Leu286, amide-π stacked interaction with Gly116, and *T*-shaped π-π interaction with Trp231. Furthermore, the heptyl moiety of **9g** was involved in an alkyl-alkyl interaction with Leu125 and a π-alkyl interaction with Tyr128.

Docking results for **9h** (Fig. 4E and F) were similar to those observed for **9g.** Interestingly, one more hydrogen bond could be seen between the carbonyl group of **9h** with Gly117 (2.9 Å) of the oxyanion cavity, one key residue which stabilized the negative charge in the process of substrate hydrolysis by BChE. The terminal methyl of the heptyl moiety formed π-alkyl interactions with Tyr332 and Trp430, and it was also involved in an alkyl-alkyl interaction with Ala328, indicating the importance of the heptyl moiety for the BChE affinity of **9h**. Additionally, this complex was further stabilized by two π-π *T*-shaped interactions (among the B ring, Trp231 and Phe329) and an alkyl-alkyl interaction (the methyl group of the pyran ring and Ala277).

As discussed above, the binding of both **9g** and **9h** with the catalytic triad had more conformation stabilizing interactions, which were absent from the **8**-*h*BChE complex. These differences explained higher BChE inhibitory potential of compounds **9g** and **9h** over **8**. For **9h**, the interaction, formed by the methyl group of the pyran ring and Ala277 and disappeared in the **9g**-*h*BChE complex**,** should be the key factor contributing to its stronger potential over **9g**. Overall, the docking results agree with the different inhibitory potency observed in experiments.

2.6. Cytotoxicity

The promising compounds **9g** and **9h** were further evaluated for their cytotoxic effect as well as **8**, and tacrine was chosen as reference drug. PC12 cells were treated with above compounds at three different concentrations (1, 5, 25 μM, due to their solubility) for 48 h and the cell viability was determined using MTT Assay. As shown in Fig. 5, no obvious cytotoxicity was observed at a concentration of 1 μM for four tested compounds. With increased concentration to 5 μM, **9g** and **9h** induced a decrease of cell viability (64.2% and 66.2%, respectively) similar to tacrine (64.2%) and **8** (66.4%). At the concentrations of 25 μM, **9g** induced slightly decrease of cell viability (53.6%) while **9h** induced moderate decrease of cell viability (43.3%) than tacrine (58.7%). it may be considered that the **9h** exhibited little more cytotoxicity than **9g**. On the other hand, both of them had higher safety

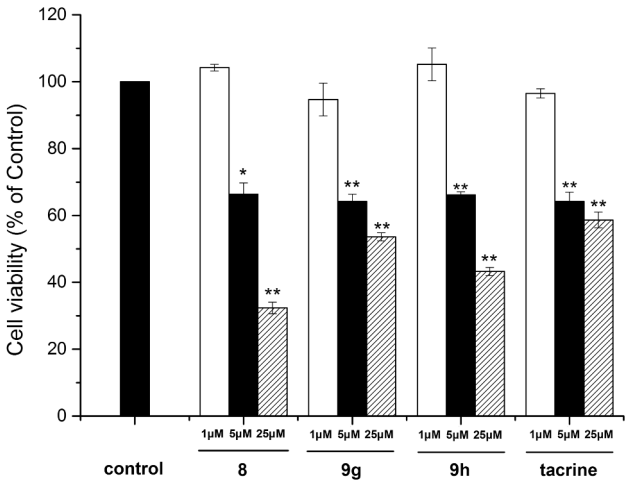


Fig. 5. *In vitro* cytotoxicity of compounds **8**, **9g** and **9h** on PC12 cell line. Data were expressed as mean ± SD (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$ vs Ctrl.

than **8** (32.3%) at the concentrations of 25 μM. These results revealed that **9g** and **9h** had a therapeutic safety range similar to tacrine [37].

3. Conclusion

In conclusion, 41 derivatives based on a naturally occurring pyranoisoflavone have been designed and synthesized, and their inhibition potency against two cholinesterases was evaluated. Twenty-six of them demonstrated good inhibitory activities on BChE. Compared to the parent compound **8**, seven derivatives displayed higher inhibitory potential. The loss of pyran ring led to a significant loss of BChE inhibition activity, while the introduction of various substituents in B ring had little impact on the activity. The introduction of carbamates in B ring significantly promoted the BChE inhibitory activity while retaining good selectivity. Among all active derivatives, **9g** (IC_{50} = 222 nM) and **9h** (IC_{50} = 93 nM) presented the strongest BChE inhibitory potential with high selectivity (SI = 1339 and 836, respectively). Subsequent kinetic analysis revealed that both compounds acted as mixed-type BChE inhibitors. Docking simulation showed they directly interacted with the catalytic triad of enzyme. The docking result helped to explain the difference in their activity. A cytotoxicity test on PC12 cells demonstrated **9g** and **9h** had a therapeutic safety range similar to tacrine.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

All reagents were purchased from commercial sources without further purification. Flash column chromatography was performed with silica gel 60 (100–200 and 200–300 mesh) using petroleum ether and ethyl acetate as the eluents. Precoated plates of silica gel F_{254} for TLC (detected by UV light) were purchased from Qingdao Marine Chemical Ltd. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on Bruker AVANCE III-400 or III-600 spectrometers in DMSO-d_6 or $CDCl_3$ with tetramethylsilane as internal standard. Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), double doublet (dd), triplet (t), and multiplet (m). Mass spectra were obtained by Waters Quattro Premier XE triquadrupole mass spectrometer with an ESI Source (Waters Corporation, USA), while High Resolution Mass spectra were obtained on a Q-TOF Priemier mass spectrometer (Micromass, Manchester, UK) with electron spray ionization (ESI) as the ion source. Melting points were measured by a YRT-3 device (Xintianguang Corporation, Tianjin, China). The purity of the synthesized compounds were determined by HPLC-ELSD and confirm ≥95% purity. [HPLC-ELSD, Alltech Lab Alliance Model 201 ultraviolet detector, equipped with Alltech ELSD 6000 detector and Cromasil C_{18} column (250 mm × 4.6 mm, 5 μm), mobile phase: methanol/0.1% formic acid (70/30–100/0), 1 mL/min.]

4.1.2. 3-Iodo-7-((tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one (4)

DHP (12 mL, 155 mmol) was mixed with CH_2Cl_2 (64 mL); the solution was dropwise added to a mixture of **1** (6.086 g, 40 mmol) and PPTS (372 mg) in CH_2Cl_2 (128 mL). After 4 h stirring at room temperature, the reaction mixture was washed with saturated $NaHCO_3$ solution and extracted three times with CH_2Cl_2. After drying with anhydrous $NaSO_4$, the filtered CH_2Cl_2 extract was concentrated *in vacuo* to give compound **2** as a white solid. Compound **2** was dissolved in DMF/DMA (6.13 mL, 46.2 mmol) and heated for 3 h under reflux; then, the reaction mixture was concentrated to give compound **3** as a red solid. The crude compound **3**, pyridine (3.54 mL, 44 mmol), and I_2 (20.3 g, 80 mmol) were added into $CHCl_3$ (50 mL) and stirred for 12 h at room temperature. The saturated $Na_2S_2O_3$ solution was added and stirred for 30 min at room temperature. The reaction mixture was extracted with CH_2Cl_2. After drying with anhydrous Na_2SO_4, the CH_2Cl_2 filtrate was

concentrated *in vacuo*. The resulting mixture was further purified by flash chromatography to yield a white solid **4** (11.12 g, 74.70%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.55–1.78 (m, 3H), 1.89–2.03 (m, 3H), 3.63–3.67 (m, 1H), 3.80–3.86 (m, 1H), 5.54–5.55 (m, 1H), 7.09–7.12 (m, 2H), 8.14 (d, 1H, J = 9.6 Hz), 8.22 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 18.4, 25.1, 30.1, 62.2, 87.1, 96.7, 103.3, 116.3, 116.7, 128, 157.5, 157.8, 161.9, 172.9.

*4.1.3. 7-Hydroxy-3-iodo-4H-chromen-4-one (**5**)*

Compound **4** (4.47 g, 12 mmol) was dissolved in a mixed solution of CH_3OH (120 mL) and THF (120 mL). Then, p-toluenesulfonic acid (228 mg, 1.2 mmol) was added into the mixture at room temperature. After 1 h stirring at 60 °C, the cooled mixture was concentrated *in vacuo* and dissolved in ethyl acetate (500 mL), the resulting solution was washed with saturated brine. The ethyl acetate extract was dried over anhydrous Na_2SO_4 solution and concentrated *in vacuo* to provide compound **5** as a white solid (3.21 g, 92.8%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.86 (t, J = 2 Hz, 1H), 6.93–6.96 (m, 1H), 7.90 (dd, J = 1.2 Hz, 8.8 Hz, 1H), 8.68 (d, J = 2.4 Hz, 1H), 10.93 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 18.4, 25.1, 30.1, 62.2, 87.1, 96.7, 103.3, 116.3, 116.7, 128.0, 157.5, 157.8, 161.9, 172.9.

*4.1.4. 3-Iodo-8,8-dimethylpyrano[2,3-f]chromen-4(8H)-one (**7**)*

3-Methylbut-2-enal (7.72 mL, 80 mmol), $KHSO_4$ (0.545 g, 4 mmol), and $(EtO)_3CH$ (13.3 mL, 80 mmol) were successively added to EtOH (24 mL) at 0 °C, keep stirring for 30 min, and then stirred for another 30 min at room temperature. The resulting mixture was filtered and washed with EtOH (5 mL). K_2CO_3 (1.106 g, 8 mmol) was added and stirred for 2 h at room temperature. Then, the resulting mixture was filtered and concentrated *in vacuo* to provide **6** as a colorless liquid. The crude **6** was used in next step without further purification.

Compound **5** (2.304 g, 8 mmol), the crude **6** (6 mL), and 3-picoline (195 μL, 2 mmol) were successively added to xylene (46 mL) under stirring at room temperature. Then the mixture was heated under reflux for 24 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The resulting residue was purified by flash chromatography and washed with petroleum ether to provide a yellow solid **7.** (1.565 g, 55.2%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.49 (s, 6H), 5.72 (d, J = 10.0 Hz, 1H), 6.73 (d, J = 10.0 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 8.23 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 78.1, 87.3, 109.2, 114.8, 115.8, 116.1, 127.1, 130.8, 152.4, 157.0, 157.8, 172.8.

*4.1.5. General procedure for the preparation of compounds **8**, **8a–8u***

Compound **7** (106 mg, 0.3 mmol) was dissolved in premixed solution of DME (1.8 mL) and H_2O (1.8 mL). Then, Na_2CO_3 (95 mg, 0.9 mmol), aryl boronic acid (0.36 mmol), and Pd/C (16 mg, 5 mol %) were added. After 1 h stirring at 45 °C, the reaction mixture was filtered, and the cake was washed with H_2O (4 mL) and CH_2Cl_2 (6 mL). The aqueous phase was then extracted twice with CH_2Cl_2, **8a–8u** were obtained from the CH_2Cl_2 extracts by flash chromatography.

*4.1.5.1. 3-(3,4-Dimethoxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f] chromen-4-one (**8**)*. The corresponding aryl boronic acid was (3,4-dimethoxyphenyl) boronic acid. White solid, yield, 73.4%; m.p. 169–171 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 6H), 5.73 (d, J = 10.0 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 6.88 (d, J = 9.1 Hz, 1H), 7.14–7.19 (m, 1H), 7.19–7.23 (m, 1H), 7.34–7.40 (m, 1H), 7.47–7.51 (m, 1H), 8.00 (d, J = 1.2 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.4, 78.0, 109.5, 115.4, 116.1, 118.5, 119.8, 120.1, 124.3, 127.0, 130.2, 130.6, 132.4, 152.6, 154.0, 157.7, 159.3, 161.8, 175.3. MS (ESI): m/z calcd. $C_{22}H_{21}O_5$ $[M+H]^+$ 365.14, found 365.08. HPLC purity: 96.0%.

*4.1.5.2. 3-(2-Fluorophenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8a**)*. The corresponding aryl boronic acid was (2-fluorophenyl) boronic acid. White solid, yield, 42%; m.p. 162–163 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 6H), 5.73 (d, J = 10.0 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 6.88 (d, J = 9.1 Hz, 1H), 7.14–7.19 (m, 1H), 7.19–7.23 (m, 1H), 7.34–7.40 (m, 1H), 7.47–7.51 (m, 1H), 8.00 (d, J = 1.2 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.4, 78.0, 109.5, 115.4, 116.1, 118.5, 119.8, 120.1, 124.3, 127.0, 130.2, 130.6, 132.4, 152.6, 154.0, 157.7, 159.3, 161.8, 175.3. HR-TOF-MS (positive mode): m/z calcd. $C_{20}H_{16}FO_3$ $[M+H]^+$ 323.1083, found 323.1082. HPLC purity: 98.3%.

*4.1.5.3. 3-(3-Fluorophenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8b**)*. The corresponding aryl boronic acid was (3-fluorophenyl) boronic acid. White solid, yield, 70%; m.p. 161–162 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.51 (s, 6H), 5.73 (d, J = 10.0 Hz, 1H), 6.81 (d, J = 10.0 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 7.06–7.09 (m, 1H), 7.32–7.34 (m, 2H), 7.37–7.41 (m, 1H), 7.99 (s, 1H), 8.07 (d, J = 8.8 Hz 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 78.0, 109.4, 115.0, 115.3, 115.6, 116.2, 118.4, 124.2, 124.6, 126.9, 130.0, 130.6, 134.2, 152.5, 157.6, 161.6, 164.1, 175.4. HR-TOF-MS (positive mode): m/z calcd. $C_{20}H_{16}FO_3$ $[M+H]^+$ 323.1083, found 323.1079. HPLC purity: 96.7%.

*4.1.5.4. 3-(4-Fluorophenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8c**)*. The corresponding aryl boronic acid was (4-fluorophenyl) boronic acid. White solid, yield, 61%; m.p. 167–169 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.51 (s, 6H), 5.73 (d, J = 10.0 Hz), 6.81 (d, J = 10.0 Hz, 1H), 6.87 (d, J = 8.8 Hz, 1H), 7.12 (t, J = 8.7 Hz, 2H), 7.53 (dd, J = 5.4, 8.4 Hz, 2H), 7.95 (s, 1H), 8.06 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 77.9, 109.3, 115.0, 115.5, 115.7, 118.4, 124.4, 126.8, 128.0, 130.5, 130.8, 130.9, 152.2, 152.5, 157.6, 161.6, 164.1, 175.7. HR-TOF-MS (positive mode): m/z calcd. $C_{20}H_{16}FO_3$ $[M+H]^+$ 323.1083, found 323.1080. HPLC purity: 99.5%.

*4.1.5.5. 3-(2-Hydroxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8d**)*. The corresponding aryl boronic acid was (2-hydroxyphenyl) boronic acid. Yellow solid, yield, 44%; m.p. 149–151 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 6H), 5.76 (d, J = 10.0 Hz, 1H), 6.84 (d, J = 10.0 Hz, 1H), 6.93–6.99 (m, 2H), 7.10 (dd, J = 1.2, 8.0 Hz, 1H), 7.17 (dd, J = 1.6, 7.6 Hz, 1H), 7.33–7.37 (m, 1H), 8.11–8.14 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.4, 78.3, 109.1, 114.7, 116.4, 117.2, 119.8, 120.8, 120.9, 125.0, 126.9, 129.7, 130.6, 130.8, 152.4, 155.2, 156.7, 158.3, 178.8. HR-TOF-MS (positive mode): m/z calcd. $C_{20}H_{17}O_4$ $[M+H]^+$ 321.1127, found 321.1129. HPLC purity: 99.2%.

*4.1.5.6. 3-(3-Hydroxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f] chromen-4-one (**8e**)*. The corresponding aryl boronic acid was (3-hydroxyphenyl)boronic acid. Yellow solid, yield, 41%; m.p. 220–221 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.50 (s, 6H), 5.73 (d, J = 10.0 Hz, 1H), 5.80 (s, 1H), 6.81 (d, J = 10.0 Hz, 1H), 6.85–6.89 (m, 2H), 7.02 (d, J = 7.6 Hz, 1H), 7.20 (dd, J = 2, 2.4 Hz, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.99 (s, 1H), 8.08 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 27.6, 77.9, 109.0, 114.1, 114.8, 115.0, 116.1, 117.9, 119.5, 123.7, 126.1, 129.1, 131.4, 133.0, 151.6, 153.7, 156.6, 157.0, 174.4. MS (ESI): m/z calcd. $C_{20}H_{17}O_4^+$ $[M+H]^+$ 321.11, found 321.24. HPLC purity: 97.3%.

*4.1.5.7. 3-(4-Hydroxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f] chromen-4-one (**8f**)*. The corresponding aryl boronic acid was (4-hydroxyphenyl)boronic acid. Yellow solid, yield, 36%; m.p. 249–251 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 6H), 5.72 (d, J = 10.0 Hz, 1H), 6.81 (d, J = 10.0 Hz, 1H), 6.85–6.90 (m, 3H), 7.41–7.44 (m, 2H), 7.94 (s, 1H), 8.07 (d, J = 8.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 28.3, 64.5, 64.6, 77.4, 77.8, 109.3, 115.1, 115.3, 117.4, 118.1, 118.5, 122.3, 124.7, 125.3, 126.9, 130.4, 143.5, 143.8, 152.0, 152.4, 157.4, 175.9. MS (ESI): m/z calcd. $C_{20}H_{17}O_4^+$ $[M+H]^+$ 321.11, found 321.27. HPLC purity: 98.0%.

*4.1.5.8. 3-(2-Methoxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f] chromen-4-one (**8g**)*. The corresponding aryl boronic acid was (2-methoxyphenyl)boronic acid. White solid, yield, 53%; m.p. 159–160 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 6H), 3.80 (s, 3H), 5.71 (d, J = 10.0 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 6.85 (d, J = 8.8 Hz, 1H), 6.97–7.04 (m, 2H), 7.32 (dd, J = 1.6, 7.2 Hz, 1H), 7.34–7.39 (m, 1H), 7.93 (s, 1H), 8.05 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.2, 55.9, 78.5, 109.4, 111.3, 115.2, 115.2, 118.6, 120.7, 121.1, 122.5, 126.9, 129.8, 130.3, 131.9, 152.5, 153.6, 157.3, 157.7, 175.6. MS (ESI): *m/z* calcd. $C_{21}H_{19}O_4$ $[M+H]^+$ 335.13, found 335.23. HPLC purity: 98.0%.

*4.1.5.9. 3-(3-Methoxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f] chromen-4-one (**8h**)*. The corresponding aryl boronic acid was (3-methoxyphenyl)boronic acid. White solid, yield, 81%; m.p. 135–137 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 6H), 3.85 (s, 3H), 5.73 (d, J = 10.0 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 6.87 (d, J = 8.8 Hz, 1H), 6.92–6.95 (m, 1H), 7.11 (d, J = 7.6 Hz, 1H), 7.15 (dd, J = 1.6, 2.4 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.98 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 55.4, 77.9, 109.3, 114.2, 114.6, 115.0, 115.4, 118.5, 121.4, 125.0, 126.9, 129.6, 130.5, 133.4, 152.4, 152.5, 157.5, 159.7, 175.7. MS (ESI): *m/z* calcd $C_{21}H_{19}O_4$ $[M+H]^+$ 335.13, found 335.21. HPLC purity: 98.1%.

*4.1.5.10. 3-(4-Methoxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f] chromen-4-one (**8i**)*. The corresponding aryl boronic acid was (4-methoxyphenyl)boronic acid. White solid, yield, 68%; m.p. 142–144 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.50 (s, 6H), 3.84 (s, 3H), 5.72 (d, J = 10.2 Hz, 1H), 6.81 (d, J = 9.6 Hz, 1H), 6.86 (d, J = 9.0 Hz, 1H), 6.96–6.98 (m, 2H), 7.48–7.51 (m, 2H), 7.94 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 55.5, 77.8, 109.3, 114.1, 115.1, 115.3, 118.5, 124.4, 124.8, 126.9, 130.3, 130.4, 151.9, 152.5, 157.4, 159.7, 176.0. MS (ESI): *m/z* calcd. $C_{21}H_{19}O_4$ $[M+H]^+$ 335.13, found 335.21. HPLC purity: 99.1%.

*4.1.5.11. 3-(Benzo[d][1,3]dioxol-5-yl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8j**)*. The corresponding aryl boronic acid was benzo[*d*][1,3]dioxol-5-ylboronic acid. White solid, yield, 71%; m.p. 153–155 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.50 (s, 6H), 5.72 (d, J = 10.0 Hz, 1H), 6.00 (s, 2H), 6.81 (d, J = 10.0 Hz, 1H), 6.87 (dd, J = 4.0, 8.0 Hz, 2H), 6.97 (dd, J = 1.8, 8.4 Hz, 1H), 7.09 (d, J = 1.2 Hz, 1H), 7.93 (s, 1H), 8.06 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 77.9, 101.3, 108.5, 109.3, 109.9, 115.1, 115.4, 118.4, 122.5, 125.0, 125.8, 126.9, 130.4, 147.8, 147.8, 152.0, 152.5, 157.5, 175.9. MS (ESI): *m/z* calcd. $C_{21}H_{17}O_5$ $[M+H]^+$ 349.11, found 349.22. HPLC purity: 98.0%.

*4.1.5.12. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8k**)*. The corresponding aryl boronic acid was (2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)boronic acid. White solid, yield, 76%; m.p. 185–187 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.52 (s, 6H), 4.28 (s, 4H), 5.72 (d, J = 10.0 Hz, 1H), 6.81 (d, J = 10.0 Hz, 1H), 6.86 (d, J = 9.0 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 7.03 (dd, J = 2.4, 8.4 Hz, 1H), 7.10 (d, J = 1.8 Hz, 1H), 7.92 (s, 1H), 8.06 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 64.5, 64.6, 77.8, 109.3, 115.1, 115.3, 117.4, 118.1, 118.5, 122.3, 124.7, 125.3, 126.9, 130.4, 143.5, 143.8, 152.0, 152.4, 157.4, 175.9. MS (ESI): *m/z* calcd $C_{22}H_{19}O_5$ $[M+H]^+$ 363.12, found 363.24. HPLC purity: 99.4%.

*4.1.5.13. Methyl 3-(8,8-dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)benzoate (**8l**)*. The corresponding aryl boronic acid was (3-(methoxycarbonyl)phenyl)boronic acid. White solid, yield, 72%; m.p. 169–170 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 6H), 3.93 (s, 3H), 5.74 (d, J = 10.0 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 6.88 (dd, J = 0.4, 8.8 Hz, 1H), 7.52 (t, J = 8.0 Hz, 1H), 7.82–7.83 (m, 1H), 8.03 (s, 1H), 8.05–8.08 (m, 2H), 8.19 (t, J = 1.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 52.3, 78.0, 109.4, 115.0, 115.6, 118.4, 124.4, 126.8, 128.7, 129.4, 129.9, 130.6, 130.6, 132.4, 133.9, 152.5, 152.7, 157.6, 167.0, 175.5. HR-TOF-MS (positive mode): *m/z* calcd. $C_{22}H_{19}O_5$ $[M+H]^+$ 363.1232, found 363.1229. HPLC purity: 99.3%.

*4.1.5.14. 3-(2,3-Dimethoxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f] chromen-4-one (**8m**)*. The corresponding aryl boronic acid was (2,3-dimethoxyphenyl)boronic acid. White solid, yield, 39%; m.p. 129–131 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.51 (s, 6H), 3.74 (s, 3H), 3.90 (s, 3H), 5.72 (d, J = 9.6 Hz, 1H), 6.83 (d, J = 10.2 Hz, 1H), 6.86 (d, J = 9.0 Hz, 1H), 6.95–6.98 (m, 2H), 7.11 (t, J = 7.8 Hz, 1H), 7.96 (s, 1H), 8.06 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 56.0, 60.9, 77.8, 109.4, 112.8, 115.2, 115.2, 118.6, 121.9, 123.7, 124.0, 126.2, 126.8, 130.4, 147.4, 152.5, 153.0, 153.8, 157.4, 175.9. HR-TOF-MS (positive mode): *m/z* calcd. $C_{22}H_{21}O_5^+$ $[M+H]^+$ 365.1389, found 365.1388. HPLC purity: 97.1%.

*4.1.5.15. 8,8-Dimethyl-3-(4-(trifluoromethyl)phenyl)-4H,8H-pyrano[2,3-f]chromen-4-one (**8n**)*. The corresponding aryl boronic acid was (2,3-dimethoxyphenyl)boronic acid. White solid, yield, 47%; m.p. 193–194 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.51 (s, 6H), 5.74 (d, J = 9.6 Hz, 1H), 6.81 (d, J = 10.2 Hz, 1H), 6.89 (d, J = 9.0 Hz, 1H), 7.69 (s, 4H), 8.01 (s, 1H), 8.07 (d, J = 9.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 78.0, 114.9, 115.7, 118.4, 124.2, 125.5, 126.8, 129.4, 130.1, 130.4, 130.7, 135.8, 152.5, 28.3, 152.8, 157.8, 175.4. MS (ESI): *m/z* calcd. $C_{21}H_{16}F_3O_3$ $[M+H]^+$ 373.11, found 373.21. HPLC purity: 97.9%.

*4.1.5.16. 3-(4-Acetylphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8o**)*. The corresponding aryl boronic acid was (4-acetylphenyl) boronic acid. White solid, yield, 55.4%; m.p. 188–190 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 6H), 2.64 (s, 3H), 5.74 (d, J = 10.0 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 8.4 Hz, 2H), 8.02–8.04 (m, 3H); 8.07 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 26.8, 28.3, 78.0, 109.4, 114.9, 115.6, 118.4, 124.3, 126.8, 128.6, 129.2, 130.6, 136.6, 137.1, 152.4, 152.9, 157.7, 175.3, 197.9. MS (ESI): *m/z* calcd. $C_{22}H_{19}O_4$ $[M+H]^+$ 347.13, found 347.22. HPLC purity: 99.6%.

*4.1.5.17. 4-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl) benzonitrile (**8p**)*. The corresponding aryl boronic acid was (4-cyanophenyl)boronic acid. White solid, yield, 60.8%; m.p. 205–206 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 6H), 5.75 (d, J = 10.0 Hz, 1H), 6.81 (d, J = 10.0 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 7.69–7.74 (m, 4H), 8.02 (s, 1H), 8.06 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 78.1, 109.4, 111.8, 114.8, 115.8, 118.2, 118.9, 123.7, 126.8, 129.6, 130.8, 132.3, 137.0, 152.4, 153.1, 157.9, 175.0. MS (ESI): *m/z* calcd. $C_{22}H_{19}O_4$ $[M+H]^+$ 330.11, found 330.15. HPLC purity: 98.3%.

*4.1.5.18. 3-(2,3-Difluorophenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f] chromen-4-one (**8q**)*. The corresponding aryl boronic acid was (2,3-difluorophenyl)boronic acid. White solid, yield, 61%; m.p. 137–139 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 6H), 5.74 (d, J = 10.0 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 7.11–7.25 (m, 3H), 8.01 (d, J = 1.6 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 78.0, 109.5, 114.9, 115.7, 117.3, 118.3, 119.2, 122.0, 124.0, 126.8, 126.9, 130.7, 147.5, 149.8, 152.5, 154.1, 157.8, 174.9. HR-TOF-MS (positive mode): *m/z* calcd. $C_{20}H_{15}F_2O_3$ $[M+H]^+$ 341.0989, found 341.0984. HPLC purity: 98.9%.

*4.1.5.19. 3-(2,5-Difluorophenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f] chromen-4-one (**8r**)*. The corresponding aryl boronic acid was (2,5-difluorophenyl)boronic acid. White solid, yield, 46.3%; m.p. 182–183 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 6H), 5.74 (d, J = 10.0 Hz, 1H), 6.81 (d, J = 10.0 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H),

7.02–7.14 (m, 2H), 7.27–7.30 (m, 1H), 8.03 (d, J = 1.6 Hz, 1H), 8.05 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 78.0, 109.5, 114.9, 115.7, 116.4, 116.9, 118.3, 118.6, 118.9, 119.0, 126.8, 130.7, 152.4, 154.3, 155.1, 157.7, 159.7, 174.8. HR-TOF-MS (positive mode): m/z calcd. $C_{20}H_{15}F_2O_3$ $[M+H]^+$ 341.0989, found 341.0990. HPLC purity: 97.9%.

*4.1.5.20. 3-(2-Fluoro-5-methoxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8s**)*. The corresponding aryl boronic acid was (2-fluoro-5-methoxyphenyl)boronic acid. White solid, yield, 54%; m.p. 132–134 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 6H), 3.81 (s, 3H), 5.73 (d, J = 10.0 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 6.85–6.89 (m, 2H), 8.01 (d, J = 1.6 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 56.0, 77.9, 109.4, 115.0, 115.5, 115.5, 116.4, 116.7, 118.4, 119.9, 120.0, 126.8, 130.6, 152.4, 154.1, 155.6, 156.0, 157.6, 175.1. HR-TOF-MS (positive mode): m/z calcd. $C_{21}H_{18}FO_4$ $[M+H]^+$ 353.1189, found 353.1186. HPLC purity: 97.2%.

*4.1.5.21. 3-(2-Fluoro-3-methoxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8t**)*. The corresponding aryl boronic acid was (2-fluoro-3-methoxyphenyl)boronic acid. White solid, yield, 43%; m.p. 168–170 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 6H), 3.92 (s, 3H), 5.73 (d, J = 10.0 Hz, 1H), 6.82 (d, J = 10.0 Hz, 1H), 6.87 (d, J = 8.8 Hz, 1H), 6.98–7.97 (m, 2H), 7.11–7.15 (m, 1H), 7.99 (d, J = 1.2 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 56.5, 77.9, 109.4, 113.5, 115.0, 115.5, 118.4, 119.8, 120.5, 123.5, 123.8, 126.8, 130.5, 148.1, 148.2, 152.5, 154.0, 157.6, 175.1. HR-TOF-MS (positive mode): m/z calcd. $C_{21}H_{18}FO_4$ $[M+H]^+$ 353.1189, found 353.1186. HPLC purity: 98.8%.

*4.1.5.22. 3-(2-Fluoro-3-hydroxyphenyl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8u**)*. The corresponding aryl boronic acid was (2-fluoro-3-hydroxyphenyl)boronic acid. Yellow solid, yield, 60%; m.p. 214–216 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.51 (s, 6H), 5.73 (d, J = 10.0 Hz, 1H), 5.89 (s, 1H), 6.81 (d, J = 10.0 Hz, 1H), 6.87–6.92 (m, 2H), 6.97–7.02 (m, 1H), 7.06 (t, J = 8.0 Hz, 1H), 7.96 (s, 1H), 8.07 (d, J = 8.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 27.6, 77.9, 109.1, 114.1, 115.2, 117.5, 117.8, 120.1, 120.6, 120.7, 121.4, 123.9, 125.9, 131.5, 145.0, 145.1, 151.7, 154.5, 156.8, 173.6. HR-TOF-MS (positive mode): m/z calcd. $C_{20}H_{16}FO_4$ $[M+H]^+$ 339.1033, found 339.1028. HPLC purity: 99.2%.

*4.1.6. General procedure for the preparation of compounds **8v** and **8w***

Na_2CO_3 (32 mg, 0.3 mmol), heteroaryl boronic acids (0.12 mmol), and tetrakis(triphenylphosphine)palladium (6 mg, 5 mol %) were added to a solution of **7** (35 mg, 0.1 mmol) in THF (3 mL) and H_2O (0.4 mL). After 6 h stirring at 60 °C, the mixture was concentrated *in vacuo* and purified by flash chromatography to give **8v** and **8w**.

*4.1.6.1. 3-(5-Fluoropyridin-3-yl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8v**)*. The corresponding heteroaryl boronic acid was (5-fluoropyridin-3-yl)boronic acid. Yellow solid, yield, 61.2%; m.p. 169–170 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 6H), 5.75 (d, J = 10.0 Hz, 1H), 6.8 (d, J = 10.0 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 7.84–7.87 (s, 1H), 8.05 (d, J = 8.8 Hz, 1H), 8.07 (s, 1H), 8.48 (d, J = 2.8 Hz, 1H), 8.52 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 78.1, 109.5, 114.8, 115.9, 118.1, 120.8, 124.1, 126.8, 129.7, 130.8, 137.7, 144.4, 152.5, 152.7, 158.0, 160.5, 175.1. HR-TOF-MS (positive mode): m/z calcd. $C_{19}H_{15}FNO_3$ $[M+H]^+$ 324.1036, found 324.1037. HPLC purity: 99.1%.

*4.1.6.2. 3-(5-Methoxypyridin-3-yl)-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**8w**)*. The corresponding heteroaryl boronic acid was (5-fluoropyridin-3-yl)boronic acid. Yellow solid, yield, 40.8%; mp 159–161 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 6H), 3.90 (s, 3H), 5.74 (d, J = 10.0 Hz, 1H), 6.80 (d, J = 10.0 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 7.61 (t, J = 2.0 Hz, 1H), 8.04 (s, 1H), 8.06 (d, J = 8.8 Hz, 1H), 8.27 (s, 1H), 8.32 (d, J = 2.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 55.8, 78.0, 109.4, 114.8, 115.8, 118.2, 121.3, 121.7, 126.7, 128.8, 130.7, 137.8, 140.7, 152.5, 152.5, 155.4, 157.8, 175.5. HR-TOF-MS (positive mode): m/z calcd. $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1236, found 336.1234. HPLC purity: 99.4%.

*4.1.7. General procedure for the preparation of compounds **9a**-**9e***

Phenol (64.0 mg, 0.2 mmol) and potassium carbonate (44.0 mg, 0.32 mmol) were added into a mixture of dimethylformamide (1.5 mL) and acetonitrile (0.6 mL). Then, carbamoyl chloride (0.22 mmol) in acetonitrile (0.3 mL) was added dropwise. The reaction mixture was stirred and refluxed at 95 °C for 3 h. After cooling to room temperature, 20 mL of water was added and the crude mixture was extracted three times with ethyl acetate. The collected organic layer was washed three times with saturated salt solution, dried over Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography to provide **9a-9e.**

*4.1.7.1. 2-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl dimethylcarbamate (**9a**)*. The corresponding phenol was **8d**, and the corresponding carbamoyl chloride was N,*N*-dimethylcarbamoyl chloride. Yellow solid, yield, 52%; m.p. 61–63 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.51 (s, 6H), 2.89 (s, 6H), 5.73 (d, J = 10.2 Hz, 1H), 6.82 (d, J = 10.2 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 7.24–7.27 (m, 2H), 7.32 (dd, J = 1.2, 7.2 Hz, 1H), 7.39–7.42 (m, 1H), 7.91 (s, 1H), 8.04 (d, J = 9.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 36.5, 36.7, 77.9, 109.4, 115.0, 115.3, 118.3, 122.5, 123.2, 125.4, 125.5, 126.7, 129.7, 130.4, 131.5, 150.0, 152.5, 153.4, 154.8, 157.4, 175.1. HR-TOF-MS (positive mode): m/z calcd. $C_{23}H_{22}NO_5^+$ (M^+) 392.1498, found 392.1503. HPLC purity: 98.9%.

*4.1.7.2. 3-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl dimethylcarbamate (**9b**)*. The corresponding phenol was **8e**, and the corresponding carbamoyl chloride was N,N-dimethylcarbamoyl chloride. White solid, yield, 65.0%; m.p. 234–236 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.51 (s, 6H), 3.01 (s, 3H), 3.11 (s, 3H), 5.73 (d, J = 10.2 Hz, 1H), 6.81 (d, J = 9.6 Hz, 1H), 6.87 (dd, J = 0.6, 9.0 Hz, 1H), 7.13–7.15 (m, 1H), 7.36–7.38 (m, 2H), 7.41 (t, 7.8 Hz, 1H), 8.00 (s, 1H), 8.06 (d, J = 9.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 36.6, 36.8, 77.9, 109.3, 115.0, 115.5, 118.4, 121.7, 122.6, 124.4, 125.7, 126.8, 129.4, 130.5, 133.3, 151.6, 152.4, 152.8, 155.0, 157.5, 175.6. HR-TOF-MS (positive mode): m/z calcd. $C_{23}H_{22}NO_5^+$ (M^+) 392.1498, found 392.1493. HPLC purity: 97.6%.

*4.1.7.3. 4-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl dimethylcarbamate (**9c**)*. The corresponding phenol was **8f**, and the corresponding carbamoyl chloride was N,N-dimethylcarbamoyl chloride. White solid, yield, 62.7%; m.p. 212–213 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.50 (s, 6H), 3.03 (s, 3H), 3.12 (s, 3H), 5.73 (d, J = 10.2 Hz), 6.81 (d, J = 10.2 Hz, 1H), 6.87 (d, J = 9.0 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.96 (s, 1H), 8.06 (d, J = 9.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 36.6, 36.9, 77.9, 109.4, 115.1, 115.4, 118.5, 121.9, 124.6, 126.8, 129.0, 130.0, 130.5, 151.6, 152.4, 152.5, 155.0, 157.5, 175.8. HR-TOF-MS (positive mode): m/z calcd. $C_{23}H_{22}NO_5$ $[M+H]^+$ 392.1498, found 392.1494. HPLC purity: 98.9%.

*4.1.7.4. 2-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl ethyl(methyl)carbamate (**9d**)*. The corresponding phenol was **8d**, and the corresponding carbamoyl chloride was *N*-ethyl-*N*-methylcarbamoyl chloride. Yellow solid, yield, 56.3%; m.p. 65–67 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.88–0.92 (m, 3H), 1.50 (s, 6H), 2.78 (d, J = 16.8 Hz, 3H), 3.16–3.24 (m, 2H), 5.65 (d, J = 10.0 Hz, 1H), 6.73 (d, J = 10.0 Hz, 1H), 6.78 (d, J = 8.8 Hz, 1H), 7.15–7.20 (m, 2H), 7.25 (d, J = 7.6 Hz, 1H), 7.31–7.35 (m, 1H), 7.83 (s, 1H), 7.96 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.6, 28.3, 34.0, 44.0, 77.8, 109.4, 115.0, 115.3, 118.3, 122.5,

123.1, 125.4, 125.5, 126.6, 129.7, 130.5, 131.5, 150.0, 152.5, 153.5, 154.4, 157.4, 175.1. HR-TOF-MS (positive mode): *m/z* calcd. $C_{24}H_{23}NO_5Na$ $[M+Na]^+$ 428.1474, found 428.1472. HPLC purity: 98.2%.

*4.1.7.5. 3-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl ethyl(methyl)carbamate (**9e**)*. The corresponding phenol was **8e**, and the corresponding carbamoyl chloride was *N*-ethyl-*N*-methylcarbamoyl chloride. White solid, yield, 30.76%; m.p. 178–180 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.17–1.26 (m, 3H), 1.50 (s, 6H), 3.03 (d, *J* = 34.4 Hz, 3H), 3.38–3.51 (m, 2H), 5.72 (d, *J* = 10.0 Hz, 1H), 6.8 (d, *J* = 10.0 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 7.35–7.41 (m, 3H), 8.00 (s, 1H), 8.05 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 13.0, 28.3, 34.2, 44.2, 77.9, 109.3, 115.0, 115.5, 118.4, 121.7, 122.7, 124.5, 125.7, 126.9, 129.4, 130.5, 133.3, 151.6, 152.5, 152.8, 154.6, 157.6, 175.7. HR-TOF-MS (positive mode): *m/z* calcd. $C_{24}H_{23}NO_5Na$ $[M+Na]^+$ 428.1474, found 428.1474. HPLC purity: 99.3%.

*4.1.8. 4-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl ethyl(methyl)carbamate (**9f**)*

64.0 mg (0.2 mmol) of **8f** and potassium carbonate (83.0 mg, 0.32 mmol) were stirred in acetonitrile (3 mL). *N*-ethyl-*N*-methylcarbamoyl Chloride (29.0 mg, 26 μL) was added dropwise. After 12 h stirring at room temperature, The reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography to provide a white solid **9f** (27 mg, 33.7%); m.p. 206–207 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.17–1.26 (m, 3H), 1.49 (s, 6H), 3.04 (d, *J* = 2.4 Hz, 3H), 3.39–3.51 (m, 2H), 5.72 (d, *J* = 10.0 Hz, 1H), 6.8 (d, *J* = 10.0 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 1H), 7.17 (dd, *J* = 2.8, 8.0 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.95 (s, 1H), 8.05 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 13.0, 28.2, 34.2, 44.2, 77.9, 109.3, 115.0, 115.4, 118.4, 121.9, 124.5, 126.8, 128.9, 130.0, 130.4, 151.6, 152.4, 152.4, 154.5, 157.4, 175.8. HR-TOF-MS (positive mode): *m/z* calcd. $C_{24}H_{23}NO_5Na$ $[M+Na]^+$ 428.1474, found 428.1476. HPLC purity: 99.5%.

*4.1.9. General procedure for the preparation of compounds **9g**-**9n***

To a solution of phenol (0.2 mmol) in CH_2Cl_2 (3 mL), triethylamine (31 μL, 0.22 mmol) was added at room temperature. After 15 min stirring at room temperature, isocyanate (0.22 mmol) was added. The mixture was stirred at room temperature for 6 h and concentrated *in vacuo*. The resulting residue was purified by flash chromatography to provide **9g**-**9n**.

*4.1.9.1. 2-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl heptylcarbamate (**9g**)*. The corresponding phenol was **8d**, and the corresponding isocyanate was 1-isocyanatoheptane. White solid, yield, 56%; m.p. 89–91 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 0.78 (t, *J* = 6.8 Hz, 3H), 1.11–1.31 (m, 10H), 1.43 (s, 6H), 3.03 (q, *J* = 6.8 Hz, 2H), 5.02 (t, *J* = 5.2 Hz, 1H), 5.65 (d, *J* = 10.0 Hz, 1H), 6.72 (d, *J* = 10.0 Hz, 1H), 6.78 (d, *J* = 8.8 Hz, 1H), 7.14–7.20 (m, 2H), 7.24 (dd, *J* = 1.6, 7.2 Hz, 1H), 7.30–7.35 (m, 2H), 7.83 (s, 1H), 7.95 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 14.2, 22.7, 26.6, 28.3, 28.9, 29.7, 31.8, 41.3, 77.9, 109.4, 115.0, 115.4, 118.2, 122.3, 123.0, 125.6, 125.7, 126.7, 129.8, 130.4, 131.7, 149.7, 152.5, 153.6, 154.7, 157.5, 175.3. HR-TOF-MS (positive mode): *m/z* calcd. $C_{28}H_{32}NO_5$ $[M+H]^+$ 462.2280, found 462.2274. HPLC purity: 99.1%.

*4.1.9.2. 3-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl heptylcarbamate (**9h**)*. The corresponding phenol was **8e**, and the corresponding isocyanate was 1-isocyanatoheptane. White solid, yield, 65%; m. p. 107–109 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 0.89 (t, *J* = 6.4 Hz, 3H), 1.29–1.33 (m, 8H), 1.50–1.58 (m, 8H), 3.25 (q, *J* = 6.4 Hz, 2H), 5.01 (s, 1H), 5.72 (d, *J* = 10.0 Hz, 1H), 6.8 (d, *J* = 10.0 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 1H), 7.14–7.15 (m, 1H), 7.36–7.42 (m, 3H), 7.98 (s, 1H), 8.06 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 14.2, 22.7, 26.9, 28.3, 29.1, 30.0, 31.9, 41.4, 77.9, 109.3, 115.0, 115.5, 118.4, 121.5, 122.4, 124.4, 125.9, 126.8, 129.4, 130.5, 133.3, 151.2, 152.4, 152.7, 154.7, 157.5, 175.5. HR-TOF-MS (positive mode): *m/z* calcd. $C_{28}H_{32}NO_5$ $[M+H]^+$ 462.2280, found 462.2275. HPLC purity: 96.8%.

*4.1.9.3. 4-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl heptylcarbamate (**9i**)*. The corresponding phenol was **8f**, and the corresponding isocyanate was 1-isocyanatoheptane. White solid, yield, 70.4%; m.p. 140–142 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 0.82 (t, *J* = 6.8 Hz, 3H), 1.18–1.29 (m, 8H), 1.43 (s, 6H), 1.47–1.54 (m, 2H), 3.2 (q, *J* = 6.8 Hz, 2H), 4.99 (s, 1H), 5.66 (d, *J* = 10.0 Hz, 1H), 6.74 (d, *J* = 10.0 Hz, 1H), 6.8 (d, *J* = 8.8 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 2H),7.88 (s, 1H), 7.99 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 14.2, 22.7, 26.9, 28.3, 29.1, 30.0, 31.9, 41.4, 77.9, 109.4, 115.0, 115.4, 118.5, 121.8, 124.6, 126.8, 129.0, 130.1, 130.5, 151.2, 152.4, 152.5, 154.6, 157.5, 175.8. HR-TOF-MS (positive mode): *m/z* calcd. $C_{28}H_{32}NO_5$ $[M+H]^+$ 462.2280, found 462.2277. HPLC purity: 99.5%.

*4.1.9.4. 2-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl phenethylcarbamate (**9j**)*. The corresponding phenol was **8d**, and the corresponding isocyanate was (2-isocyanatoethyl)benzene. White solid, yield, 60%; m.p. 123–125 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.50 (s, 6H), 2.72 (t, *J* = 6.4 Hz, 2H), 3.41 (q, *J* = 6.4 Hz, 2H), 5.06 (s, 1H), 5.73 (d, *J* = 10.0 Hz, 1H), 6.82 (d, *J* = 10.0 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 7.07 (d, *J* = 7.2 Hz, 2H), 7.16–7.22 (m, 4H), 7.26–7.34 (m, 2H), 7.41 (t, *J* = 7.2 Hz, 1H), 7.91 (m, 1H), 8.04 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 28.3, 36.1, 42.5, 77.9, 109.4, 115.0, 115.4, 118.3, 122.4, 123.1, 125.5, 125.8, 126.6, 126.8, 128.7, 128.8, 129.8, 130.6, 131.8, 138.6, 149.6, 152.6, 153.6, 154.6, 157.5, 175.3. HR-TOF-MS (positive mode): *m/z* calcd. $C_{29}H_{26}NO_5$ $[M+H]^+$ 468.1811, found 468.1804. HPLC purity: 99.8%.

*4.1.9.5. 3-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl phenethylcarbamate (**9k**)*. The corresponding phenol was **8e**, and the corresponding isocyanate was (2-isocyanatoethyl)benzene. White solid, yield, 82%; m.p. 145–147 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.51 (s, 6H), 2.89 (t, *J* = 6.8 Hz, 2H), 3.53 (q, *J* = 6.4 Hz, 2H), 5.11 (s, 1H), 5.73 (d, *J* = 10.0 Hz, 2H), 6.81 (d, *J* = 10.0 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 7.13 (d, *J* = 6.4 Hz, 1H), 7.23–7.27 (m, 3H), 7.32–7.48 (m, 5H), 7.99 (s, 1H), 8.06 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 28.3, 36.1, 42.5, 77.9, 109.3, 115.0, 115.5, 118.4, 121.4, 122.4, 124.4, 125.9, 126.8, 126.9, 128.9, 129.0, 129.4, 130.5, 133.3, 138.7, 151.1, 152.4, 152.7, 154.6, 157.5, 175.5. HR-TOF-MS (positive mode): *m/z* calcd. $C_{29}H_{26}NO_5$ $[M+H]^+$ 468.1811, found 468.1808. HPLC purity: 99.9%.

*4.1.9.6. 4-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl phenethylcarbamate (**9l**)*. The corresponding phenol was **8f**, and the corresponding isocyanate was (2-isocyanatoethyl)benzene. White solid, yield, 83%; m.p. 174–176 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.42 (s, 6H), 2.82 (t, *J* = 6.8 Hz, 2H), 3.47 (q, *J* = 6.8 Hz, 2H), 5.06 (t, *J* = 5.6 Hz, 1H), 5.65 (d, *J* = 10.0 Hz, 1H), 6.73 (d, *J* = 10.0 Hz, 1H), 6.79 (d, *J* = 8.8 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 2H), 7.15–7.19 (m, 3H), 7.26 (t, *J* = 7.2 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.87 (s, 1H), 7.98 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 28.3, 36.1, 42.5, 77.9, 109.4, 115.0, 115.5, 118.5, 121.8, 124.6, 126.8, 126.9, 128.9, 129.0, 129.1, 130.1, 130.5, 138.7, 151.1, 152.4, 152.5, 154.6, 157.5, 175.8. HR-TOF-MS (positive mode): *m/z* calcd. $C_{29}H_{26}NO_5$ $[M+H]^+$ 468.1811, found 468.1804. HPLC purity: 99.6%.

*4.1.9.7. 3-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl (4-isopropylphenyl)carbamate (**9m**)*. The corresponding phenol was **8e**, and the corresponding isocyanate was 1-isocyanato-4-isopropylbenzene. White solid, yield, 51%; m.p. 184–186 °C; ^{1}H NMR

(DMSO-d_6, 400 MHz) δ: 1.24 (d, J = 6.8 Hz, 6H), 1.51 (s, 6H), 2.83–2.93 (m, 1H), 5.73 (d, J = 10.0 Hz, 1H), 6.81 (d, J = 10.0 Hz, 1H), 6.87 (d, J = 8.8 Hz, 1H), 6.99 (s, 1H), 7.18–7.20 (m, 3H), 7.36–7.43 (m, 5H), 8.0 (d, J = 2.8 Hz, 1H), 8.07 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 24.0, 27.7, 32.8, 77.9, 109.0, 114.1, 115.1, 117.8, 118.6, 121.5, 122.3, 122.7, 125.8, 126.1, 126.6, 129.1, 131.5, 133.2, 136.3, 143.1, 150.3, 151.6, 151.7, 154.3, 156.7, 174.3. HR-TOF-MS (positive mode): m/z calcd. $C_{30}H_{28}NO_5$ $[M+H]^+$ 482.1967, found 482.1969. HPLC purity: 98.7%.

*4.1.9.8. 4-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl (4-isopropylphenyl)carbamate (**9n**)*. The corresponding phenol was **8f**, and the corresponding isocyanate was 1-isocyanato-4-isopropylbenzene. White solid, yield, 48%; m.p. 183–185 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.16 (d, J = 7.2 Hz, 6H), 1.43 (s, 6H), 2.77–2.84 (m, 1H), 5.65 (d, J = 10.0 Hz, 1H), 6.73 (d, J = 10.0 Hz, 1H), 6.79 (d, J = 8.8 Hz, 1H), 7.10–7.18 (m, 5H), 7.31 (d, J = 7.6 Hz, 2H), 7.49 (d, J = 7.6 Hz, 2H), 7.87 (s, 1H), 8.0 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 24.2, 28.3, 33.7, 77.9, 109.4, 115.0, 115.5, 118.4, 119.1, 121.8, 121.9, 124.5, 126.8, 127.1, 129.4, 130.2, 130.5, 135.2, 144.7, 150.7, 151.8, 152.5, 157.5, 175.9. HR-TOF-MS (positive mode): m/z calcd. $C_{30}H_{28}NO_5$ $[M+H]^+$ 482.1967, found 482.1962. HPLC purity: 98.2%.

*4.1.9.9. 3-(8,8-Dimethyl-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)-2-fluorophenyl heptylcarbamate (**9o**)*. The corresponding phenol was **8u**, and the corresponding isocyanate was 1-isocyanatoheptane. White solid, yield, 74.7%; m.p. 113–115 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.88 (t, J = 6.8 Hz, 3H), 1.27–1.34 (m, 8H), 1.50 (s, 6H), 1.53–1.59 (m, 2H), 3.26 (q, J = 6.4 Hz), 5.17 (s, 1H), 5.73 (d, J = 10.0 Hz, 1H), 6.81 (d, J = 10.0 Hz, 1H), 6.87 (d, J = 8.8 Hz, 1H), 7.15–7.24 (m, 2H), 7.31–7.35 (m, 1H), 8.02 (s, 1H), 8.05 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 14.2, 22.7, 26.8, 28.3, 29.1, 29.9, 31.9, 41.6, 77.9, 109.4, 115.0, 115.5, 118.3, 119.4, 121.0, 123.8, 124.2, 126.8, 128.8, 130.5, 139.0, 151.3, 152.5, 153.8, 154.2, 157.6, 175.0. HR-TOF-MS (positive mode): m/z calcd. $C_{28}H_{31}FNO_5$ $[M+H]^+$ 480.2186, found 480.2185. HPLC purity: 99.0%.

*4.1.10. General procedure for the preparation of compounds **11a** and **11b***

Compound **5** (144 mg, 0.5 mmol) and potassium carbonate (111 mg, 0.8 mmol) were added into a mixture of dimethylformamide (1.5 mL) and acetonitrile (0.6 mL). Then, carbamoyl chloride (0.68 mmol) in acetonitrile (0.3 mL) was added dropwise. The reaction mixture was refluxed at 95 °C for 3 h. After cooling to room temperature, the reaction mixture was neutralized with hydrochloric acid. Excess reducing agent was quenched by careful addition of water (20 mL), the mixture was extracted three times with ethyl acetate. The organic layer was washed three times with saturated salt solution, dried with Na_2SO_4, filtered, and then concentrated. The residue was washed with 5 mL methanol and filtered to give the crude compounds **10a** and **10b**. The crude was used in the next step without further purification.

Na_2CO_3 (95 mg, 0.9 mmol), (3,4-dimethoxyphenyl) boronic acid (0.36 mmol), and Pd/C (16 mg, 5 mol %) were added to a solution of the crude compounds **10a**/**10b** (0.3 mmol) in DME (1.8 mL) and H_2O (1.8 mL). The resulting mixture was stirred for 1 h at 45 °C and filtered. The cake was successively washed with H_2O (4 mL) and CH_2Cl_2 (6 mL). Then, the aqueous phase was extracted twice with CH_2Cl_2. After drying with anhydrous Na_2SO_4, the CH_2Cl_2 layer was filtered and concentrated *in vacuo*. The residue was purified by flash chromatography to give **11a** and **11b**.

*4.1.10.1. 3-(3,4-Dimethoxyphenyl)-4-oxo-4H-chromen-7-yl dimethylcarbamate (**11a**)*. The corresponding carbamoyl chloride was N,N-dimethylcarbamoyl chloride. White solid, yield over two steps, 51%; m.p. 164–166 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.05 (s, 3H), 3.14 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 6.93 (d, J = 8.0 Hz, 1H), 7.05 (dd, J = 2.0, 8.4 Hz, 1H), 7.18–7.21 (m, 2H), 7.33 (d, J = 2.4 Hz, 1H),7.99 (s, 1H), 8.29 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 36.7, 37.0, 56.1, 77.6, 110.8, 111.3, 112.5, 119.7, 121.2, 121.8, 124.5, 125.2, 127.6, 148.9, 149.3, 152.9, 153.8, 155.6, 156.8, 176.0. HR-TOF-MS (positive mode): m/z calcd $C_{20}H_{20}NO_6$ $[M+H]^+$ 370.1291, found 370.1289. HPLC purity: 99.4%.

*4.1.10.2. 3-(3,4-Dimethoxyphenyl)-4-oxo-4H-chromen-7-yl ethyl(methyl) carbamate (**11b**)*. The corresponding carbamoyl chloride was N-ethyl-N-methylcarbamoyl chloride. White solid, yield over two steps, 56%; m.p. 129–131 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.20–1.29 (m, 3H), 3.02 (s, 3H), 3.10 (s, 3H), 3.41–3.53 (m, 2H), 3.91 (s, 3H), 3.93 (s, 3H), 6.93 (d, J = 8.0 Hz, 1H), 7.06 (dd, J = 2.0, 8.0 Hz, 1H), 7.18–7.21 (m, 2H), 7.34 (dd, J = 2.4, 6 Hz, 1H), 8.00 (s, 1H), 8.29 (d, J = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.0, 34.3, 44.4, 56.1, 110.8, 111.3, 112.5, 119.7, 121.2, 121.8, 124.5, 125.2, 127.6, 148.9, 149.3, 152.9, 153.4, 155.6, 156.8, 176.0. HR-TOF-MS (positive mode): m/z calcd. $C_{21}H_{22}NO_6$ $[M+H]^+$ 384.1447, found 384.1442. HPLC purity: 99.5%.

4.2. EeAChE and eqBChE inhibition assays

ChE inhibitory activities were determined according to the procedure reported in our previous study [38].

4.3. ADME prediction and molecular docking study

Seven compounds with potential BChE inhibitory activity (IC_{50} < 2 μM) were further analyzed with QikProp v. 5.5 (Schrodinger) to predict their ADME properties.

The molecular docking program GOLD 5.0 was used. First, the preprocessing of human butyrylcholinesterase (PDB code: 1P0I) and the compounds was performed with Discovery Studio 2016 client software because BChE from the equine serum has a 94% homology and 90% sequence identity with BChE from the human serum [39]. The procedure included removing H_2O, adding hydrogens and assigning a CHARMM-like force field. Subsequently, the binding site of the ligand to the BChE protein was set as a sphere including the residues in the active site of human butyrylcholinesterase and slight manual adjustments were made. The “Number of dockings” parameter was set to 10 without using early termination option, and the “genetic algorithm parameter” was set to “Automatic”. The “GoldScore” was selected to evaluate the docking pose, and the settings for other parameters remained default.

4.4. Kinetic studies

The kinetic studies were carried out under the same test conditions. Five concentrations of the substrate (from 0.05 to 0.8 mM) were selected for the test in combination with four concentrations of the inhibitors (0 ~ 0.4 μM and 0 ~ 0.15 μM for **9g** and **9h,** respectively). The kinetic parameters and apparent inhibition constants were calculated by the “Enzyme kinetics” module of Prism.

4.5. Cytotoxicity assays

The cell viability of PC12 cells treated by **8**, **9g**, **9h** and tacrine was determined by the MTT cell proliferation assay. In brief, PC12 cells ($4–5 \times 10^3$) were added into 96-well plates and incubated for 24 h. Subsequently, the cells were treated with various concentrations of the compounds for 48 h. MTT solution (10 μL) was added into every well at the concentration of 10 mg/mL. Then, the plate was incubated for another 2.5 h at 37 °C. The supernatant was aspirated from each well, and 150 μL of dimethyl sulfoxide was added per well for 15–20 min. The absorption was measured at 570 nm with a Spectra MAX M5 microplate spectrophotometer (Molecular Devices).

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.102949.

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