Bioorganic & Medicinal Chemistry 21 (2013) 7406–7417

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Inhibition of cholinesterase and monoamine oxidase-B activity by Tacrine–Homoisoflavonoid hybrids

Yang Sun [a], Jianwen Chen [a], Xuemin Chen [b], Ling Huang [a,*], Xingshu Li [a,*]

[a] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China
[b] Shenzhen Salubris Pharmaceuticals Co., Ltd, Shenzhen 518102, China

ARTICLE INFO

Article history:
Received 9 August 2013
Revised 19 September 2013
Accepted 19 September 2013
Available online 1 October 2013

Keywords:
Alzheimer's disease
Cholinesterase
Monoamine oxidase-B

ABSTRACT

A series of Tacrine–Homoisoflavonoid hybrids were designed, synthesised and evaluated as inhibitors of cholinesterases (ChEs) and human monoamine oxidases (MAOs). Most of the compounds were found to be potent against both ChEs and MAO-B. Among these hybrids, compound **8b**, with a 6 carbon linker between tacrine and (*E*)-7-hydroxy-3-(4-methoxybenzylidene)chroman-4-one, proved to be the most potent against AChE and MAO-B with IC_{50} values of 67.9 nM and 0.401 μM, respectively. This compound was observed to cross the blood–brain barrier (BBB) in a parallel artificial membrane permeation assay for the BBB (PAMPA-BBB). The results indicated that compound **8b** is an excellent multifunctional promising compound for development of novel drugs for Alzheimer's disease (AD).

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to the most common form of dementia in elderly people.[1] The pathogenesis of the disease is complex; decades after Alzheimer's original description, there is still limited progress in defining the pathogenesis for AD.[2] Although several diverse hallmarks, such as β-amyloid (Aβ) deposits, τ (tau)—protein aggregation, oxidative stress and low levels of acetylcholine (ACh) have been thought to play significant roles in the pathophysiology of AD, acetylcholinesterase inhibitors (AChEIs) are the main drugs used clinically for the treatment of this disease.[3] The drug design strategy of AChEIs is based on the cholinergic hypothesis, which proposes that insufficient cholinergic neurotransmission in AD is responsible for the progressive loss of cognition and memorycapacities.[4] Currently, there are four AChEIs (i.e., tacrine, donepezil, rivastigmine, and galantamine) that have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of AD, with tacrine being the first. Although the therapeutic benefit of tacrine is limited by its liver toxicity and is not used in US and many other countries, this drug has been widely used as a scaffold for the development of new multifunctional agents with additional biological properties aside from AChE inhibition.[5]

Monoamine oxidases (MAOs; EC 1.4.3.4), which are found in the outer mitochondrial membrane, are responsible for the regulation and metabolism of monoamine neurotransmitters in the brain and peripheral tissues.[6] Recent studies have revealed that MAOs are associated with psychiatric and neurological disorders, including depression, Parkinson's disease (PD) and AD. Because inhibition of MAOs could increase the level of neurotransmitters in the central nervous system, development of MAO inhibitors has become an important approach to the treatment of such illnesses.[7] Two isoforms of MAO (MAO-A and MAO-B) have been identified in humans.[8] MAO-A inhibitors are used as antidepressants and antianxiety agents in the clinic, while MAO-B inhibitors are used as therapeutics for AD and PD.[9] Moreover, MAO-B activity increases in association with gliosis, which can result in higher levels of H_2O_2 and oxidative free radicals.[10] Thus, MAO-B inhibitors are potential candidates as anti-AD drugs due to their regulation of neurotransmitters and capacity to inhibit oxidative damage in the central nervous system.

More recently, a novel dual AChE and MAO-B inhibitor, ladostigil (Fig. 1), was developed by combining the carbamate moiety of rivastigmine (an AChE inhibitor) and the propargyl group of rasagiline (an MAO inhibitor, Fig. 1).[11] This multi-functional anti-AD drug was recently approved for Phase II clinical trials by the FDA; this outcome demonstrates the benefit of developing drugs that simultaneously target AChE and MAO-B.[12] In light of this drug design strategy, we combined tacrine (an AChE inhibitor) and homoisoflavonoids (which are known to be efficient MAO-B selective inhibitors[13]) by carbon spacers of different lengths (Fig. 2) to yield a series of Tacrine–Homoisoflavonoid hybrids. In this study, we describe the preparation and preliminary in vitro screening of these hybrids as inhibitors of ChEs and MAOs. To estimate the efficacy of these hybrids, the permeability of the blood–brain barrier

* Corresponding authors. Tel./fax: +86 20 3994 3051 (L.H.); tel./fax: +86 20 3994 3050 (X.S.).
E-mail addresses: Huangl72@mail.sysu.edu.cn (L. Huang), lixsh@mail.sysu.edu.cn (X. Li).

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.09.050

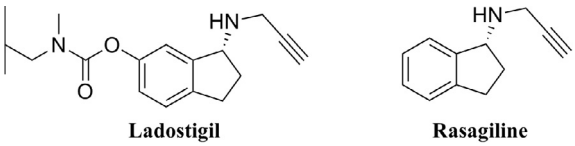


Figure 1. Chemical structures of Ladostigil and Rasagiline.

(BBB) was assayed by the parallel artificial membrane permeation assay for the BBB (PAMPA-BBB) method.

2. Results and discussion

2.1. Chemistry

The synthetic routes for **8a**–**8p** and **10** are shown in Scheme 1. First, tacrine (**2a**) and 6-chlorotacrine (**2b**) were synthesised using cyclohexanone and 2-aminobenzonitrile or 2-amino-4-chlorobenzonitrile as the starting materials. These two compounds were then reacted with different dibromoalkanes to yield intermediate **3a**–**3g**. Synthesis of the homoisoflavonoid intermediate began with resorcinol (**4**), which was reacted with 3-chloropropionic acid via Fries rearrangement to yield compound **5**. Following cyclization of compound **5** in aqueous NaOH, the key intermediate 7-hydroxychroman-4-one (**6**) was obtained. The aldol reaction of **6** with differently substituted benzaldehydes was catalyzed by piperidine to afford compounds **7a**–**7j**. The ^{1}H NMR spectrums of **7a**–**7j** showed that the olefinic proton (=CH) in (*E*)-isomers appeared as a singlet in the range of δ 7.56 and 8.32, confirming that a single stereo isomer (*E*) was obtained. Finally, the two intermediates **3a**–**3g** and **7a**–**7j** were refluxed in acetone to afford the desired compounds **8a**–**8p**.

To investigate the necessity of the double bond in compound **7a**, we synthesised the single bond compound **10**. This consisted of hydrogenation of **7a** in the presence of 10% Pd/C to afford compound **9**, which was subsequently reacted with **3b** in refluxing acetone to obtain the desired compound **10**.

2.2. In vitro inhibition of AChE and BuChE

The AChE (*Electrophorus electricus*, *ee*AChE) and BuChE (equine serum) inhibitory effects of the hybrids were determined by the spectroscopic method described by Ellman et al.[14] using tacrine as the standard. The results summarised in Table 1 showed that most of these compounds have good inhibitory activity against AChE and BuChE compared to tacrine, with IC_{50} values in the nanomolar range. In particular, compound **8b**, with a *para*-methoxy group substituent at the homoisoflavonoid moiety, provided the most potent inhibition activity for AChE (IC_{50} = 67.9 nM).

The IC_{50} values of hybrids revealed that the length of the alkyl chain has an effect on the inhibition of AChE. When the length of the alkyl chain was changed from 5 to 8, the activity against AChE did not change significantly (Table 1, **8a**, **8b** and **8c**, *n* = 5, 6 and 8, IC_{50} = 78.3, 67.9 and 68.5 nM, respectively). However, the activity decreased dramatically when the chain length increased to 9 or 10 (Table 1, **8d** and **8e**, *n* = 9 and 10, IC_{50} = 168.9 and 887.9 nM, respectively). These results suggested that an alkyl chain length of 5–8 between the two pharmacophore is suitable. Moreover, changes in substituent at the homoisoflavonoid moiety did not affect the inhibition activity obviously, except in the case of the diethylamine group (**8j**, IC_{50} = 693.3 nM). It is surprising that the 6-chlorotacrine derivatives (**8o** and **8p**) displayed weaker activity than the tacrine derivatives, which was inconsistent with some other 6-chlorotacrine derivatives in literatures. Meanwhile, homoisoflavonoid **7a**, a parent compound of hybrids, did not show any activity against ChEs, which could be concluded that the tacrine moiety plays a crucial role in inhibiting ChEs. Notably, only compound **8e** exhibited higher selectivity for BuChE than AChE, which indicated that most of these hybrids could be useful as dual AChE/BChE inhibitors for treatment of AD.

2.3. In vitro inhibition of *h*AChE

Considering human AChE (*h*AChE) is more appropriate, we also evaluated the inhibition activity of compounds **8a**, **8b**, **8c** and **8h** against *h*AChE. The IC_{50} values were listed in Table 2. The results indicated that all the four tested compounds are potent to inhibit the *h*AChE, with IC_{50} values at nanomolar range. Among the tested hybrids, **8a** showed the best inhibition activity against *h*AChE, with an IC_{50} of 98.0 nM, which was 2.9-fold better than tacrine (IC_{50} = 285.0 nM). Similar to *ee*AChE inhibition activity, the length of the alkyl chain did not affect the activity obviously. In addition, hybrid **8h** (IC_{50} = 105.3 nM), with a fluoro group at the *para*-position of homoisoflavonoid moiety, provided better activity than hybrid **8b** (IC_{50} = 156.8 nM), with a methoxy group at the

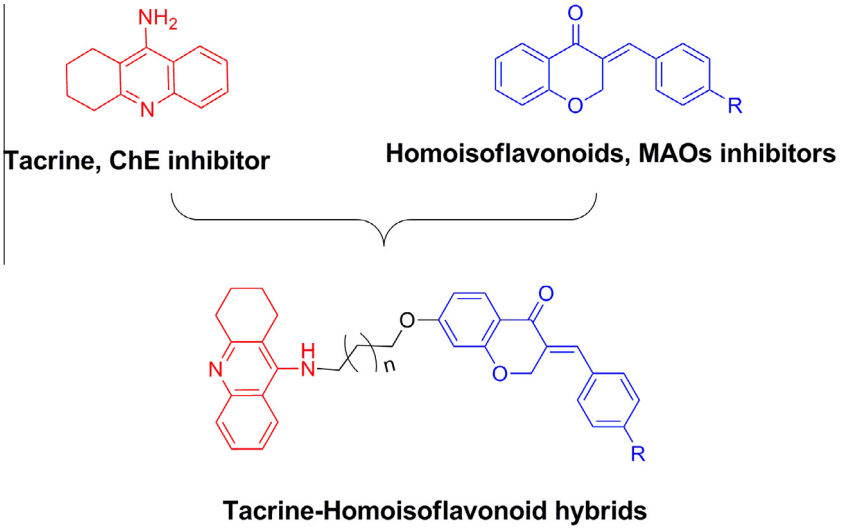
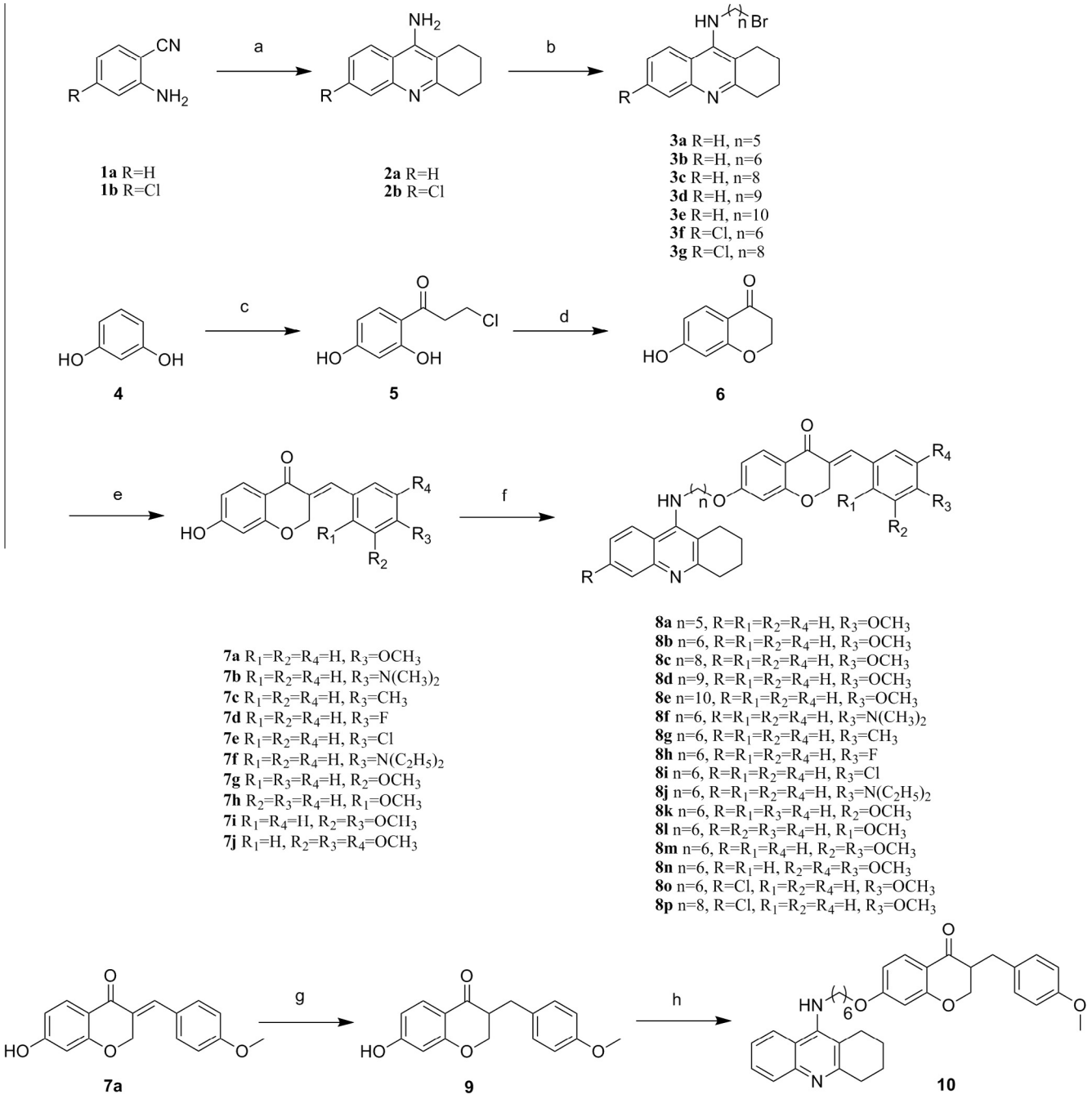


Figure 2. Design strategy for Tacrine–Homoisoflavonoid hybrids.



Scheme 1. Reagents and conditions: (a) (i) cyclohexanone, $POCl_3$, 120 °C, (ii) cyclohexanone, $ZnCl_2$, 140 °C; (b) different dibromoalkanes, KOH, CH_3CN; (c) 3-chloropropionic acid, trifluoromethanesulfonic acid; (d) NaOH, 0 °C; (e) substituted benzaldehyde, piperidine, 80 °C; (f) Compounds **3a**–**3g**, K_2CO_3, acetone, reflux; (g) Pd/C, H_2; (h) Compound **3b**, K_2CO_3, acetone, reflux.

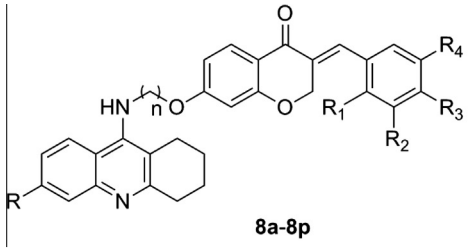
para-position of homoisoflavonoid moiety. However, considering the results in inhibiting MAO-B, compound **8b** seems to be a better dual AChE and MAO-B inhibitor, so we choose **8b** for kinetic study.

2.4. Kinetic study of AChE

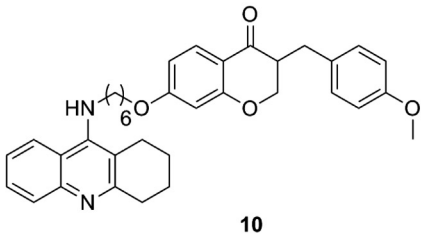
The inhibition type for *ee*AChE was investigated by graphical analysis of steady state inhibition data (Fig. 3) using compound **8b** as a typical example. Reciprocal plots (Lineweaver–Burk plots) showed both increasing slopes (decreased V_{max}) and increasing intercepts (higher K_m) at increasing concentration of the inhibitor, indicating a mixed-type inhibition. This result revealed that compound **8b** was able to bind to both the catalytic anionic site (CAS) and the peripheral anionic site (PAS) of AChE.

Recent study shows that the PAS region of the AChE involved in the interaction with Aβ peptide, which may induce the deposition of neurotoxic Aβ fibrils.[15] It is possible that the mixed-type inhibitor might be able to decrease amyloid plaque formation and to alleviate cognitive deficits in AD patients. Donepezil, a mixed-type inhibitor, exhibited some extent of effect on decreasing AChE induced Aβ aggregation (around 22%), which can be attributed to its higher affinity for the PAS.[16] Compound **8b**, with a long linker

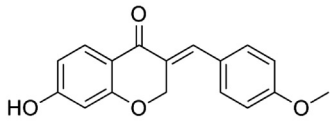
Table 1
Inhibition of ChEs and MAOs by Tacrine–Homoisoflavonoid hybrids **8a**–**8p** and **10**, (*E*)-7-hydroxy-3-(4-methoxybenzylidene)chroman-4-one (**7a**), Tacrine (**2a**)



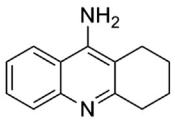
8a-8p



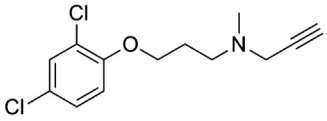
10



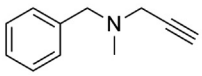
7a



2a (Tacrine)



Clorgyline



Pargyline

Compd	*n*	R	R_1	R_2	R_3	R_4	IC_{50} ± SD (nM)[a]			MAO-A[b] inhibition (%)	IC_{50} MAO-B[a] (μM)
							AChE[c]	BuChE[d]	BuChE/AChE Selectivity		
8a	5	H	H	H	OCH_3	H	78.3 ± 6.7	23.6 ± 0.3	0.31	58.4 ± 1.4	1.16 ± 0.01
8b	6	H	H	H	OCH_3	H	67.9 ± 4.1	33.0 ± 2.7	0.49	48.3 ± 0.6	0.401 ± 0.011
8c	8	H	H	H	OCH_3	H	68.5 ± 7.6	93.1 ± 4.1	1.35	64.8 ± 3.3	0.518 ± 0.010
8d	9	H	H	H	OCH_3	H	168.9 ± 9.4	30.9 ± 1.3	0.18	60.2 ± 1.3	0.437 ± 0.039
8e	10	H	H	H	OCH_3	H	887.9 ± 6.4	6.2 ± 0.5	0.007	69.6 ± 1.2	3.43 ± 0.22
8f	6	H	H	H	$N(CH_3)_2$	H	115.4 ± 5.8	65.7 ± 4.3	0.57	31.5 ± 1.5	1.03 ± 0.02
8g	6	H	H	H	CH_3	H	129.1 ± 0.3	54.6 ± 3.1	0.43	60.5 ± 1.3	1.24 ± 0.16
8h	6	H	H	H	F	H	70.1 ± 0.2	44.2 ± 1.9	0.63	58.4 ± 2.3	1.09 ± 0.09
8i	6	H	H	H	Cl	H	100.5 ± 1.0	44.5 ± 4.4	0.45	23.3 ± 1.2	15.4 ± 0.35
8j	6	H	H	H	$N(C_2H_5)_2$	H	693.3 ± 48.0	235.2 ± 14.6	0.34	22.4 ± 1.2	[e]
8k	6	H	H	OCH_3	H	H	141.6 ± 4.5	47.1 ± 0.6	0.33	54.1 ± 1.9	2.20 ± 0.38
8l	6	H	OCH_3	H	H	H	151.4 ± 1.0	29.9 ± 3.0	0.20	70.3 ± 2.3	1.79 ± 0.04
8m	6	H	H	OCH_3	OCH_3	H	116.8 ± 5.4	34.7 ± 2.6	0.30	51.6 ± 3.1	19.73 ± 0.08
8n	6	H	H	OCH_3	OCH_3	OCH_3	106.1 ± 2.4	108.8 ± 6.1	1.02	25.7 ± 0.6	[e]
8o	6	Cl	H	H	OCH_3	H	123.1 ± 4.7	25.9 ± 0.5	0.21	15.3 ± 0.3	1.36 ± 0.07
8p	8	Cl	H	H	OCH_3	H	188.3 ± 11.8	612.5 ± 44.8	3.26	33.1 ± 2.3	1.56 ± 0.01
10	—	—	—	—	—	—	91.7 ± 5.1	27.4 ± 2.6	0.29	78.2 ± 4.2	1.11 ± 0.01
7a	—	—	—	—	—	—	>100,000	>100,000	—	24.1 ± 1.2	1.06 ± 0.01
2a	—	—	—	—	—	—	111.0 ± 2.5	19.2 ± 1.4	0.17	31.2 ± 5.1	[f]
Clorgyline	—	—	—	—	—	—	—	—	—	4.1 ± 0.2 nM[g]	nt.
Pargyline	—	—	—	—	—	—	—	—	—	nt.[h]	0.188 ± 0.016
Ladostigil	—	—	—	—	—	—	—	—	—	nt.	37.1 ± 3.1

[a] Data are the mean ± SD of three independent experiments.
[b] Test concentration is 100 μM.
[c] AChE from electric eel.
[d] BuChE from horse serum.
[e] Inactive at 20 μM.
[f] Inactive at 100 μM (highest concentration tested).
[g] The IC_{50} of Clorgyline.
[h] nt. = not tested.

between tacrine and homoisoflavonoid, can simultaneously interact with the CAS and PAS of AChE, which has potential activity to AChE-induced Aβ aggregation.

Table 2
Inhibition of human AChE by **8a**, **8b**, **8c**, **8h** and tacrine (**2a**)

Compd	*n*	R	R_1	R_2	R_3	R_4	IC_{50} ± SD[a] (nM) *h*-AChE[b]
8a	5	H	H	H	OCH_3	H	98.0 ± 5.9
8b	6	H	H	H	OCH_3	H	156.8 ± 12.9
8c	8	H	H	H	OCH_3	H	121.6 ± 11.6
8h	6	H	H	H	F	H	105.3 ± 9.4
2a	—	—	—	—	—	—	285.0 ± 29.4

[a] Data are the mean ± SD of three independent experiments.
[b] AChE (EC 3.1.1.7) from human erythrocytes.

2.5. In vitro inhibition of *h*MAO-A and *h*MAO-B

To further study the multipotent biological profile of the target compounds, the inhibitory activity against *h*MAO-A and *h*MAO-B (recombinant human enzyme) was determined[17] and compared with that of ladostigil, which was an *h*MAO-B inhibitor approved to carry out phase II clinical trial by FDA. The results were depicted in Table 1. Most of these hybrids exhibited selective inhibition to *h*MAO-B in the micromolar range. A structure–activity relationship analysis showed that hybrids with a methoxy group in the R_3 position were favorable for inhibitory activity against *h*MAO-B. For example, compound **8b**, featuring a methoxy group in R_3 position, displayed the most potent inhibitory activity with an IC_{50} of 0.401 μM, which was approximately 2.5-fold activity as the lead

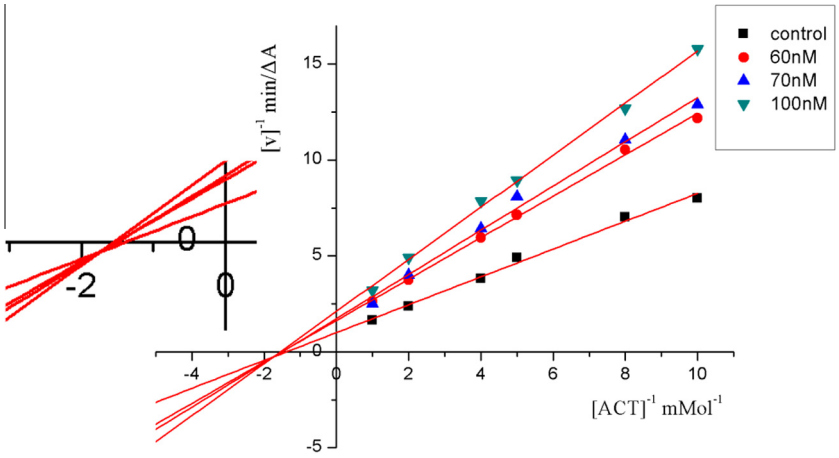


Figure 3. Steady state inhibition of AChE-catalyzed ACh hydrolysis by compound **8b**.

compound **7a** (IC_{50} = 1.06 μM). All other analogues gave weaker activities when the methoxygroup was substituted by other groups or at other positions. Dramatically, compound **8n**, which featured three methoxygroups at the R_2, R_3 and R_4 positions, provided almost no inhibitory activity; the result can be attributed to steric hindrance. In order to investigate whether the double bond is necessary in inhibiting *h*MAO-B, we prepared compound **10**, the reduction product of **8b**. The IC_{50} value of compound **10** had increased to 1.11 μM, which indicated that the double bond is crucial to the activity.

Based on the results of ChEs and MAOs inhibitory activity, we chose compound **8b** (*ee*AChE: IC_{50} = 67.9 nM; *h*AChE: IC_{50} = 158.8 nM; BuChE: IC_{50} = 33.0 nM; MAO-B: IC_{50} = 0.401 μM) as a promising multi-functional inhibitor for further study.

2.6. Reversibility and irreversibility of *h*MAO-B inhibition

To determine the reversibility or irreversibility of the hybrids' inhibitory activity, **8b** and pargyline were used for study according to a literature method.[17] Pargyline, with known irreversibility for the *h*MAO-B inhibitor, was used as a reference compound.[18] Data shown in Table 3 indicated that *h*MAO-B inhibition of **8b** is irreversible as evidenced by the lack of enzyme activity restoration after repeated washing. A similar result was obtained for pargyline, a well-known irreversible *h*MAO-B inhibitor.

2.7. In vitro blood–brain barrier permeation assay

Brain penetration is very important for successful anti-AD drugs.[19] To evaluate the potential for these hybrids to cross the blood–brain barrier (BBB), we used a parallel artificial membrane permeation assay for BBB (PAMPA-BBB), which was described by Di et al.[20] Assay validation was made by comparing experimental permeabilities of 13 commercial drugs with reported values (Table 4).

A plot of experimental data versus bibliographic values gave a good linear correlation, P_e (exp.) = 1.4574 P_e (bibl.) −1.0773 (R^2 = 0.9427). From this equation and considering the limit established by Di et al. for blood–brain barrier permeation, we established that compounds with permeability values over 4.7×10^{-6} cm s^{-1} should be able to cross the BBB. Six compounds (**8a**, **8b**, **8c**, **8h**, **8i** and **10**) that exhibited good activity against AChE and MAO-B were chosen as the test compounds. The results sum-

Table 4
Permeability ($P_e \times 10^{-6}$ cm s^{-1}) in the PAMPA-BBB assay for 13 commercial drugs, used in the experiment validation

Commercial drugs	Bibl[a]	PBS:EtOH (70:30)[b]
Testosterone	17	22.3 ± 1.4
Verapamil	16	21.2 ± 1.9
Desipramine	12	16.4 ± 1.2
Progesterone	9.3	17.7 ± 1.2
Promazine	8.8	14.3 ± 0.5
Chlorpromazine	6.5	6.0 ± 0.3
Clonidine	5.3	5.1 ± 0.3
Piroxicam	2.5	0.24 ± 0.01
Hydrocortisone	1.9	0.65 ± 0.01
Lomefloxacin	1.1	0.37 ± 0.02
Atenolol	0.8	0.78 ± 0.02
Ofloxacin	0.8	0.37 ± 0.02
Theophylline	0.1	0.26 ± 0.01

[a] Taken from Ref. 20
[b] Data are the mean ± SD of three independent experiments.

Table 3
Reversibility and irreversibility of *h*MAO-B inhibition of hybrid **8b** and Pargyline[a]

Compd	% *h*MAO-B inhibition[b]	
	Before washing	After repeated washing
8b (400 nM)	47.06 ± 2.73	33.47 ± 1.03
8b (1 μM)	78.39 ± 0.97	67.50 ± 2.41
Pargyline (200 nM)	60.3 ± 6.25	56.6 ± 5.56

[a] Pargyline is an irreversible *h*MAO-B inhibitor.
[b] Data are the mean ± SD of three independent experiments.

Table 5
Permeability ($P_e \times 10^{-6}$ cm s^{-1}) in the PAMPA-BBB assay for Tacrine–Homoisoflavonoid hybrids and their predictive penetration in the CNS

Compd	Permeability[a] ($P_e \times 10^{-6}$ cm s^{-1})	Prediction
8a	6.6 ± 0.6	CNS +
8b	6.8 ± 0.6	CNS +
8c	4.9 ± 0.4	CNS +
8h	4.0 ± 0.3	CNS +/−
8i	1.1 ± 0.05	CNS −
10	2.8 ± 0.2	CNS +/−

[a] Data are the mean (n = 3) ± SD.

marised in Table 5 indicated that three of the hybrids (**8a**, **8b**, and **8c**) should be able to cross the BBB to target the enzyme in the central nervous system, two of them (**8h** and **10**) were located in the uncertain area, and one (**8i**) was classified as low BBB permeation.

3. Conclusion

We developed a new series of Tacrine–Homoisoflavonoid hybrids as dual inhibitors capable of targeting ChEs and MAOs. Most of these compounds were potent ChEIs with IC_{50} values in the nanomolar range. These hybrids were also potent and selective *h*MAO-B inhibitors and interact irreversibly with *h*MAO-B. Compared to lead compound **7a**, several of these hybrids (**8a**, **8b**, **8c**) exhibited greater potency to inhibit *h*MAO-B. Among the hybrids, compound **8b**, with a methoxygroup in *para*-position of the homoisoflavonoid moiety and a six carbon linker between the two pharmacophores, provided the best results with an IC_{50} of 67.9 nM for *ee*AChE, 158.8 nM for *h*AChE and 0.401 μM for *h*MAO-B. The PAMPA-BBB assay indicated that compound **8b** is able to cross the BBB. Based on these results, compound **8b** is a promising candidate to target both ChEs and *h*MAO-B for AD treatment and warrants further study.

4. Experimental section

4.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker BioSpin GmbH spectrometer at 400.132 and 100.614 MHz, respectively, using TMS as the internal standard. Coupling constants are given in Hz. High-resolution mass spectra were obtained using a Shimadzu LCMS-IT-TOF mass spectrometer. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. All reactions were monitored by thin layer chromatography using silica gel. The purity of compounds **8a**–**8p**, **10** (greater than 95%) were confirmed by HPLC (Agilent Technologies 1200 series system, TC-C18 column (4.6 × 250 mm, 5 μm), eluted with methanol/water (0.1%TFA), 70:30, at a flow rate of 0.8 mL/min).

4.1.1. Synthesis of 2a (1,2,3,4-tetrahydroacridin-9-amine)

To a stirred solution of 2-aminobenzonitrile (6.8 g, 50 mmol) and cyclohexanone (4.9 g, 50 mmol) at 0 °C, phosphorusoxychloride (41.5 mL) was added in dropwise. The solution was warmed to 120 °C for 2 h. The mixture was cooled to room temperature and the phosphorusoxychloride was evaporated under reduced pressure. The residue was diluted with ethyl acetate, neutralised with an aqueous solution of K_2CO_3 to pH 7, and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine, dried over K_2CO_3, and evaporated to dryness under reduced pressure. The residue was recrystallised in acetone to obtain a yellow solid (6.4 g) in 65% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.12 (d, J = 8.4 Hz, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.51–7.43 (m, 1H), 7.26 (ddd, J = 8.1, 6.8, 1.2 Hz, 1H), 6.29 (s, 2H), 2.81 (t, J = 5.7 Hz, 2H), 2.54 (t, J = 5.9 Hz, 2H), 1.87–1.75 (m, 4H). LC/MS (ESI): m/z 199.1 $[M+H]^+$.

4.1.2. Synthesis of 2b (6-chloro-1,2,3,4-tetrahydroacridin-9-amine)

Cyclohexanone (36 mL), 2-amino-4-chlorobenzonitrile (4.6 g, 30 mmol) and zinc chloride (4.1 g, 30 mmol) were mixed in a flask and the mixture was heated to 120 °C for 3 h. Following cooling to room temperature and evaporation of the solvent, the residue was diluted with ethyl acetate (30 mL) and the solid was collected by filtration. A 10% aqueous solution of NaOH (50 mL) was added. After stirring for 2 h, the mixture was filtered, and the filter cake was washed with water and extracted with methanol. The combined extracts were concentrated to afford the desired product (4.7 g) in 67% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.17 (d, J = 9.0 Hz, 1H), 7.62 (d, J = 2.0 Hz, 1H), 7.28 (s, 1H), 6.46 (s, 2H), 2.81 (d, J = 5.5 Hz, 2H), 2.52 (s, 2H), 1.80 (d, J = 4.7 Hz, 4H). LC/MS (ESI): m/z 233.2 $[M+H]^+$.

4.1.3. General procedure for the synthesis of 3a–3g

Compound **2a** or **2b** (2 mmol) and KOH (0.34 g, 6 mmol) were dissolved in acetonitrile. The solution was stirred for 1 h under argon at room temperature. Then, different dibromoalkanes (4 mmol) were added to the mixture. After stirring for 48 h, the mixture was filtered and evaporated under reduced pressure. The residue was chromatographed on silica gel, with petroleum ether/ethyl acetate, 10:1, plus 10 mL triethylamine per 1000 mL as the eluent to afford the compounds.

4.1.4. *N*-(5-Bromononyl)-1,2,3,4-tetrahydroacridin-9-amine (3a)

Compound **2a** (1,2,3,4-tetrahydroacridin-9-amine) was treated with 1,5-dibromopentane according to the general procedure to give the desired product **3a** as a yellow oil, 13% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.5 Hz, 1H), 7.91 (d, J = 8.5 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 3.93 (s, 1H), 3.49 (dd, J = 13.0, 6.5 Hz, 2H), 3.40 (t, J = 6.6 Hz, 2H), 3.06 (s, 2H), 2.72 (s, 2H), 1.94–1.84 (m, 6H), 1.71–1.65 (m, 2H), 1.58–1.52 (m, 2H). LC/MS (ESI): m/z 347.2 $[M+H]^+$.

4.1.5. *N*-(6-Bromononyl)-1,2,3,4-tetrahydroacridin-9-amine (3b)

Compound **2a** (1,2,3,4-tetrahydroacridin-9-amine) was treated with 1,6-dibromohexane according to the general procedure to give the desired product **3b** as a yellow oil, 22% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (t, J = 7.9 Hz, 2H), 7.60–7.54 (m, 1H), 7.36 (t, J = 7.6 Hz, 1H), 4.03 (s, 1H), 3.51 (d, J = 4.4 Hz, 2H), 3.40 (t, J = 6.7 Hz, 2H), 3.08 (s, 2H), 2.72 (s, 2H), 1.93 (dt, J = 6.5, 3.4 Hz, 4H), 1.89–1.84 (m, 2H), 1.73–1.67 (m, 2H), 1.51–1.43 (m, 4H). LC/MS (ESI): m/z 361.2 $[M+H]^+$.

4.1.6. *N*-(8-Bromononyl)-1,2,3,4-tetrahydroacridin-9-amine (3c)

Compound **2a** (1,2,3,4-tetrahydroacridin-9-amine) was treated with 1,8-dibromooctane according to the general procedure to give the desired product **3c** as a yellow oil, 26% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (dd, J = 8.5, 0.8 Hz, 1H), 7.90 (dd, J = 8.5, 0.7 Hz, 1H), 7.54 (ddd, J = 8.3, 6.8, 1.3 Hz, 1H), 7.33 (ddd, J = 8.2, 6.8, 1.2 Hz, 1H), 3.93 (s, 1H), 3.47 (dd, J = 12.8, 6.9 Hz, 2H), 3.39 (t, J = 6.8 Hz, 2H), 3.09–3.01 (m, 2H), 2.70 (s, 2H), 1.94–1.88 (m, 4H), 1.86–1.80 (m, 2H), 1.68–1.61 (m, 2H), 1.43–1.36 (m, 4H), 1.34–1.29 (m, 4H). LC/MS (ESI): m/z 389.2 $[M+H]^+$.

4.1.7. *N*-(9-Bromononyl)-1,2,3,4-tetrahydroacridin-9-amine (3d)

Compound **2a** (1,2,3,4-tetrahydroacridin-9-amine) was treated with 1,9-dibromononane according to the general procedure to give the desired product **3d** as a yellow oil, 29% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.5 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.55 (t, J = 7.1 Hz, 1H), 7.34 (t, J = 7.2 Hz, 1H), 4.04 (s, 1H), 3.50 (dd, J = 12.5, 6.4 Hz, 2H), 3.40 (t, J = 6.8 Hz, 2H), 3.07 (s, 2H), 2.70 (s, 2H), 1.91 (d, J = 3.1 Hz, 4H), 1.87–1.80 (m, 2H), 1.70–1.62 (m, 2H), 1.39 (s, 4H), 1.30 (s, 6H). LC/MS (ESI): m/z 403.2 $[M+H]^+$.

4.1.8. *N*-(10-Bromodecyl)-1,2,3,4-tetrahydroacridin-9-amine (3e)

Compound **2a** (1,2,3,4-tetrahydroacridin-9-amine) was treated with 1,10-dibromodecane according to the general procedure to

give the desired product **3e** as a yellow oil, 44% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.5 Hz, 1H), 7.94 (d, J = 8.5 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 4.01 (s, 1H), 3.50 (s, 2H), 3.41 (t, J = 6.8 Hz, 2H), 3.08 (s, 2H), 2.72 (s, 2H), 1.97–1.91 (m, 4H), 1.88–1.82 (m, 2H), 1.71–1.63 (m, 2H), 1.41 (d, J = 5.8 Hz, 4H), 1.30 (s, 8H). LC/MS (ESI): m/z 417.2 $[M+H]^+$.

4.1.9. *N*-(6-Bromohexyl)-6-chloro-1,2,3,4-tetrahydroacridin-9-amine (3f)

Compound **2b** (6-chloro-1,2,3,4-tetrahydroacridin-9-amine) was treated with 1,6-dibromohexane according to the general procedure to give the desired product **3f** as a yellow oil, 23% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94–7.86 (m, 2H), 7.32–7.27 (m, 1H), 3.93 (s, 1H), 3.49 (d, J = 6.1 Hz, 2H), 3.41 (td, J = 6.6, 1.3 Hz, 2H), 3.04 (s, 2H), 2.68 (s, 2H), 1.93 (d, J = 1.4 Hz, 4H), 1.89–1.85 (m, 2H), 1.71–1.66 (m, 2H), 1.51–1.42 (m, 4H). LC/MS (ESI): m/z 395.2 $[M+H]^+$.

4.1.10. *N*-(8-Bromooctyl)-6-chloro-1,2,3,4-tetrahydroacridin-9-amine (3g)

Compound **2b** (6-chloro-1,2,3,4-tetrahydroacridin-9-amine) was treated with 1,8-dibromooctane according to the general procedure to give the desired product **3g** as a yellow oil, 12% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84–7.76 (m, 2H), 7.18 (d, J = 2.9 Hz, 1H), 3.85 (s, 1H), 3.44–3.37 (m, 2H), 3.32 (t, J = 6.7 Hz, 2H), 2.95 (s, 2H), 2.60 (s, 2H), 1.84 (d, J = 2.9 Hz, 4H), 1.76 (d, J = 7.2 Hz, 2H), 1.57 (d, J = 7.1 Hz, 2H), 1.37–1.30 (m, 8H). LC/MS (ESI): m/z 423.2 $[M+H]^+$.

4.1.11. Synthesis of 3-chloro-1-(2,4-dihydroxyphenyl)propan-1-one (5)

To a stirred mixture of resorcinol (2.2 g, 20 mmol) and 3-chloropropionic acid (2.2 g, 20 mmol), trifluoromethanesulphonic acid (10 g, 66.4 mmol) was added in one portion. The solution was warmed to 80 °C, stirred for 1 h, then cooled to room temperature, and poured into water (100 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. Concentration in vacuo gave 1.9 g of an orange solid (47% yield) which was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.48 (s, 1H), 7.63 (d, J = 8.6 Hz, 1H), 6.47–6.38 (m, 2H), 3.91 (t, J = 6.7 Hz, 2H), 3.40 (t, J = 6.7 Hz, 2H). LC/MS (ESI): m/z 201.2 $[M+H]^+$.

4.1.12. Synthesis of 7-hydroxychroman-4-one (6)

To a stirred solution of 2 M NaOH (20 mL) at 0 °C, **5** (1.9 g, 9.5 mmol) was added in one portion. The solution was warmed to room temperature for 2 h and then cooled to 0 °C. The pH was adjusted to 2 with 6 M H_2SO_4. The mixture was extracted with ethyl acetate (3 × 50 mL), washed with brine twice, dried over Na_2SO_4 and concentrated in vacuo to give a brown solid. Recrystallisation from water gave the product (1.0 g, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 8.4 Hz, 1H), 6.55 (d, J = 8.6 Hz, 1H), 6.42 (s, 1H), 4.52 (s, 2H), 2.78 (s, 2H). LC/MS (ESI): m/z 165.1 $[M+H]^+$.

4.1.13. General procedure for the synthesis of 7a–7j

A mixture of **6** (0.2 g, 1.22 mmol), substituted benzaldehyde (1.76 mmol), and piperidine (0.25 mL) was heated at 80 °C for 2 h. Upon cooling to room temperature, the mixture was diluted with water (50 mL), acidified with HCl, and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent afforded the residue, which was purified by column chromatography over silica gel using petroleum ether and ethyl acetate (10:1) as eluent to yield the homoisoflavonoid products (23–61% yield).

4.1.14. (*E*)-7-Hydroxy-3-(4-methoxybenzylidene)chroman-4-one (7a)

7-Hydroxychroman-4-one (**6**) was treated with 4-methoxybenzaldehyde according to the general procedure to give the desired product **7a** as a yellow solid, 44% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.64 (s, 1H), 7.73 (d, J = 8.7 Hz, 1H), 7.63 (s, 1H), 7.40 (d, J = 8.6 Hz, 2H), 7.04 (d, J = 8.6 Hz, 2H), 6.54 (dd, J = 8.6, 2.0 Hz, 1H), 6.31 (d, J = 1.9 Hz, 1H), 5.35 (s, 2H), 3.81 (s, 3H). LC/MS (ESI): m/z 283.1 $[M+H]^+$.

4.1.15. (*E*)-3-(4-(Dimethylamino)benzylidene)-7-hydroxychroman-4-one (7b)

7-Hydroxychroman-4-one (**6**) was treated with 4-(dimethylamino)benzaldehyde according to the general procedure to give the desired product **7b** as a red solid, 33% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.58 (s, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.58 (s, 1H), 7.29 (d, J = 8.6 Hz, 2H), 6.76 (d, J = 8.6 Hz, 2H), 6.56 – 6.49 (m, 1H), 6.31 (t, J = 4.2 Hz, 1H), 5.37 (s, 2H), 2.97 (d, J = 8.5 Hz, 6H). LC/MS (ESI): m/z 296.2 $[M+H]^+$.

4.1.16. (*E*)-7-Hydroxy-3-(4-methylbenzylidene)chroman-4-one (7c)

7-Hydroxychroman-4-one (**6**) was treated with 4-methylbenzaldehyde according to the general procedure to give the desired product **7c** as a yellow solid, 31% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.73 (s, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.71 (s, 1H), 7.37 (q, J = 8.4 Hz, 4H), 6.62 (dd, J = 8.7, 2.1 Hz, 1H), 6.38 (d, J = 2.2 Hz, 1H), 5.41 (d, J = 1.6 Hz, 2H), 2.42 (s, 3H). LC/MS (ESI): m/z 267.2 $[M+H]^+$.

4.1.17. (*E*)-3-(4-Fluorobenzylidene)-7-hydroxychroman-4-one (7d)

7-Hydroxychroman-4-one (**6**) was treated with 4-fluorobenzaldehyde according to the general procedure to give the desired product **7d** as a orange solid, 24% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.69 (s, 1H), 7.75 (d, J = 8.7 Hz, 1H), 7.66 (s, 1H), 7.49 (dd, J = 8.6, 5.6 Hz, 2H), 7.31 (t, J = 8.8 Hz, 2H), 6.55 (dd, J = 8.7, 2.2 Hz, 1H), 6.32 (d, J = 2.2 Hz, 1H), 5.33 (d, J = 1.7 Hz, 2H). LC/MS (ESI): m/z 271.2 $[M+H]^+$.

4.1.18. (*E*)-3-(4-Chlorobenzylidene)-7-hydroxychroman-4-one (7e)

7-Hydroxychroman-4-one (**6**) was treated with 4-chlorobenzaldehyde according to the general procedure to give the desired product **7e** as a yellow solid, 24% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.72 (s, 1H), 7.76 (dd, J = 8.7, 4.1 Hz, 1H), 7.66 (s, 1H), 7.58–7.52 (m, 2H), 7.50–7.42 (m, 2H), 6.59–6.54 (m, 1H), 6.36–6.30 (m, 1H), 5.34 (s, 2H). LC/MS (ESI): m/z 287.2 $[M+H]^+$.

4.1.19. (*E*)-3-(4-(Diethylamino)benzylidene)-7-hydroxychroman-4-one (7f)

7-Hydroxychroman-4-one (**6**) was treated with 4-(diethylamino)benzaldehyde according to the general procedure to give the desired product **7f** as a yellow solid, 24% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.53 (s, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.56 (s, 1H), 7.28 (d, J = 8.7 Hz, 2H), 6.73 (d, J = 8.7 Hz, 2H), 6.52 (dd, J = 8.6, 2.1 Hz, 1H), 6.31 (d, J = 2.0 Hz, 1H), 5.38 (s, 2H), 3.43 (d, J = 6.9 Hz, 2H), 3.39 (d, J = 7.0 Hz, 2H), 1.12 (t, J = 6.9 Hz, 6H). LC/MS (ESI): m/z 324.2 $[M+H]^+$.

4.1.20. (*E*)-7-Hydroxy-3-(3-methoxybenzylidene)chroman-4-one (7g)

7-Hydroxychroman-4-one (**6**) was treated with 3-methoxybenzaldehyde according to the general procedure to give the desired product **7g** as a yellow solid, 34% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.69 (s, 1H), 7.76 (d, J = 8.6 Hz, 1H), 7.66 (s, 1H), 7.40

(t, J = 7.7 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 8.1 Hz, 2H), 6.57 (d, J = 8.5 Hz, 1H), 6.33 (s, 1H), 5.36 (s, 2H), 3.80 (s, 3H). LC/MS (ESI): m/z 283.1 $[M+H]^+$.

4.1.21. (*E*)-7-Hydroxy-3-(2-methoxybenzylidene)chroman-4-one (7h)

7-Hydroxychroman-4-one (**6**) was treated with 2-methoxybenzaldehyde according to the general procedure to give the desired product **7h** as a yellow solid, 40% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.67 (s, 1H), 7.80–7.72 (m, 2H), 7.45 (t, J = 7.8 Hz, 1H), 7.16 (d, J = 7.4 Hz, 1H), 7.13 (d, J = 8.3 Hz, 1H), 7.04 (t, J = 7.5 Hz, 1H), 6.56 (d, J = 8.7 Hz, 1H), 6.32 (s, 1H), 5.21 (s, 2H), 3.85 (s, 3H). LC/MS (ESI): m/z 283.1 $[M+H]^+$.

4.1.22. (*E*)-3-(3,4-Dimethoxybenzylidene)-7-hydroxychroman-4-one (7i)

7-Hydroxychroman-4-one (**6**) was treated with 3,4-dimethoxybenzaldehyde according to the general procedure to give the desired product **7i** as a yellow solid, 44% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.77 (dd, J = 8.6, 3.1 Hz, 1H), 7.67 (s, 1H), 7.06 (d, J = 7.3 Hz, 2H), 6.99 (d, J = 7.8 Hz, 1H), 6.58 (dd, J = 5.7, 2.2 Hz, 1H), 6.35 (d, J = 2.2 Hz, 1H), 5.41 (s, 2H), 3.83 (d, J = 2.7 Hz, 6H). LC/MS (ESI): m/z 313.2 $[M+H]^+$.

4.1.23. (*E*)-7-Hydroxy-3-(3,4,5-trimethoxybenzylidene)chroman-4-one (7j)

7-Hydroxychroman-4-one (**6**) was treated with 3,4,5-trimethoxybenzaldehyde according to the general procedure to give the desired product **7j** as a yellow solid, 61% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.68 (s, 1H), 7.75 (d, J = 8.7 Hz, 1H), 7.64 (s, 1H), 6.73 (s, 2H), 6.56 (dd, J = 8.7, 2.2 Hz, 1H), 6.33 (d, J = 2.1 Hz, 1H), 5.42 (s, 2H), 3.83 (s, 6H), 3.72 (s, 3H). LC/MS (ESI): m/z 343.3 $[M+H]^+$.

4.1.24. Synthesis of 7-hydroxy-3-(4-methoxybenzyl)chroman-4-one (9)

To a stirred solution of (*E*)-7-hydroxy-3-(4-methoxybenzylidene)chroman-4-one (**7a**, 0.1 g) in THF at 0 °C was added 10% Pd/C. The solution was charged with H_2 at 180 psi for 4 h. The mixture was filtered and evaporated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 5:1) to give a white solid **9** (90% yield). ^{1}H NMR (400 MHz, DMSO) δ 10.56 (s, 1H), 7.64 (d, J = 8.6 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 6.50 (dd, J = 8.6, 1.7 Hz, 1H), 6.30 (d, J = 1.8 Hz, 1H), 4.29 (dd, J = 11.4, 4.5 Hz, 1H), 4.09 (dd, J = 11.1, 9.2 Hz, 1H), 3.73 (s, 3H), 3.04 (dd, J = 14.0, 4.9 Hz, 1H), 2.93–2.85 (m, 1H), 2.59 (dd, J = 13.9, 9.5 Hz, 1H). LC/MS (ESI): m/z 285.0 $[M+H]^+$.

4.1.25. General procedure for the synthesis of 8a–8p and 10

A mixture of **7a**–**7j** or **9** (0.2 mmol) and K_2CO_3 (0.4 mmol) was dissolved in acetone and stirred at room temperature for 30 min. Compounds **3a**–**3f** (0.2 mmol) were added to the mixture and then refluxed for 12 h. The mixture was filtered and evaporated to remove the solvent. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 5:1, plus 10 mL triethylamine per 1000 mL).

4.1.26. (*E*)-3-(4-Methoxybenzylidene)-7-(5-(1,2,3,4-tetrahydroacridin-9-ylamino)pentyloxy)chroman-4-one (8a)

(*E*)-7-Hydroxy-3-(4-methoxybenzylidene)chroman-4-one (**7a**) was treated with **3a** (*N*-(5-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8a** as a yellow oil, 91% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.11 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.7 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.67 (s, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.33 (t, J = 7.5 Hz, 1H), 7.05 (d, J = 8.4 Hz, 2H), 6.63 (d, J = 8.9 Hz, 1H), 6.50 (s, 1H), 5.40 (s, 3H), 4.00 (t, J = 6.0 Hz, 2H), 3.83 (s, 3H), 3.41 (d, J = 6.7 Hz, 2H), 2.89 (s, 2H), 2.71 (s, 2H), 1.80 (d, J = 4.8 Hz, 4H), 1.73–1.66 (m, 2H), 1.65–1.57 (m, 2H), 1.43 (d, J = 6.9 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ 179.53, 164.85, 162.35, 160.26, 157.88, 150.26, 146.89, 135.49, 132.15, 128.88, 128.44, 128.23, 127.75, 126.37, 123.11, 122.93, 120.20, 115.75, 115.01, 114.18, 110.43, 101.11, 67.93, 67.63, 55.19, 47.83, 33.45, 30.23, 28.09, 24.99, 22.77, 22.69, 22.37; HRMS (ESI) m/z calcd for $C_{35}H_{36}N_2O_4$, 549.2748; found, 549.2726. Purity: 99.2% (by HPLC).

4.1.27. (*E*)-3-(4-Methoxybenzylidene)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8b)

(*E*)-7-Hydroxy-3-(4-methoxybenzylidene)chroman-4-one (**7a**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8b** as a yellow oil, 80% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 8.2 Hz, 1H), 7.66 (s, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.41 (d, J = 8.8 Hz, 2H), 7.31 (t, J = 7.6 Hz, 1H), 7.04 (d, J = 8.7 Hz, 2H), 6.64 (dd, J = 8.8, 2.3 Hz, 1H), 6.49 (d, J = 2.3 Hz, 1H), 5.39 (d, J = 1.6 Hz, 2H), 5.32 (t, J = 6.3 Hz, 1H), 3.98 (t, J = 6.4 Hz, 2H), 3.82 (s, 3H), 3.39 (d, J = 7.1 Hz, 2H), 2.88 (t, J = 6.1 Hz, 2H), 2.70 (t, J = 5.9 Hz, 2H), 1.87–1.73 (m, 4H), 1.71–1.61 (m, 2H), 1.58–1.54 (m, 2H), 1.40–1.30 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.63, 164.93, 162.42, 160.31, 157.78, 150.39, 146.71, 135.56, 132.23, 128.92, 128.50, 128.06, 127.87, 126.38, 123.16, 122.98, 120.11, 115.69, 115.00, 114.27, 110.58, 101.17, 67.98, 67.65, 55.28, 47.71, 33.35, 30.39, 28.22, 25.91, 25.05, 24.99, 22.66, 22.35; HRMS (ESI) m/z calcd for $C_{36}H_{38}N_2O_4$, 563.2904; found, 563.2903. Purity: 97.4% (by HPLC).

4.1.28. (*E*)-3-(4-Methoxybenzylidene)-7-(8-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8c)

(*E*)-7-Hydroxy-3-(4-methoxybenzylidene)chroman-4-one (**7a**) was treated with **3c** (*N*-(8-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8c** as a yellow oil, 80% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, J = 8.4 Hz, 1H), 7.79 (dd, J = 8.8, 1.0 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.67 (s, 1H), 7.53–7.48 (m, 1H), 7.42 (d, J = 8.2 Hz, 2H), 7.35–7.29 (m, 1H), 7.06 (d, J = 7.9 Hz, 2H), 6.67 (d, J = 8.9 Hz, 1H), 6.53 (s, 1H), 5.41 (s, 2H), 5.38 (s, 1H), 4.01 (t, J = 6.2 Hz, 2H), 3.83 (s, 3H), 3.40–3.36 (m, 2H), 2.89 (t, J = 5.9 Hz, 2H), 2.70 (t, J = 5.6 Hz, 2H), 1.85–1.75 (m, 4H), 1.71–1.64 (m, 2H), 1.59–1.51 (m, 2H), 1.36–1.24 (m, 8H); ^{13}C NMR (101 MHz, DMSO) δ 179.59, 164.94, 162.41, 160.30, 157.77, 150.30, 146.77, 135.52, 132.21, 128.92, 128.50, 128.12, 127.81, 126.39, 123.11, 122.97, 120.12, 115.64, 115.00, 114.25, 110.56, 101.17, 68.09, 67.66, 55.27, 47.73, 33.39, 30.45, 28.58, 28.53, 28.30, 26.15, 25.20, 25.00, 22.68, 22.37; HRMS (ESI) m/z calcd for $C_{38}H_{42}N_2O_4$, 591.3217; found, 591.3244. Purity: 99.4% (by HPLC).

4.1.29. (*E*)-3-(4-Methoxybenzylidene)-7-(9-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8d)

(*E*)-7-Hydroxy-3-(4-methoxybenzylidene)chroman-4-one (**7a**) was treated with **3d** (*N*-(9-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8d** as a yellow oil, 83% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.72–7.64 (m, 2H), 7.50 (t, J = 7.6 Hz, 1H), 7.41 (d, J = 8.8 Hz, 2H), 7.32 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 8.7 Hz, 2H), 6.66 (dd, J = 8.8, 2.3 Hz, 1H), 6.52 (d, J = 2.3 Hz, 1H), 5.40 (d, J = 1.6 Hz, 2H), 5.35 (t, J = 6.4 Hz, 1H), 4.00 (t, J = 6.5 Hz, 2H), 3.82 (s, 3H), 3.41–3.36 (m, 2H), 2.89 (t, J = 6.0 Hz, 2H), 2.70 (t, J = 5.8 Hz, 2H), 1.86–1.76 (m, 4H), 1.71–1.62 (m, 2H), 1.57–1.50 (m, 2H), 1.34–1.20 (m, 10H);

^{13}C NMR (101 MHz, DMSO) δ 179.65, 164.95, 162.39, 160.29, 157.60, 150.54, 146.46, 135.56, 132.19, 128.93, 128.44, 127.96, 127.78, 126.34, 123.16, 122.99, 119.94, 115.46, 114.96, 114.24, 110.55, 101.13, 68.08, 67.62, 55.25, 47.81, 33.17, 30.46, 28.72, 28.51, 28.44, 28.28, 26.12, 25.23, 24.92, 22.61, 22.26; HRMS (ESI) *m*/*z* calcd for $C_{39}H_{44}N_2O_4$, 605.3374; found, 605.3379. Purity: 99.8% (by HPLC).

4.1.30. (*E*)-3-(4-Methoxybenzylidene)-7-(10-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8e)

(*E*)-7-Hydroxy-3-(4-methoxybenzylidene)chroman-4-one (**7a**) was treated with **3e** (*N*-(10-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8e** as a yellow oil, 75% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.09 (d, *J* = 8.4 Hz, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.65 (s, 1H), 7.49 (t, *J* = 7.4 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.04 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.7 Hz, 1H), 6.51 (s, 1H), 5.39 (s, 2H), 5.35 (t, *J* = 5.5 Hz, 1H), 4.00 (t, *J* = 6.0 Hz, 2H), 3.81 (s, 3H), 3.37 (d, *J* = 4.4 Hz, 2H), 2.88 (s, 2H), 2.69 (s, 2H), 1.78 (s, 4H), 1.69–1.63 (m, 2H), 1.55–1.48 (m, 2H), 1.33 (s, 2H), 1.23–1.14 (m, 10H); ^{13}C NMR (101 MHz, DMSO) δ 179.51, 164.92, 162.37, 160.27, 157.76, 150.26, 146.83, 135.47, 132.16, 128.89, 128.44, 128.16, 127.74, 126.37, 123.04, 122.93, 120.15, 115.61, 115.00, 114.20, 110.47, 101.11, 68.07, 67.64, 55.22, 47.77, 33.42, 30.54, 30.50, 28.80, 28.77, 28.64, 28.35, 26.22, 25.31, 25.00, 22.69, 22.38; HRMS (ESI) *m*/*z* calcd for $C_{40}H_{46}N_2O_4$, 619.3530; found, 619.3537. Purity: 99.7% (by HPLC).

4.1.31. (*E*)-3-(4-(Dimethylamino)benzylidene)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one. (8f)

(*E*)-3-(4-(Dimethylamino)benzylidene)-7-hydroxychroman-4-one (**7b**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8f** as a yellow oil, 88% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, *J* = 8.5 Hz, 1H), 7.76 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.60 (s, 1H), 7.50 (t, *J* = 7.2 Hz, 1H), 7.33 (s, 1H), 7.29 (d, *J* = 8.9 Hz, 2H), 6.76 (d, *J* = 8.9 Hz, 2H), 6.61 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.47 (d, *J* = 2.2 Hz, 1H), 5.40 (s, 2H), 5.32 (t, *J* = 6.2 Hz, 1H), 3.97 (t, *J* = 6.4 Hz, 2H), 3.39 (d, *J* = 6.3 Hz, 2H), 2.98 (s, 6H), 2.88 (t, *J* = 6.1 Hz, 2H), 2.70 (t, *J* = 5.8 Hz, 2H), 1.79 (d, *J* = 5.7 Hz, 4H), 1.68–1.62 (m, 2H), 1.59–1.53 (m, 2H), 1.34 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.42, 164.61, 162.16, 157.83, 150.96, 150.28, 146.80, 136.52, 132.41, 128.78, 128.15, 127.82, 125.33, 123.15, 122.97, 121.18, 120.15, 115.73, 115.27, 111.65, 110.27, 101.13, 67.99, 67.92, 47.73, 33.41, 30.45, 30.41, 28.25, 25.93, 25.07, 25.00, 22.67, 22.37; HRMS (ESI) *m*/*z* calcd for $C_{37}H_{41}N_3O_3$, 576.3221; found, 576.3242. Purity: 95.6% (by HPLC).

4.1.32. (*E*)-3-(4-Methylbenzylidene)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8g)

(*E*)-7-Hydroxy-3-(4-methylbenzylidene)chroman-4-one (**7c**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8g** as a yellow oil, 42% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.09 (d, *J* = 8.3 Hz, 1H), 7.78 (d, *J* = 8.8 Hz, 1H), 7.70–7.66 (m, 2H), 7.52–7.47 (m, 1H), 7.35–7.29 (m, 5H), 6.65 (d, *J* = 8.8 Hz, 1H), 6.50 (s, 1H), 5.38 (s, 2H), 5.35 (d, *J* = 6.2 Hz, 1H), 3.99 (t, *J* = 6.3 Hz, 2H), 3.43 (s, 2H), 2.88 (t, *J* = 5.8 Hz, 2H), 2.70 (t, *J* = 5.7 Hz, 2H), 2.36 (s, 3H), 1.79 (d, *J* = 5.7 Hz, 4H), 1.68–1.63 (m, 2H), 1.58–1.53 (m, 2H), 1.34 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.71, 165.04, 162.54, 157.73, 150.37, 146.63, 139.48, 135.73, 131.08, 130.25, 129.89, 129.36, 128.98, 127.99, 127.92, 123.20, 123.01, 120.08, 115.67, 114.96, 110.70, 101.22, 68.03, 67.58, 47.70, 33.31, 30.37, 28.21, 25.89, 25.05, 24.98, 22.65, 22.33, 20.93; HRMS (ESI) *m*/*z* calcd for $C_{36}H_{38}N_2O_3$, 547.2955; found, 547.2949. Purity: 98.4% (by HPLC).

4.1.33. (*E*)-3-(4-Fluorobenzylidene)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8h)

(*E*)-3-(4-Fluorobenzylidene)-7-hydroxychroman-4-one (**7d**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8h** as a yellow oil, 36% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, *J* = 8.6 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 5.5 Hz, 2H), 7.55–7.47 (m, 3H), 7.33 (t, *J* = 8.6 Hz, 3H), 6.66 (d, *J* = 8.8 Hz, 1H), 6.52 (s, 1H), 5.38 (s, 2H), 5.36 (s, 1H), 4.00 (t, *J* = 6.4 Hz, 2H), 3.40 (d, *J* = 6.6 Hz, 2H), 2.89 (t, *J* = 5.6 Hz, 2H), 2.71 (t, *J* = 5.4 Hz, 2H), 1.80 (d, *J* = 5.5 Hz, 4H), 1.71–1.64 (m, 2H), 1.60–1.54 (m, 2H), 1.36 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.63, 165.11, 163.71, 162.55, 161.23 (d, *J* = 250.48 Hz), 157.46, 150.66, 146.26, 134.57, 132.56, 132.48 (d, *J* = 8.08 Hz), 130.49, 130.38, 130.35 (d, *J* = 3.03 Hz), 129.00, 128.12, 127.57, 123.26, 123.05, 119.84, 115.84, 115.62 (d, *J* = 22.22 Hz), 115.43, 114.83, 110.75, 101.17, 68.01, 67.39, 47.76, 33.03, 30.36, 28.17, 25.85, 25.00, 24.89, 22.56, 22.20; HRMS (ESI) *m*/*z* calcd for $C_{35}H_{35}N_2O_3F$, 551.2704; found, 551.2702. Purity: 98.2% (by HPLC).

4.1.34. (*E*)-3-(4-Chlorobenzylidene)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8i)

(*E*)-3-(4-Chlorobenzylidene)-7-hydroxychroman-4-one (**7e**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8i** as a yellow oil, 47% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.68 (s, 2H), 7.58–7.46 (m, 5H), 7.35–7.29 (m, 1H), 6.66 (d, *J* = 8.9 Hz, 1H), 6.52 (s, 1H), 5.37 (s, 3H), 4.00 (t, *J* = 6.5 Hz, 2H), 3.41 (s, 2H), 2.89 (t, *J* = 6.0 Hz, 2H), 2.71 (t, *J* = 5.5 Hz, 2H), 1.79 (s, 4H), 1.70–1.64 (m, 2H), 1.60–1.54 (m, 2H), 1.36 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.48, 165.16, 162.60, 157.81, 150.29, 146.76, 134.28, 134.15, 132.74, 131.87, 131.29, 129.01, 128.73, 128.12, 127.84, 123.15, 122.97, 120.13, 115.72, 114.83, 110.81, 101.21, 68.05, 67.41, 47.72, 33.38, 30.40, 28.21, 25.91, 25.05, 25.00, 22.67, 22.36; HRMS (ESI) *m*/*z* calcd for $C_{35}H_{35}N_2O_3Cl$, 567.2409; found, 567.2426. Purity: 96.1% (by HPLC).

4.1.35. (*E*)-3-(4-(Diethylamino)benzylidene)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8j)

(*E*)-3-(4-(Diethylamino)benzylidene)-7-hydroxychroman-4-one (**7f**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8j** as a yellow oil, 43% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, *J* = 8.3 Hz, 1H), 7.75 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.58 (s, 1H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 2H), 6.73 (d, *J* = 8.4 Hz, 2H), 6.63 (t, *J* = 8.3 Hz, 1H), 6.48 (s, 1H), 5.42 (s, 2H), 5.38 (s, 1H), 3.98 (t, *J* = 6.3 Hz, 2H), 3.40 (d, *J* = 6.8 Hz, 6H), 2.89 (t, *J* = 5.6 Hz, 2H), 2.70 (t, *J* = 5.5 Hz, 2H), 1.79 (s, 4H), 1.66 (s, 2H), 1.59–1.54 (m, 2H), 1.35 (s, 4H), 1.11 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, DMSO) δ 179.39, 164.56, 162.14, 157.81, 150.31, 148.48, 146.75, 136.56, 132.86, 128.76, 128.11, 127.85, 124.74, 123.17, 122.98, 120.41, 120.13, 115.73, 115.32, 111.08, 110.25, 101.14, 68.05, 67.92, 47.72, 43.71, 33.38, 30.40, 28.25, 25.92, 25.07, 24.99, 22.67, 22.37, 12.36; HRMS (ESI) *m*/*z* calcd for $C_{39}H_{45}N_3O_3$, 604.3534; found, 604.3546. Purity: 99.2% (by HPLC).

4.1.36. (*E*)-3-(3-Methoxybenzylidene)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8k)

(*E*)-7-Hydroxy-3-(3-methoxybenzylidene)chroman-4-one (**7g**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the de-

sired product **8k** as a yellow oil, 54% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 8.2 Hz, 2H), 7.52–7.47 (m, 1H), 7.40 (t, J = 7.8 Hz, 1H), 7.34–7.29 (m, 1H), 7.02 (d, J = 7.4 Hz, 1H), 6.98 (d, J = 8.1 Hz, 2H), 6.65 (dd, J = 8.6, 1.9 Hz, 1H), 6.51 (d, J = 2.0 Hz, 1H), 5.38 (s, 2H), 5.36 (d, J = 6.2 Hz, 1H), 3.99 (t, J = 6.3 Hz, 2H), 3.80 (s, 3H), 3.39 (d, J = 6.9 Hz, 2H), 2.88 (t, J = 5.9 Hz, 2H), 2.70 (t, J = 5.7 Hz, 2H), 1.79 (d, J = 5.6 Hz, 4H), 1.69–1.64 (m, 2H), 1.59–1.53 (m, 2H), 1.34 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.61, 165.09, 162.60, 159.28, 157.86, 150.24, 146.85, 135.61, 135.22, 130.92, 129.75, 128.99, 128.21, 127.77, 123.12, 122.94, 122.20, 120.18, 115.75, 115.30, 115.27, 114.91, 110.70, 101.19, 68.02, 67.53, 55.15, 47.74, 33.44, 30.42, 28.23, 25.93, 25.06, 25.00, 22.69, 22.39; HRMS (ESI) m/z calcd for $C_{36}H_{38}N_2O_4$, 563.2904; found, 563.2879. Purity: 95.7% (by HPLC).

4.1.37. (*E*)-3-(2-Methoxybenzylidene)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8l)

(*E*)-7-Hydroxy-3-(2-methoxybenzylidene)chroman-4-one (**7h**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8l** as a yellow oil, 38% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.08 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 7.1 Hz, 2H), 7.69 (d, J = 8.3 Hz, 1H), 7.51–7.46 (m, 1H), 7.43 (t, J = 7.8 Hz, 1H), 7.34–7.28 (m, 1H), 7.14 (d, J = 7.5 Hz, 1H), 7.09 (d, J = 8.3 Hz, 1H), 7.02 (d, J = 7.3 Hz, 1H), 6.62 (d, J = 8.8 Hz, 1H), 6.46 (s, 1H), 5.35 (d, J = 5.6 Hz, 1H), 5.21 (s, 2H), 3.94 (t, J = 6.1 Hz, 2H), 3.82 (s, 3H), 3.37 (d, J = 6.4 Hz, 2H), 2.87 (s, 2H), 2.67 (s, 2H), 1.77 (s, 4H), 1.63 (s, 2H), 1.54 (s, 2H), 1.32 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.88, 165.04, 162.68, 157.87, 157.76, 150.27, 146.82, 131.66, 131.42, 130.40, 130.33, 128.98, 128.17, 127.81, 123.15, 122.96, 122.41, 120.25, 120.15, 115.74, 115.04, 111.31, 110.70, 101.22, 68.01, 67.77, 55.51, 47.72, 33.41, 30.45, 28.21, 25.90, 25.04, 25.00, 22.67, 22.37; HRMS (ESI) m/z calcd for $C_{36}H_{38}N_2O_4$, 563.2904; found, 563.2911. Purity: 98.2% (by HPLC).

4.1.38. (*E*)-3-(3,4-Dimethoxybenzylidene)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (8m)

(*E*)-3-(3,4-Dimethoxybenzylidene)-7-hydroxychroman-4-one (**7i**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8m** as a yellow oil, 38% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.11 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 8.7 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.67 (s, 1H), 7.52 (d, J = 7.2 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.07 (d, J = 7.6 Hz, 2H), 7.00 (d, J = 8.4 Hz, 1H), 6.65 (d, J = 8.8 Hz, 1H), 6.51 (s, 1H), 5.44 (s, 2H), 5.37 (s, 1H), 4.00 (t, J = 6.3 Hz, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.40 (d, J = 6.6 Hz, 2H), 2.89 (t, J = 5.6 Hz, 2H), 2.71 (t, J = 5.4 Hz, 2H), 1.85–1.76 (m, 4H), 1.70–1.64 (m, 2H), 1.61–1.52 (m, 2H), 1.36 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.56, 164.90, 162.39, 157.86, 150.31, 150.10, 148.59, 146.84, 135.96, 128.91, 128.62, 128.18, 127.78, 126.59, 123.66, 123.11, 122.94, 120.17, 115.72, 115.01, 113.81, 111.50, 110.46, 101.14, 67.95, 67.68, 55.49, 47.86, 33.42, 30.47, 28.25, 25.94, 25.07, 24.99, 22.68, 22.37; HRMS (ESI) m/z calcd for $C_{37}H_{40}N_2O_5$, 593.3010; found, 593.3009. Purity: 95.9% (by HPLC).

4.1.39. (*E*)-7-(6-(1,2,3,4-Tetrahydroacridin-9-ylamino)hexyloxy)-3-(3,4,5-trimethoxybenzylidene)chroman-4-one (8n)

(*E*)-7-Hydroxy-3-(3,4,5-trimethoxybenzylidene)chroman-4-one (**7j**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8n** as a yellow oil, 48% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, J = 8.5 Hz, 1H), 7.79 (d, J = 8.7 Hz, 1H), 7.71–7.63 (m, 2H), 7.53–7.47 (m, 1H), 7.32 (t, J = 7.6 Hz, 1H), 6.74 (s, 2H), 6.66 (d, J = 8.8 Hz, 1H), 6.51 (s, 1H), 5.46 (s, 2H), 5.36 (t, J = 6.1 Hz, 1H), 4.00 (t, J = 6.9 Hz, 2H), 3.83 (s, 6H), 3.72 (s, 3H), 3.40 (d, J = 6.9 Hz, 2H), 2.89 (t, J = 5.9 Hz, 2H), 2.71 (t, J = 5.6 Hz, 2H), 1.80 (d, J = 5.4 Hz, 4H), 1.70–1.64 (m, 2H), 1.60–1.54 (m, 2H), 1.36 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.60, 165.03, 162.55, 157.84, 152.81, 150.32, 146.80, 138.70, 136.04, 130.00, 129.40, 128.99, 128.15, 127.80, 123.13, 122.95, 120.15, 115.73, 114.95, 110.58, 107.83, 101.20, 68.00, 67.62, 60.07, 55.99, 47.84, 33.40, 30.45, 28.23, 25.92, 25.06, 24.99, 22.67, 22.37; HRMS (ESI) m/z calcd for $C_{38}H_{42}N_2O_6$, 623.3116; found, 623.3131. Purity: 97.7% (by HPLC).

4.1.40. (*E*)-7-(6-(6-Chloro-1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)-3-(4-methoxybenzylidene)chroman-4-one (8o)

(*E*)-7-Hydroxy-3-(4-methoxybenzylidene)chroman-4-one (**7a**) was treated with **3f** (*N*-(6-bromohexyl)-6-chloro-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8o** as a yellow oil, 84% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.14 (d, J = 9.1 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.70 (d, J = 2.2 Hz, 1H), 7.67 (s, 1H), 7.42 (d, J = 8.8 Hz, 2H), 7.32 (dd, J = 9.1, 2.3 Hz, 1H), 7.06 (d, J = 8.8 Hz, 2H), 6.65 (dd, J = 8.8, 2.3 Hz, 1H), 6.51 (d, J = 2.3 Hz, 1H), 5.55 (t, J = 6.2 Hz, 1H), 5.41 (s, 2H), 4.00 (t, J = 6.4 Hz, 2H), 3.83 (s, 3H), 3.44–3.39 (m, 2H), 2.88 (t, J = 6.0 Hz, 2H), 2.68 (t, J = 5.7 Hz, 2H), 1.79 (d, J = 5.5 Hz, 4H), 1.70–1.64 (m, 2H), 1.61–1.53 (m, 2H), 1.40–1.29 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 179.47, 164.83, 162.31, 160.23, 159.20, 150.42, 147.51, 135.44, 132.40, 132.11, 128.85, 128.39, 126.61, 126.34, 125.24, 123.22, 118.44, 115.69, 114.96, 114.15, 110.38, 101.04, 67.90, 67.60, 55.16, 47.81, 33.42, 30.42, 28.24, 25.91, 25.04, 24.89, 22.50, 22.18; HRMS (ESI) m/z calcd for $C_{36}H_{37}N_2O_4Cl$, 597.2515; found, 597.2507. Purity: 98.4% (by HPLC).

4.1.41. (*E*)-7-(8-(6-Chloro-1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)-3-(4-methoxybenzylidene)chroman-4-one (8p)

(*E*)-7-Hydroxy-3-(4-methoxybenzylidene)chroman-4-one (**7a**) was treated with **3g** (*N*-(8-bromohexyl)-6-chloro-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **8p** as a yellow oil, 75% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.12 (d, J = 9.1 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.71–7.64 (m, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 9.1 Hz, 1H), 7.04 (d, J = 8.5 Hz, 2H), 6.65 (d, J = 8.7 Hz, 1H), 6.51 (s, 1H), 5.54 (s, 1H), 5.39 (s, 2H), 3.99 (t, J = 6.4 Hz, 2H), 3.81 (s, 3H), 3.42–3.39 (m, 2H), 2.86 (s, 2H), 2.66 (s, 2H), 1.77 (s, 4H), 1.70–1.62 (m, 2H), 1.53 (s, 2H), 1.31 (s, 2H), 1.23 (s, 6H); ^{13}C NMR (101 MHz, DMSO) δ 179.40, 164.89, 162.38, 160.28, 158.99, 150.59, 147.44, 135.38, 132.47, 131.98, 128.80, 128.46, 126.43, 126.39, 125.15, 123.12, 118.42, 115.54, 115.03, 114.06, 110.28, 100.97, 67.97, 67.60, 55.02, 47.86, 33.30, 30.96, 30.49, 28.61, 28.36, 26.16, 25.24, 24.90, 22.53, 22.20; HRMS (ESI) m/z calcd for $C_{38}H_{41}N_2O_4Cl$, 625.2828; found, 625.2832. Purity: 96.3% (by HPLC).

4.1.42. 3-(4-Methoxybenzyl)-7-(6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyloxy)chroman-4-one (10)

7-Hydroxy-3-(4-methoxybenzyl)chroman-4-one (**9**) was treated with **3b** (*N*-(6-bromononyl)-1,2,3,4-tetrahydroacridin-9-amine) according to the general procedure to give the desired product **10** as a colorless oil, 66% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.09 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 4.6 Hz, 1H), 7.67 (d, J = 5.1 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.34–7.28 (m, 1H), 7.14 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 6.59 (dd, J = 8.7, 2.0 Hz, 1H), 6.48 (d, J = 2.1 Hz, 1H), 5.35 (s, 1H), 4.31 (dd, J = 11.4, 4.4 Hz, 1H), 4.12 (dd, J = 11.3, 9.1 Hz, 1H), 3.95 (t, J = 6.4 Hz, 2H), 3.71 (s, 3H), 3.41 (s, 2H), 3.03 (dd, J = 13.9, 5.0 Hz, 1H), 2.94–2.90 (m, 1H), 2.88 (t, J = 5.5 Hz, 2H), 2.69 (t, J = 5.8 Hz, 2H), 2.59 (dd, J = 13.9, 9.4 Hz, 1H), 1.78 (d, J = 5.5 Hz, 4H), 1.68–1.61 (m, 2H), 1.58–1.52 (m,

2H), 1.33 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 191.56, 164.80, 162.99, 157.89, 157.75, 150.30, 146.89, 130.13, 129.86, 128.38, 128.23, 127.75, 123.10, 122.94, 120.21, 115.74, 113.81, 113.75, 110.06, 101.00, 69.53, 67.90, 54.88, 47.87, 46.31, 33.45, 30.87, 30.48, 28.23, 25.93, 25.06, 25.01, 22.68, 22.38; HRMS (ESI) m/z calcd for $C_{36}H_{40}N_2O_4$, 565.3061; found, 565.3061. Purity: 99.4% (by HPLC).

4.2. Biological activity

4.2.1. In vitro inhibition studies on AChE and BuChE[14]

Acetylcholinesterase (AChE, E.C. 3.1.1.7, from electric eel), human acetylcholinesterase (*h*AChE, EC 3.1.1.7, from human erythrocytes), butylcholinesterase (BuChE, E.C. 3.1.1.8, from equine serum), 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine chloride (ATC), and butylthiocholine chloride (BTC) were purchased from Sigma–Aldrich. Compounds were dissolved in DMSO (10 mM) and diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to the desired final concentration. All the compounds are soluble at the tested concentration. DMSO was diluted to a concentration of less than 0.01%, and no inhibitory action on either AChE or BuChE was detected in separate prior experiments at this concentration.

The in vitro AChE assay was performed as follows: all assays were conducted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) using a Shimadzu UV-2450 spectrophotometer. Enzyme solutions were prepared to give 2.0 U/mL in 2 mL aliquots. The assay medium (1 mL) consisted of phosphate buffer (pH 8.0), 50 μL of 0.01 M DTNB, 10 μL of enzyme, and 50 μL of 0.01 M substrate (ACh chloride solution). Test compounds were added to the assay solution and pre-incubated with the enzyme at 37 °C for 15 min, followed by addition of substrate. The activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals at 37 °C. Calculations were performed according to the method of the equation in Ellman et al. Each concentration was assayed in triplicate.

The in vitro BuChE and *h*AChE assay was performed similarly to the method described above.

4.2.2. Kinetic characterization of AChE inhibition

Kinetic characterization of AChE was performed using a reported method. Test compound was added into the assay solution and pre-incubated with the enzyme at 37 °C for 15 min, followed by addition of substrate. Kinetic characterization of the hydrolysis of ATC catalyzed by AChE was performed spectrometrically at 412 nm. A parallel control with no inhibitor in the mixture allowed the change in activities to be measured at various times. The plots were assessed by a weighted least square analysis that assumed the variance of V to be a constant percentage of V for the entire data set. The slopes of these reciprocal plots were subsequently plotted against the concentration of the inhibitors in a weighted analysis, and K_i was determined as the ratio of the replot intercept to the replot slope.

4.2.3. Inhibition of MAO activity[17]

The potential effects of the test compounds on *h*MAO activity were investigated by measuring their effects on the production of H_2O_2 from *p*-tyramine, using the Amplex Red MAO assay kit (Molecular Probes, Inc.) and recombinant human MAO-A or MAO-B (Sigma–Aldrich) according to published procedures. Compounds were dissolved in DMSO (10 mM) and diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to the desired final concentration. All the compounds are soluble at the tested concentration. Adequate amounts of recombinant *h*MAO-A or *h*MAO-B (Sigma–Aldrich) were acquired and adjusted to 12.5 μg/mL for *h*MAO-A and 75 μg/mL for *h*MAO-B. Test drugs (20 μL) and MAO (80 μL) were incubated at 37 °C for 15 min in a flat-black-bottom 96-well microtest plate in dark. The reaction was started by adding 200 μM Amplex Red reagent, 2 U/mL horseradish peroxidase, and 2 mM *p*-tyramine for *h*MAO-A or 2 mM benzylamine for *h*MAO-B, at 37 °C for 20 min. The reaction was quantified in a multidetection microplate fluorescence reader based on the fluorescence generated (excitation, 545 nm; emission, 590 nm).

The specific fluorescence emission was calculated after subtraction of the background activity, which was determined from vials containing all components except the *h*MAO isoforms, which were replaced by a sodium phosphate buffer solution.

4.2.4. Reversibility and irreversibility experiments[17]

To evaluate whether compound **8b** is a reversible or irreversible *h*MAO-B inhibitor, an effective centrifugation ultrafiltration method (so-called 'repeated washing') was used. The experiment was performed according a reported method.[13] Adequate amounts of recombinant monoamine oxidase with or without test drugs were incubated at 37 °C for 15 min. An aliquot from this incubation was stored at 4 °C for later use in the measurement of MAO activity. Another aliquot was transferred to an Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-30 membrane (Millipore) and centrifuged (4 °C, 9000*g*, 20 min) 3 times. The enzyme was obtained and used for the subsequent measurement of activity using a method similar to that described above (the section 'Inhibition of MAO activity'). The corresponding values of percent *h*MAO-B inhibition were separately calculated for samples with and without repeated washing.

4.2.5. In vitro blood–brain barrier permeation assay.[20]

Brain penetration of compounds was evaluated using a parallel artificial membrane permeation assay (PAMPA) in a similar manner as described by Di et al. Commercial drugs were purchased from Sigma and Alfa Aesar. The porcine brain lipid (PBL) was obtained from Avanti Polar Lipids. The donor microplate (PVDF membrane, pore size 0.45 mm) and the acceptor microplate were both from Millipore. The 96-well UV plate (COSTAR®) was from Corning Incorporated. The acceptor 96-well microplate was filled with 300 μL of PBS/EtOH (7:3), and the filter membrane was impregnated with 4 μL of PBL in dodecane (20 mg/mL). Compounds were dissolved in DMSO at 5 mg/mL and diluted 50-fold in PBS/EtOH (7:3) to achieve a concentration of 100 mg/mL, 200 μL of which was added to the donor wells. The acceptor filter plate was carefully placed on the donor plate to form a sandwich, which was left undisturbed for 10 h at 25 °C. After incubation, the donor plate was carefully removed and the concentration of compound in the acceptor wells was determined using a UV plate reader (Flexstation® 3). Every sample was analyzed at five wavelengths, in four wells, in at least three independent runs, and the results are given as the mean ± standard deviation. In each experiment, 13 quality control standards of known BBB permeability were included to validate the analysis set.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 21302235), Distinguished Young Talents in Higher Education of Guangdong (2012LYM_003), Ph.D. Programs Foundation of Ministry of Education of China (20120171120045), and the Guangdong Engineering Research Center of Chiral Drugs.

References and notes

1. de Blennow, K.; Leon, M. J.; Zetterberg, H. *Lancet* **2006**, *368*, 387.
2. Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741.
3. (a) Scarpini, E.; Schelterns, P.; Feldman, H. *Lancet Neurol.* **2003**, *2*, 539; (b) Winkler, J.; Thal, L. J.; Gage, F. H.; Fisher, L. J. *J. Mol. Med.* **1998**, *76*, 555; (c) Cummings, J. L. *Am. J. Psychiatry* **2000**, *157*, 4.

4. Francis, P. T.; Palmer, A. M.; Snape, M.; Wilcock, G. K. *J. Neurol. Neurosurg. Psychiatry* **1999**, *66*, 137.
5. Villarroyaa, M.; García, A. G.; Marco, J. L. *Curr. Pharm. Des.* **2004**, *10*, 3177.
6. Walker, W. H.; Kearney, E. B.; Seng, R. L.; Singer, T. P. *Eur. J. Biochem.* **1971**, *24*, 328.
7. (a) Binda, C.; Newton-Vinson, P.; Hubalek, F.; Edmondson, D. E.; Mattevi, A. *Nat. Struct. Biol.* **2002**, *9*, 22; (b) Edmondson, D. E.; DeColibus, L.; Binda, C.; Li, M.; Mattevi, A. *J. Neural Trans. (Vienna, Austria: 1996)* **2007**, *114*, 703.
8. Bach, A. W.; Lan, N. C.; Johnson, D. L.; Abell, C. W.; Bembenek, M. E.; Kwan, S. W.; Seeburg, P. H.; Shih, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4934.
9. Saura, J.; Luque, J. M.; Da Cesura, A. M.; Prada, M.; Chan-Palay, V.; Huber, G.; Loffler, J.; Richards, J. G. *Neuroscience* **1994**, *62*, 15.
10. Nebbioso, M.; Pascarella, A.; Cavallotti, C.; Pescosolido, N. *Int. J. Exp. Pathol* **2012**, *93*, 401.
11. Sterling, J.; Herzig, Y.; Goren, T.; Finkelstein, N.; Lerner, D.; Goldenberg, W.; Miskolczi, I.; Molnar, S.; Rantal, F.; Tamas, T.; Toth, G.; Zagyva, A.; Zekany, A.; Lavian, G.; Gross, A.; Friedman, R.; Razin, M.; Huang, W.; Krais, B.; Chorev, M.; Youdim, M. B.; Weinstock, M. *J. Med. Chem.* **2002**, *45*, 5260.
12. Youdim, M.; Amit, T.; Bar-Am, O.; Weinreb, O.; Yogev-Falach, M. *Neurotox. Res.* **2006**, *10*, 181.
13. Desideri, N.; Bolasco, A.; Fioravanti, R.; Proietti Monaco, L.; Orallo, F.; Yáñez, M.; Ortuso, F.; Alcaro, S. *J. Med. Chem.* **2011**, *54*, 2155.
14. Ellman, G. L.; Courtney, K. D.; Andres, B. J.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
15. Inestrosa, N. C.; Alvarez, A.; Perez, C. A.; Moreno, R. D.; Vicente, M.; Linker, C.; Casanueva, O. I.; Soto, C.; Garrido, J. *Neuron* **1996**, *16*, 881.
16. Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. *Biochem. Pharmacol.* **2003**, *65*, 407.
17. Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Bizzarri, B.; Granese, A.; Carradori, S.; Yáñez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. *J. Med. Chem.* **2009**, *52*, 1935.
18. Izumi, N. I. T.; Kitaichi, Y.; Kato, A.; Inoue, T.; Koyama, T. *Eur. J. Pharmacol.* **2007**, *565*, 105.
19. (a) Clark, D. E.; Pickett, S. D. *Drug Discovery Today* **2000**, *5*, 49; (b) Kerns, E. H.; Di, L. *Drug Discovery Today* **2003**, *8*, 316.
20. Di, L.; Kerns, E. H.; Fan, K.; McConnell, O. J.; Carter, G. T. E. *J. Med. Chem.* **2003**, *38*, 223.