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ARTICLE

Discovery of boron-containing compounds as Aβ aggregation inhibitors and antioxidants for the treatment of Alzheimer's disease

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A novel series of boron-containing compounds were designed, synthesized and evaluated as multi-target-directed ligands against Alzheimer's disease. Biological activity results demonstrated that these compounds possessed a significant ability to inhibit self-induced Aβ aggregation (20.5-82.8%, 20 μM) and to act as potential antioxidants (oxygen radical absorbance capacity assay using fluorescein (ORAC-FL) values of 2.70-5.87). In particular, compound **17h** is a potential lead compound for AD therapy (IC_{50} = 3.41 μM for self-induced Aβ aggregation; ORAC-FL value = 4.55). Compound **17h** also functions as a metal chelator. These results indicated that boron-containing compounds could be new structural scaffolds for the treatment of AD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the progressive impairment of higher cognitive function, memory loss, and altered behavior. [1] The pathological hallmarks of AD include low levels of acetylcholine (ACh), β-amyloid (Aβ) deposits, τ-protein aggregation, oxidative stress and biometal dyshomeostasis. [2] Due to the complex pathogenesis of AD, there are only five drugs approved by FDA for treatment of AD to date, which include cholinesterase inhibitors (tacrine, donepezil, rivastigmine, and galantamine)[3] and an NMDA receptor antagonist (memantine). [4] These drugs give only a modest improvement in memory and cognitive function and do not prevent progressive neurodegeneration. Thus, the development of new drugs for treatment of AD remains a challenge in the pharmaceutical community.

Senile plaques composed of extracellular amyloid beta (Aβ) peptide aggregates are a key pathological marker of AD. The "amyloid hypothesis" proposes that the production and accumulation of oligomeric aggregates of Aβ in the brain is a central event in the pathogenesis of AD and that these aggregates initiate the pathogenic cascade that ultimately leads to neuronal loss and dementia.[5] Recent studies indicate that oxidative stress is one of the earliest events in AD pathogenesis.[6] Oxidative damage present within the brain of AD patients can be observed within every class of biological macromolecules, including nucleic acids, proteins, lipids, and carbohydrates.[7] The free-radical and oxidative stress theory of aging also suggests that oxidative damage is an important player in neuronal degeneration. Therefore, antioxidant protection is important for treatment of AD as the endogenous antioxidant protection system rapidly declines. Indeed, several antioxidant compounds have demonstrated efficacy in a number of recent studies.[8]

Boron is an element that possesses potential for the development of pharmaceutical drugs. Bortezomib (Velcade),[9] a proteasome inhibitor that has shown in vitro and in vivo activity against a variety of malignancies, has been used clinically for the treatment of cancers since 2003. Several cyclic boron-containing compounds have exhibited very good biological activity. For example, tavaborole (Kerydin) [10-11] is a boron-containing small molecule antifungal agent that was approved by the FDA in 2014 for the topical treatment of onychomycosis. The benzoxaborole SCYX-7158,[12] is in clinical trials for the treatment of stage 2 human African trypanosomiasis. Some boron-containing compounds have also demonstrated inhibitory activity against the phosphodiesterase 4 enzyme (PDE4) and inflammation-related cytokine release,[13] which have been shown to impact cognition enhancement in aging and Alzheimer's disease (AD).[14]

Aspired by the 'multifunctional agents' design strategy,[15-18] our research group has a long-standing interest in the search of novel compounds with multifunctional effects and therapeutic potential in the treatment of AD.[19-21] In this paper, we describe the design, synthesis and evaluation of a series of boron-containing compounds as Aβ aggregation inhibitors, antioxidants and metal-chelating agent for the treatment of AD.

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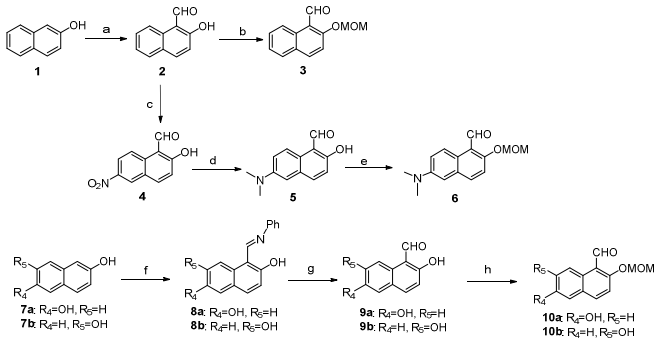
2. Results and Discussion

2.1 Chemistry

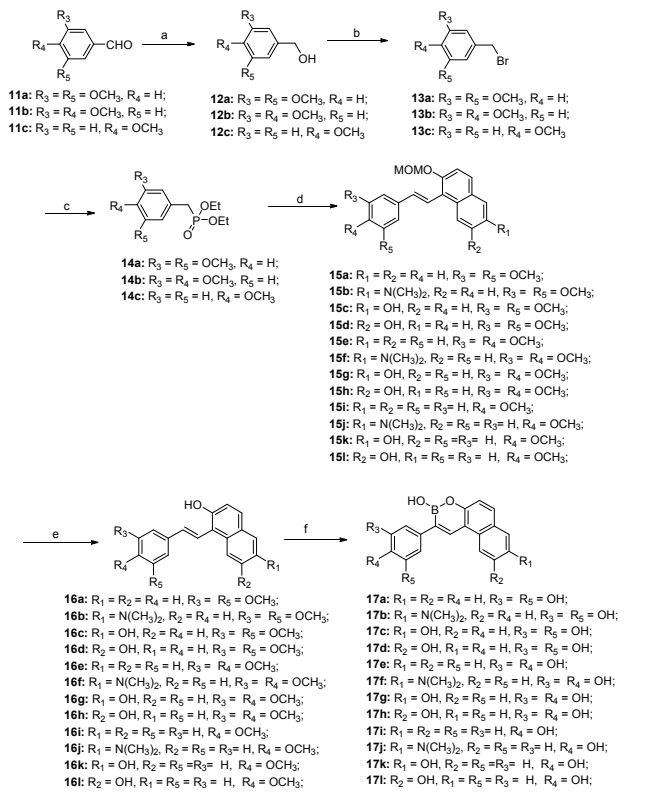
The synthetic routes for the new boron-containing compounds are shown in Schemes 1 and 2. Commercially available 2-naphthol (**1**) was treated with NaOH and chloroform to give 2-hydroxy-1-naphthaldehyde (**2**), which was then protected with a methoxymethyl (MOM) group through reaction with chloro(methoxy)methane in the presence of diisopropylethylamine to give **3**. Meanwhile, the nitration, reduction and then protection of the hydroxyl group of **1** gave amine **6**. Naphthalene-2,6-diol (**7a**) and naphthalene-2,7-diol (**7b**) were treated with N,N'-diphenylformamidine at 120 °C for 5 hours to provide **8a** and **8b**; these compounds were then treated with H_2SO_4 and then the hydroxyl groups were protected to afford compounds **10a** and **10b** (Scheme 1).



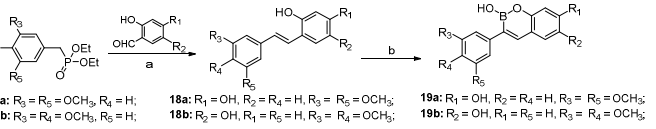
Scheme 1. Reagents and conditions: a. NaOH, $CHCl_3$, EtOH, 80-90 °C, 6h; b.CH_2Cl_2, MOMCl, i-Pr_2NEt, 0 °C, 4h; c. Fuming nitric acid, -5 °C, 20min; d. Pt/C, Formaldehyde, H_2, EtOH; e. CH_2Cl_2, MOMCl, i-Pr_2NEt, 0 °C, 4h; f. N,N'-Diphenylformamidine, Ar_2, 120 °C, 5h; g. ether, H_2SO_4, H_2O, 4d; h. CH_2Cl_2, MOMCl, i-Pr_2NEt, 0 °C, 4h.

The reduction of substituted benzaldehydes **11a-c** with sodium borohydride in methanol provided alcohols **12a-c** that were subsequently treated with PBr_3 in the presence of pyridine to give bromides **13a-c**, which were converted into Wittig reagents **14a-c** in high yield. The Wittig olefination of **14a-c** with the substituted benzaldehydes **2**, **6** and **10a-b** provided compounds **15a-l.** Finally, target compounds **17a-l** were obtained by removal of the protection groups on **15a-l** with hydrochloric acid followed by demethylation and cyclization in the presence of boron tribromide at −78 °C (Scheme 2).

Resveratrol-boron derivatives **19a-b** were also prepared according to scheme 3. The Wittig olefination of **14a-b** with the 2,4-dihydroxybenzaldehyde or 2,5-dihydroxybenzaldehyde provided compounds **18a-b**. Then, the resveratrol-boron derivatives **19a-b** were obtained by treating with BBr_3.



Scheme 2. Reagents and conditions: a. $NaBH_4$, MeOH, 0 °C; b. PBr_3, pyridine, 0 °C; c. Triethyl phosphite, 120 °C; d. 2, 6, 10a-b, CH_3ONa, 0 °C, 1 h, rt, 12 h; e. HCl, CH_3OH, 45 °C, 1h; f. BBr_3, CH_2Cl_2, -78 °C, 5h.



Scheme 3. Reagents and conditions: a. 2, 6, 10a-b, CH_3ONa, 0 °C, 1 h, rt, 12 h; b. BBr_3, CH_2Cl_2, -78 °C, 5h.

2.2 Inhibition of self-mediated $A\beta_{1-42}$ aggregation

To evaluate the inhibitory activities of boron-containing compounds **17a-l** against self-mediated $A\beta_{1-42}$ aggregation, a thioflavin-T (ThT) fluorescence binding assay[22] was performed. Curcumin, a known amyloid aggregation inhibitor, was used as a reference compound. The results shown in Table 1 indicate that almost half of the target compounds exhibited more potent Aβ aggregation inhibition (67.3%-82.8%, 20 μM) compared to curcumin (53.9%, 20 μM). Among them, compounds **17c, 17d, 17g** and **17h**, which feature two hydroxyl groups at the benzyl ring and one hydroxyl group on the naphthalene ring, exhibited the most potent inhibition activities (70.3%, 78.7%, 80.5% and 82.8%, 20 μM, respectively). However, compounds with only one hydroxyl group on the benzyl ring saw dramatically lower inhibition activities. For instance, compounds **17i** and **17j**, which possess one hydroxyl group at the benzyl ring and no substitution on the naphthalene ring, saw almost no inhibition of Aß aggregation at 20 μM. A simple analysis of the

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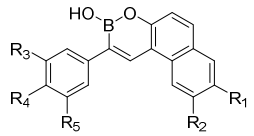
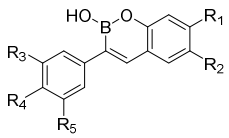
relationship between structure and activity suggests that two hydroxyl groups on the benzyl ring may play a key role in the inhibition of $A\beta_{1-42}$ aggregation. This result is consistent with previous studies that show that hydrogen bonds are crucial for interactions between polyphenols and proteins. The inhibition activity was also affected by substituent groups at naphthalene ring. Hydroxyl-substituted naphthalene rings substituents were better than unsubstituted or dimethylamino-substituted rings (the ability of compounds **17e**, **17f, 17g** and **17h** to inhibit Aβ aggregation was 43.7%, 49.5% and 82.8% at 20 μM, respectively). The substituent position on the naphthalene ring showed a slight influence on inhibition activities. In order to compare the activities with resveratrol, resveratrol- boron compounds **19a-b** were designed and synthesised. From the results, we can see that the inhibition activity of 19b was similar with resveratrol, but was much lower than compound 17h.

The complete dose-response curves of compounds with greater than 50% inhibition were evaluated. The IC_{50} values listed in Table 1 indicated that compound **17h** demonstrated the most potent inhibition of self-mediated $A\beta_{1-42}$ aggregation (IC_{50} = 3.4 μM), almost 4-fold greater than that of curcumin (IC_{50} = 13.7 μM).

Table 1. Inhibition of $A\beta_{1-42}$ aggregation and oxygen radical absorbance capacity (ORAC, Trolox equivalents) of boron - containing compounds **17a-l**, **19a-b,** Resveratrol , Curcumin, Ferulic acid and (E)-1,3-dimethoxy-5-(4-methoxystyryl)benzene.



17a - l



19a-b

Compd.	R_1	R_2	R_3	R_4	R_5	Inhibition of $A\beta_{1-42}$ aggregation(%)[a]	$A\beta_{1-42}$ IC_{50} (μM)[d]	ORAC[b]
17a	H	H	OH	H	OH	67.3 ± 4.6	12.6 ± 2.8	3.00 ± 0.15
17b	N(CH3)2	H	OH	H	OH	32.4 ± 2.4	n.t.c	5.27 ± 0.17
17c	OH	H	OH	H	OH	70.3 ± 5.4	8.9 ± 1.8	5.87 ± 0.09
17d	H	OH	OH	H	OH	78.7 ± 3.8	4.9 ± 0.7	5.34 ± 0.23
17e	H	H	OH	OH	H	43.7 ± 3.6	n.t.[c]	2.70 ± 0.03
17f	N(CH3)2	H	OH	OH	H	49.5 ± 2.8	n.t.[c]	4.88 ±0.14
17g	OH	H	OH	OH	H	80.5 ± 3.8	4.6 ± 1.1	4.05 ± 0.23
17h	H	OH	OH	OH	H	82.8 ± 3.1	3.4 ± 0.5	4.55 ± 0.20
17i	H	H	H	OH	H	0	n.t.[c]	2.06 ± 0.04
17j	N(CH3)2	H	H	OH	H	0	n.t.[c]	2.41 ± 0.11
17k	OH	H	H	OH	H	12.3 ± 1.7	n.t.[c]	3.94 ± 0.20
17l	H	OH	H	OH	H	20.5 ± 2.4	n.t.[c]	4.45 ± 0.12
19a	OH	H	OH	H	OH	50.2 ± 3.5	21.7 ± 4.0	4.84 ± 0.31
19b	H	OH	OH	OH	H	58.8 ± 2.9	17.5 ± 2.1	4.09 ± 0.27
Resveratrol	-	-	-	-	-	61.2 ± 5.2	16.8 ± 3.8	5.33 ± 0.17
Curcumin	-	-	-	-	-	53.9 ± 1.9	13.7 ± 1.9	n.t.[c]
Ferulic acid	–	–	–	–	–	n.t.[c]	n.t.[c]	3.24 ± 0.21
(E)-1,3-dimethoxy-5-(4-methoxystyryl)benzene	–	–	–	–	–	n.t.[c]	n.t.[c]	0.21 ± 0.02

[a] The Thioflavin-T fluorescence method was used. The values are expressed as the mean ± SD of at least three independent measurements. All values were obtained at a compound concentration of 20 μM. [b] The mean ± SD of the three independent experiments. The data are expressed as μmol of Trolox equivalents/μmol tested compound. [c] n.t. means not tested. [d] Mean ± SD of at least three independent measurements

2.3 Anti-oxidant activity *in vitro*

The antioxidant activity of the boron-containing compounds was determined using an oxygen radical absorbance capacity assay using fluorescein (ORAC-FL) according to the method originally described by Ou et al.[23] and modified by Dávalos.[24] The vitamin E analog Trolox was used as a standard, and the antioxidant activity was expressed as Trolox equivalents (μmol of Trolox/μmol of tested compound). The results in Table **1** indicate that all target compounds exhibited excellent antioxidant capacity with ORAC-FL values of 2.06-5.87 Trolox equivalents, which are similar or more active than ferulic acid; compound **17c** exhibited the best antioxidant activity with ORAC-FL value of 5.87.

2.4 Metal-chelating properties of compound 17h

Based on the inhibition of self-mediated $A\beta_{1-42}$ aggregation activity and anti-oxidant activity of compound **17h** ($A\beta_{1-42}$ aggregation activity: IC_{50} = 3.4 μM; ORAC-FL value of 4.55), we subjected this promising multi-functional inhibitor to further study. The metal-chelating ability of compound **17b** toward biometals such as Cu(II), Zn(II), Fe(II) and Fe(III) was investigated using UV-vis spectrometry.[25]The results in Fig. 1 show that **17b** produces maximum absorbance peaks at 234 and

331 nm. When $CuSO_4$ or $FeSO_4$ was added, the maximum absorbance at 331 nm exhibited a red shift to 355 nm or 350 nm, which suggests the formation of a **17h**–copper (II) and **17h**–iron (II) complex. However, with the addition of $Fe_2(SO_4)_3$ and $ZnCl_2$, there was no significant shift.

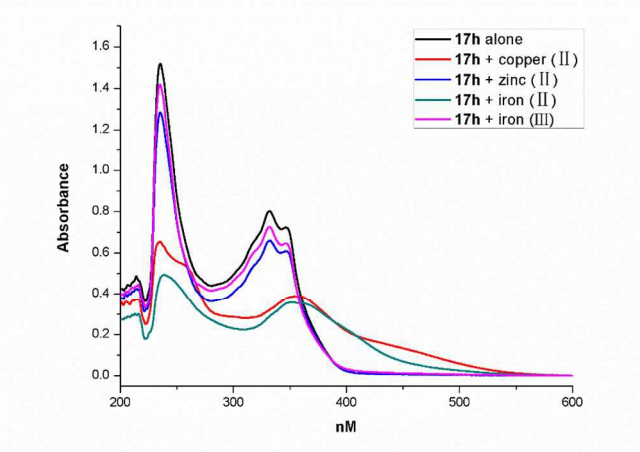


Fig. 1 UV spectra of compound **17h** (40 μM) alone and in the presence of 20 μM $CuSO_4$, $ZnCl_2$, $FeSO_4$ or $Fe_2(SO_4)_3$. All solutions were prepared using a HEPES buffer solution (20 mM HEPES, 150 mM NaCl, pH 7.4).

3. Conclusion

In summary, we have developed a novel series of boron-containing compounds, which exhibited multifunctional activity as potential anti-AD drugs, including significant ability to inhibit self-induced Aβ aggregation and to act as antioxidants and biometal chelators. Among the synthesized compounds, compound **17h** gave the greatest inhibitory potency toward self-induced Aβ aggregation (82.8%, 20 μM, IC_{50} = 3.4 μM) and good antioxidant activity (ORAC = 4.55). In addition, **17h** showed good metal-chelating ability toward biometals Cu(II) and Fe(II). These results indicate that boron–containing compounds could be new structural scaffolds for the treatment of AD. Further studies based on these results are in progress.

Experimental section

Chemistry

The ^{1}H NMR and ^{13}C NMR spectra were recorded using TMS as the internal standard on a Bruker BioSpin GmbH spectrometer at 400.132 MHz and 100.614 MHz, respectively. Coupling constants are given in Hz. LC-MS spectra were recorded on a Agilent LC-MS 6120 instrument with an ESI mass selective detector. High-resolution mass spectra were obtained using a Shimadzu LCMS-IT-TOF mass spectrometer. High-performance liquid chromatography (HPLC), which was equipped with a TC-C18 column (4.6×250 mm, 5μm), was used to determine the purity of the synthesised compounds. Flash column chromatography was performed using silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. All the reactions were monitored by thin layer chromatography using silica gel.

Synthesis procedure of 2- Hydroxy-1-Napthylaldehyde (2)

2- Hydroxy-1-Napthylaldehyde (**2**) was synthesized according to a reported procedure.[26] 2- naphthol (7.22g, 50 mmol) was dissolved in EtOH at 80-90°C, then NaOH (14.40g, 360 mmol) in 100 mL water was added drop wise to the hot solution, and the solution became darker. After half an hour, $CHCl_3$ (80 mmol) was added drop wise using a dropping funnel. The reaction mixture was stirred for six hours. Excess ethanol and chloroform were removed via the process of distillation. The dark oil produced was mixed with a considerable amount of sodium chloride. Sufficient water was added to dissolve the salt, and the oil was separated and washed with hot water. Then the solution was neutralized by dilute hydrochloric acid and extracted with chloroform. Finally, the product was purified by 60-120 silica gel by 1-2% ethyl acetate in pet ether. Yield of the product was 3.78 g (44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 9.87 (s, 1H), 8.73 (d, J = 7.9 Hz, 1H), 8.02-8.09 (m, 2H), 7.76 – 7.81 (m, 2H), 7.08 (d, J = 7.2 Hz, 1H).

Synthesis procedure of 2-(methoxymethoxy)-1-naphthaldehyde (3)

The preparation was carried out according to our previous reported procedure. [27] MOMCl (7.5 mmol) was added dropwise to an ice-cooled solution of diisopropylethylamine (10 mmol) and **2** (5 mmol) in dry CH_2Cl_2 (10 mL). After complete addition, the reaction mixture was allowed to warm to ambient temperature and stirred for 5 h. The reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine before being dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel with petrol/ethyl acetate as the elution solvent to afford 2-(methoxymethoxy)-1-naphthaldehyde (**3**) as light yellow solid, 0.93 g (yield: 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H), 8.64 (d, J = 9.2 Hz, 1H), 8.02-8.08, 7.59 – 7.68 (m, 2H), 7.01 (d, J = 9.2 Hz, 1H).

Synthesis procedure of 2-hydroxy-6-nitro-1-naphthaldehyde (4)

Compound 3 (5mmol, 0.86g) was added to Fuming Nitric acid (5.0 mL) in -5 °C and the mixture was allowed to stir at -5 °C for 20 minutes. Then the reaction mixture was poured into ice-water (20 mL). The resulting precipitate was filtered off and recrystalized by ethyl acetate to give 2-hydroxy-6-nitro-1-naphthaldehyde **(4)** as yellow powder in 58 % yield (0.63g). ^{1}H NMR (400 MHz, DMSO-d_6) δ12.21(s, 1H), 10.71 (s, 1H), 9.11 (d, J=9.1 Hz, 1H), 8.87 (s, 1H), 8.39 (d, J=9.1 Hz, 1H), 8.29 (d, J=9.1 Hz, 1H), 7.31 (d, J=9.1 Hz, 1H).

Synthesis procedure of 6-(dimethylamino)-2-hydroxy-1-naphthaldehyde (5)

Compound 4 (0.54g, 2.5 mmol) was dissolved in EtOH (25 mL). Formaldehyde (1.5 mL) and Pd/C 5% (100 mg) were added to the

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solution. The resulting mixture was stirred under hydrogen for 12 h. The reaction mixture was then filtered and evaporated. The residue was purified by flash chromatography on silica gel with petrol/ethyl acetate as the elution solvent to afford 6-(dimethylamino)-2-hydroxy-1-naphthaldehyde (5) as light yellow oil in 72 % yield (0.39g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.92 (s, 1H), 10.75 (s, 1H), 8.91 (d, J =9.1 Hz, 1H), 8.05 (d, J = 9.1 Hz, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.48 (d, J = 9.1 Hz, 1H), 7.22 (d, J = 9.1 Hz, 1H), 3.09 (s, 6H).

Synthesis procedure of 6-(dimethylamino)-2-(methoxymethoxy)-1-naphthalde -hyde (6)

MOMCl (6 mmol) was added dropwise to an ice-cooled solution of diisopropylethylamine (8 mmol) and **5** (4 mmol) in dry CH_2Cl_2 (10 mL). After complete addition, the reaction mixture was allowed to warm to ambient temperature and stirred for 5 h. The reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine before being dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel with petrol/ethyl acetate as the elution solvent to afford 2-(methoxymethoxy)-1-naphthaldehyde (**6**) as light yellow solid, 0.86 g (yield: 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.78 (d, J =9.2 Hz, 1H), 8.25 (d, J = 9.2 Hz, 1H), 7.47 (d, J = 9.2 Hz, 1H), 7.22 (d, J = 9.1 Hz, 1H), 7.08 (d, J = 9.1 Hz, 1H), 6.17(s, 3H), 3.31(s, 3H), 3.09 (s, 6H).

General Synthesis procedures of 8

The preparation was carried out according to our provious reported procedure.[28] A mixture of 2,6-dihydroxynaphtalene (**7a**, 30mmol) or 2,7-dihydroxynaphtalene (**7b**, 30mmol) and diphenyl formamidine (45 mmol) was stirred at 120°C under argon for 5 h. The progress of the reaction was monitored by TLC. The resulting mixture was cooled to room temperature and 30 ml of acetone were added, the resulting precipitate was filtered off and dried to give **8a** (5.67g; 72%) and **8b** (6.14g; 78%) as red powder. Compounds **8a** and **8b** were used in the next step without other purification.

General Synthesis procedures of 9

A solution of **8a** (20 mmol) or **8b** (20 mmol) in 5 ml of water and 4 ml of concentrated H_2SO_4 (96%) was put in a liquid-liquid extraction by upward displacement with ether during 4 days. Removal of the solvent under reduced pressure gave compound **9a** and **9b** as yellow solid.

2,6-dihydroxy-1-naphthaldehyde (9a)

yellow solid, yield:67%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (s, 1H), 11.64 (s, 1H), 10.84 (s, 1H), 8.35 (s, 1H), 7.94 (d, J = 9.2 Hz, 1H), 7.61 (d, J = 8.8 Hz, 1H), 7.32 (d, J1 = 9.2 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H)

2,6-dihydroxy-1-naphthaldehyde (9b)

yellow solid, yield:72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s, 1H), 11.90 (s, 1H), 10.79 (s, 1H), 8.86 (d, J = 2.2 Hz, 1H), 8.05 (d, J = 9.0 Hz, 1H), 7.38 (d, J = 8.8 Hz, 1H), 7.24 (dd, J1 = 9.0 Hz, J2 = 2.2 Hz, 1H), 7.18 (d, J = 8.8 Hz, 1H).

General Synthesis procedures of 10

MOMCl (15 mmol) was added dropwise to an ice-cooled solution of diisopropylethylamine (20 mmol) and **5** (10 mmol) in dry CH_2Cl_2 (20 mL). After complete addition, the reaction mixture was allowed to warm to ambient temperature and stirred for 5 h. The reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine before being dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel with petrol/ethyl acetate as the elution solvent to afford **10a** and **10b** as light yellow solid.

6-hydroxy-2-(methoxymethoxy)-1-naphthaldehyde (10a)

light yellow solid, yield 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.57 (s, 1H), 10.40 (s, 1H), 8.44 (d, J = 2.4 Hz, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.49 (d, J = 8.8 Hz, 1H), 7.28 (d, J1 = 9.0 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H)， 6.10(s, 3H), 3.25(s, 3H)

7-hydroxy-2-(methoxymethoxy)-1-naphthaldehyde (10b)

light yellow solid, yield 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.88 (s, 1H), 10.68 (s, 1H), 8.80 (d, J = 2.0 Hz, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.48 (d, J1 = 9.2 Hz, 1H), 7.27 (d, J = 8.8 Hz, 1H)， 6.14(s, 3H), 3.28(s, 3H)

General Synthesis procedures of 12

The preparation was carried out according to our previous reported procedure. [29] To a stirred solution of 3,5-dimethoxybenzaldehyde (11a), 3,4-dimethoxybenzaldehyde or 4-methoxybenzaldehyde in methanol, $NaBH_4$ was added in batches at 0^{o}C. The reaction mixture was slowly warmed to room temperature and stirred for another 2 hours. The solvent was evaporated under vacuum and the residue was extracted with EtOAc, washed by water, dried over Na_2SO_4, and concentrated to give **12a**, **12b** or **12c** as white solid. The product was used directly in the next step without further purification.

General Synthesis procedures of 13

The preparation was carried out according to our previous reported procedure. [29] PBr_3 was added dropwise to a solution of **12a**, **12b** or **12c** and pyridine in CH_2Cl_2 at 0℃. After the mixture was slowly warmed to room temperature and stirred for 4 h, the reaction was quenched by the slowly addition of ice water, extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, and concentrated to provide **13a**, **13b** or **13c** as white solid.

General Synthesis procedures of 14

The preparation was carried out according to our previous reported procedure. [4] The mixture of **13a**, **13b** or **13c** and

triethyl phosphate were heated at 120℃ for 10h. The excess triethyl phosphate was removed in vacuum and the crude product was purified by silica gel column chromatography (EtOAc/petroleum ether) to give **14a**, **14b** or **14c** compound as oil.

General procedure for the preparation of 15

Sodium methoxide (3 mmol) was added to a solution of phosphonic acid diethyl ester **14a**, **14b** or **14c** (1 mmol) in dry DMF (2 mL). The resulting mixture was stirred at room temperature for 5 min, and compounds **2**, **6**, **10a** or **10b** (1.2 mmol) was added at 0°C. The mixture was stirred at room temperature for 0.5 h and then for 12 h at 80°C. The reaction was quenched by pouring into ice-water with stirring. Reactions that gave solids were filtered and dried. Reactions that gave oils were extracted with ethyl acetate, and the ethyl acetate layer was washed with water and brine and then dried over Na_2SO_4. Filtration and evaporation of the solvent afforded the oils. The crude solids or oils were purified by flash chromatography on silica gel with petrol / ethyl acetate as the elution solvent to afford the desired product **15a** - **15l**.

(E)-1-(3,5-dimethoxystyryl)-2-(methoxymethoxy)naphthalene (15a)

Yellow solid, yield 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, J = 9.1 Hz, 1H), 7.92 (d, J = 8.6 Hz, 1H), 7.44-7.65 (m, 3H), 7.20 (d, J = 8.6 Hz, 1H), 7.08 (d, J = 8.3 Hz, 1H), 6.92 (s, 1H), 6.87 (s, 1H), 6.72 (d, J = 8.6 Hz, 1H), 6.21 (s, 1H), 4.61 (t, 2H), 3.85 (s, 6H), 3.68 (t, 2H), 3.31 (s, 3H).

(E)-5-(3,5-dimethoxystyryl)-6-(2-methoxyethoxy)-N,N-dimethylnaphthalen-2-amine (15b)

Yellow solid, yield 61%. ^{1}H NMR (400 MHz, DMSO-d_6) δ7.82 (d, J = 8.6 Hz, 1H), 7.44-7.50 (m, 2H), 7.18 (d, J = 8.7 Hz, 1H), 7.04 (d, J = 8.3 Hz, 1H), 6.90 (s, 1H), 6.85 (s, 1H), 6.67 (d, J = 8.6 Hz, 1H), 6.78 (s, 1H), 6.19 (s, 1H), 4.60 (t, 2H), 3.83 (s, 6H), 3.65 (t, 2H), 3.29 (s, 3H), 3.13 (s, 6H).

(E)-5-(3,5-dimethoxystyryl)-6-(2-methoxyethoxy)naphthalen-2-ol (15c)

Yellow solid, yield 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (d, J = 9.2 Hz, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.19 (d, J = 8.8 Hz, 1H), 7.12 (s, 1H), 7.08 (d, J = 8.3 Hz, 1H), 7.02 (s, 1H), 6.95 (s, 1H), 6.84 (s, 1H), 6.71 (d, J = 8.8 Hz, 1H), 6.24 (s, 1H), 4.59 (t, 2H), 3.84 (s, 6H), 3.62 (t, 2H), 3.30 (s, 3H).

(E)-8-(3,5-dimethoxystyryl)-7-(2-methoxyethoxy)naphthalen-2-ol (15d)

Yellow solid, yield 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.20 (d, J = 8.8 Hz, 1H), 7.01-7.12 (m, 3H), 6.90 (s, 1H), 6.85 (s, 1H), 6.74 (d, J = 8.8 Hz, 1H), 6.18 (s, 1H), 4.60 (t, 2H), 3.85 (s, 6H), 3.65 (t, 2H), 3.28 (s, 3H).

(E)-1-(3,4-dimethoxystyryl)-2-(2-methoxyethoxy)naphthalene (15e)

Yellow solid, yield 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 7.6 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.48-7.66 (m, 3H), 7.36 (s, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 8.8 Hz, 1H), 4.50 (t, 2H), 3.83 (s, 6H), 3.71 (t, 2H), 3.29 (s, 3H).

(E)-5-(3,4-dimethoxystyryl)-6-(2-methoxyethoxy)-N,N-dimethylnaphthalen-2-amine (15f)

Yellow solid, yield 61%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (d, J = 8.7 Hz, 1H), 7.48-7.50 (m, 2H), 7.34 (s, 1H), 7.30 (d, J = 8.6 Hz, 1H), 7.20 (d, J = 8.8 Hz, 1H), 6.79-6.99 (m, 3H), 6.70 (d, J = 8.8 Hz, 1H), 4.52 (t, 2H), 3.82 (s, 6H), 3.74 (t, 2H), 3.30 (s, 3H), 3.06(s, 6H).

(E)-5-(3,4-dimethoxystyryl)-6-(2-methoxyethoxy)naphthalen-2-ol (15g)

Yellow solid, yield 68%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (d, J = 9.2 Hz, 1H), 7.52 (d, J = 8.6 Hz, 1H),, 7.34 (s, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H), 7.19 (s, 1H), 7.03 (d, J = 8.6 Hz, 1H), 6.94-6.99 (m, 2H), 6.77 (d, J = 8.8 Hz, 1H), 4.42 (t, 2H), 3.82 (s, 6H), 3.75 (t, 2H), 3.31 (s, 3H).

(E)-8-(3,4-dimethoxystyryl)-7-(2-methoxyethoxy)naphthalen-2-ol (15h)

Yellow solid, yield 64%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, J = 9.2 Hz, 1H), 7.62 (d, J = 8.6 Hz, 1H), 7.32-7.35 (m, 2H), 7.21 (d, J = 8.8 Hz, 1H), 7.15 (d, J = 8.6 Hz, 1H), 6.87-6.96 (m, 3H), 6.74 (d, J = 8.8 Hz, 1H), 4.50 (t, 2H), 3.83 (s, 6H), 3.71 (t, 2H), 3.29 (s, 3H).

(E)-2-(2-methoxyethoxy)-1-(4-methoxystyryl)naphthalene (15i)

Yellow solid, yield 64%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75-7.85 (m, 5H), 7.58-7.54 (m, 1H), 7.24-7.38 (m, 3H), 7.20 (d, J = 8.6 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 6.79 (d, J = 8.2 Hz, 1H), 4.43 (t, 2H), 3.82 (s, 3H), 3.70 (t, 2H), 3.39 (s, 3H).

(E)-6-(2-methoxyethoxy)-5-(4-methoxystyryl)-N,N-dimethylnaphthalen-2-amine (15j)

Yellow solid, yield 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68-7.74 (m, 3H), 7.54-7.57 (m, 2H), 7.23 (d, J = 9.2 Hz, 1H), 7.11-7.15 (m, 2H), 6.55-6.59 (m, 2H), 6.78 (d, J = 9.2 Hz, 1H), 4.41 (t, 2H), 3.82 (s, 3H), 3.74 (t, 2H), 3.40 (s, 3H), 2.99(s, 6H).

(E)-6-(2-methoxyethoxy)-5-(4-methoxystyryl)naphthalen-2-ol (15k)

Yellow solid, yield 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72-7.79 (m, 3H), 7.67 (d, J = 8.6 Hz, 1H), 7.43 (s, 1H), 7.25 (d, J = 9.2 Hz, 1H), 7.14-7.19 (m, 2H), 6.87-6.92 (m, 2H), 6.80 (d, J = 9.2 Hz, 1H), 4.44 (t, 2H), 3.83 (s, 3H), 3.76 (t, 2H), 3.38 (s, 3H).

(E)-7-(2-methoxyethoxy)-8-(4-methoxystyryl)naphthalen-2-ol (15l)

Yellow solid, yield 49%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74-7.80 (m, 3H), 7.69 (d, J = 9.0 Hz, 1H), 7.49 (s, 1H), 7.22

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(d, *J* = 9.2 Hz, 1H), 7.15-7.19 (m, 2H), 6.94-7.00 (m, 2H), 6.78 (d, *J* = 9.2 Hz, 1H), 4.40 (t, 2H), 3.83 (s, 3H), 3.75 (t, 2H), 3.39 (s, 3H).

General procedure for the preparation of 16

A solution of compound **15a - 15l** (0.5 mmol) in methanol (5 mL) was treated with 6 M HCl (0.5 mL), and the mixture was refluxed for 3 h. The solvent was removed by evaporation, and the residue was neutralised by saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine and dried over Na_2SO_4.The solvent was removed by evaporation, and the residue was purified by flash chromatography on silica gel with petrol / ethyl acetate as the elution solvent to afford the desired product **16a - 16l.**

General procedure for the preparation of 17

BBr_3 (5-7.5 eq.) was added dropwise at -78°C under nitrogen to a solution of dried CH_2Cl_2 containing compound **16a - 16l** (0.5 mmol). The resulting solution was slowly warmed to room temperature and stirred overnight. After monitoring the reaction progress by TLC, water was added slowly. The mixture was neutralised by saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The ethyl acetate layer was washed with water then dried over Na_2SO_4.The solvent was removed by evaporation, and the residue was purified by flash chromatography on silica gel with CH_2Cl_2 / methanol as the elution solvent to afford the desired product **17a - 17l.**

5-(3-hydroxy-3H-naphtho[1,2-e][1,2]oxaborinin-2-yl)benzene-1,3-diol (17a)

Red solid, yield: 80%. ^{1}H NMR (400 MHz, MeOD) δ: 8.56 (s, 1H), 8.30 (d, *J* = 8.4 Hz, 1H), 7.79 (dd, *J* = 12.2, 8.6 Hz, 2H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.46-7.27 (m, 2H), 6.76 (d, *J* = 2.1 Hz, 2H), 6.44-5.99 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 159.3, 151.4, 144.0, 138.7, 131.9, 131.4, 130.9, 129.7, 128.0, 125.6, 122.6, 119.9, 118.4, 107.6, 102.5, 101.4. HRMS (ESI) m/z [M-H]$^-$ for $C_{18}H_{13}BO_4$ pred. 303.0834, meas. 303.0827. HPLC purity: 99.3%.

5-(8-(dimethylamino)-3-hydroxy-3H-naphtho[1,2-e][1,2]oxaborinin-2-yl)benzene-1,3-diol (17b)

Red solid, yield:60%。^{1}H NMR (400 MHz, MeOD) δ: 8.46 (s, 1H), 8.09 (d, *J* = 9.3 Hz, 1H), 7.58 (d, *J* = 8.9 Hz, 1H), 7.28-7.11 (m, 2H), 7.00-6.90 (m, 1H), 6.71 (d, *J* = 2.2 Hz, 2H), 6.23 (t, *J* = 2.2 Hz, 1H), 2.98-2.82 (m, 6H). ^{13}C NMR (101 MHz, MeOD) δ: 159.2, 149.3, 144.3, 139.1, 132.8, 129.7, 124.6, 123.4, 119.9, 118.4, 109.5, 107.6, 102.4, 41.2. HRMS (ESI) m/z [M-H]$^-$ for $C_{20}H_{18}BNO_4$ pred. 346.1256, meas. 346.1250. HPLC purity: 96.2%.

2-(3,5-dihydroxyphenyl)-3H-naphtho[1,2-e][1,2]oxaborinine-3,8-diol (17c)

Red solid, yield:73%. ^{1}H NMR (400 MHz, MeOD) δ: 8.58 (s, 1H), 8.26 (d, *J* = 8.6 Hz, 1H), 7.65 (d, *J* = 8.7 Hz, 1H), 7.37 (d, *J* = 8.6 Hz, 1H), 7.18 (d, *J* = 13.8 Hz, 2H), 6.75 (d, *J* = 2.1 Hz, 2H), 6.26 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 159.2, 157.8, 152.1, 144.2, 139.1, 133.8, 131.4, 130.9, 126.1, 117.3, 117.2, 116.8, 107.5, 105.1, 102.3. HRMS (ESI) m/z [M-H]$^-$ for $C_{18}H_{13}BO_5$ pred. 319.0783, meas. 319.0780. HPLC purity: 97.7%.

2-(3,5-dihydroxyphenyl)-3H-naphtho[1,2-e][1,2]oxaborinine-3,9-diol (17d)

Red solid, yield:78%. ^{1}H NMR (400 MHz, MeOD) δ: 8.48 (s, 1H), 7.70 (dd, *J* = 8.8, 3.3 Hz, 2H), 7.63 (d, *J* = 2.1 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 1H), 7.02 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.73 (d, *J* = 2.2 Hz, 2H), 6.26 (t, *J* = 2.2 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 159.2, 157.8, 152.1, 144.2, 139.1, 133.8, 131.4, 130.9, 126.1, 117.3, 117.2, 116.8, 107.5, 105.1, 102.3. HRMS (ESI) m/z [M-H]$^-$ for $C_{18}H_{13}BO_5$ pred. 319.0783, meas. 319.0775. HPLC purity: 98.0%.

4-(3-hydroxy-3H-naphtho[1,2-e][1,2]oxaborinin-2-yl)benzene-1,2-diol (17e)

Red solid, yield:80%。^{1}H NMR (400 MHz, MeOD) δ: 8.56 (s, 1H), 8.37 (d, *J* = 8.5 Hz, 1H), 7.80 (dd, *J* = 16.1, 8.5 Hz, 2H), 7.52-7.56 (m, 1H), 7.45-7.35 (m, 2H), 7.27 (d, *J* = 2.1 Hz, 1H), 7.13 (dt, *J* = 24.2, 12.1 Hz, 1H), 6.78 (d, *J* = 8.2 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 151.3, 146.0, 137.0, 134.0, 131.9, 131.5, 130.4, 129.7, 127.2, 125.6, 122.8, 120.7, 119.9, 118.7, 116.2, 116.1. HRMS (ESI) m/z [M-H]$^-$ for $C_{18}H_{13}BO_4$ pred. 303.0834, meas. 303.0829. HPLC purity: 96.8%.

4-(8-(dimethylamino)-3-hydroxy-3H-naphtho[1,2-e][1,2]oxaborinin-2-yl)benzene-1,2-diol (17f)

Red solid, yield:70%。^{1}H NMR (400 MHz, MeOD) δ: 8.47 (s, 1H), 8.19 (d, *J* = 9.3 Hz, 1H), 7.61 (d, *J* = 8.9 Hz, 1H), 7.32-7.23 (m, 3H), 7.15 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.00 (d, *J* = 2.5 Hz, 1H), 6.82 (d, *J* = 8.2 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 149.3, 148.9, 145.9, 145.8, 137.3, 134.2, 132.9, 129.2, 124.7, 123.5, 120.6, 119.9, 118.7, 118.3, 116.2, 116.1, 109.5, 41.3. HRMS (ESI) m/z [M-H]$^-$ for $C_{20}H_{18}BNO_4$ pred. 346.1256, meas. 346.1248. HPLC purity: 96.3%.

2-(3,4-dihydroxyphenyl)-3H-naphtho[1,2-e][1,2]oxaborinine-3,8-diol (17g)

Red solid, yield:68%。^{1}H NMR (400 MHz, MeOD) δ: 8.57 (s, 1H), 8.32 (d, *J* = 9.2 Hz, 1H), 7.65 (d, *J* = 8.9 Hz, 1H), 7.39 (d, *J* = 8.9 Hz, 1H), 7.30 (d, *J* = 2.1 Hz, 1H), 7.24-7.15 (m, 3H), 6.82 (d, *J* = 8.2 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 158.9, 153.0, 145.4, 139.4, 138.0, 133.2, 132.0, 129.9, 127.0, 118.5, 116.8, 114.2, 107.0, 104.9, 101.8. HRMS (ESI) m/z [M-H]$^-$ for $C_{18}H_{13}BO_5$ pred. 319.0783, meas. 319.0774. HPLC purity: 97.7%.

2-(3,4-dihydroxyphenyl)-3H-naphtho[1,2-e][1,2]oxaborinine-3,9-diol (17h)

Red solid, yield:75%。^{1}H NMR (400 MHz, MeOD) δ: 8.49 (s, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 2.1 Hz, 1H), 7.27 (dd, *J* = 10.7, 5.5 Hz, 2H), 7.19-7.11 (m, 1H), 7.04 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.81 (d, *J* = 8.2 Hz, 1H). δ: 159.1, 158.0, 151.8, 144.2, 138.8, 134.0, 132.1, 131.2, 127.4, 116.7, 115.0, 107.0,

105.1, 103.1. HRMS (ESI) m/z [M-H]$^-$ for $C_{18}H_{13}BO_5$ pred. 319.0783, meas. 319.0776. HPLC purity: 98.0%.

2-(4-hydroxyphenyl)-3H-naphtho[1,2-e][1,2]oxaborinin-3-ol (17i)

Red solid, yield:80%。^{1}H NMR (400 MHz, MeOD) δ: 8.48 (s, 1H), 8.30 (d, *J* = 8.5 Hz, 1H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.73 (d, *J* = 8.9 Hz, 1H), 7.62-7.56 (m, 2H), 7.50-7.54 (m, 1H), 7.44-7.37 (m, 1H), 7.32 (d, *J* = 8.9 Hz, 1H), 6.87-6.76 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ: 157.9, 150.9, 136.9, 133.3, 131.8, 131.4, 130.3, 130.0, 129.6, 127.8, 125.5, 122.8, 119.9, 118.7, 116.1. HRMS (ESI) m/z [M-H]$^-$ for $C_{18}H_{13}BO_3$ pred. 287.0885, meas. 287.0882. HPLC purity: 96.2%.

8-(dimethylamino)-2-(4-hydroxyphenyl)-3H-naphtho[1,2-e][1,2]oxaborinin-3-ol (17j)

Red solid, yield:80%。^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 9.06 (s, 1H), 8.60 (s, 1H), 8.42 (d, *J* = 9.4 Hz, 1H), 7.71 (dd, *J* = 8.6, 6.3 Hz, 2H), 7.29 (dd, *J* = 9.1, 4.1 Hz, 2H), 7.04 (d, *J* = 2.5 Hz, 1H), 6.80 (d, *J* = 8.6 Hz, 1H), 2.99 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 159.9, 148.8, 144.0, 138.4, 133.0, 131.7, 128.7, 125.0, 122.9, 119.4, 118.9, 108.2, 107.0, 101.7, 41.2. HRMS (ESI) m/z [M-H]$^-$ for $C_{20}H_{18}BNO_3$ pred. 330.1307, meas. 330.1299. HPLC purity: 96.3%.

2-(4-hydroxyphenyl)-3H-naphtho[1,2-e][1,2]oxaborinine-3,8-diol (17k)

Red solid, yield: 74%。^{1}H NMR (400 MHz, MeOD) δ: 8.50 (s, 1H), 8.24 (d, *J* = 9.0 Hz, 1H), 7.62-7.53 (m, 3H), 7.31 (d, *J* = 8.9 Hz, 1H), 7.17-7.08 (m, 2H), 6.81-6.76 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ: 158.0, 153.8, 150.4, 134.9, 133.1, 131.9, 130.8, 130.0, 129.3, 126.9, 125.1, 123.0, 120.2, 118.1, 115.3. HRMS (ESI) m/z [M-H]$^-$ for $C_{18}H_{13}BO_4$ pred. 303.0834, meas. 303.0825.. HPLC purity: 97.7%.

2-(4-hydroxyphenyl)-3H-naphtho[1,2-e][1,2]oxaborinine-3,9-diol (17l)

Red solid, yield: 80%。^{1}H NMR (400 MHz, MeOD) δ: 8.50 (s, 1H), 8.24 (d, *J* = 9.0 Hz, 1H), 7.62-7.53 (m, 3H), 7.31 (d, *J* = 8.9 Hz, 1H), 7.17-7.08 (m, 2H), 6.81-6.76 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ: 158.2, 152.1, 149.8, 134.7, 133.5, 132.0, 130.7, 130.2, 129.0, 127.0, 125.7, 122.8, 120.1, 117.9, 114.8. HRMS (ESI) m/z [M-H]$^-$ for $C_{18}H_{13}BO_4$ pred. 303.0834, meas. 303.0824. HPLC purity: 97.1%.

Biological assays

ThT assay

$A\beta_{1-42}$ (Millipore, counter ion: NaOH) was dissolved in ammonium hydroxide (1% v/v) to give a stock solution (2000 μM), which was aliquoted into small samples and stored at -80 °C.

For the inhibition of self-mediated $A\beta_{1-42}$ aggregation experiment, the Aβ stock solution was diluted with 50 mM phosphate buffer (pH 7.4) to 50 μM before use. A mixture of the peptide (10 μL, 25 μM, final concentration) with or without the tested compound (10 μL, 20 μM, final concentration) was incubated at 37 °C for 48 h. Blanks using 50 mM phosphate buffer (pH 7.4) instead of Aβ with or without inhibitors were also carried out. The sample was diluted to a final volume of 200 μL with 50 mM glycine–NaOH buffer (pH 8.0) containing thioflavin T (5 μM). Then the fluorescence intensities were recorded five minutes later (excitation, 450 nm; emission, 485 nm).[24] The percent inhibition of aggregation was calculated by the expression $(1\text{-}IF_i/IF_c) \times 100\%$, in which IF_i and IF_c are the fluorescence intensities obtained for Aβ in the presence and absence of inhibitors after subtracting the background, respectively.

For the inhibition of copper-mediated $A\beta_{1-42}$ aggregation experiment, the Aβ stock solution was diluted in 20 μM HEPES (pH 6.6) with 150 μM NaCl. The mixture of the peptide (10 μL, 25 μM, final concentration) with or without copper (10 μL, 25 μM, final concentration) and the tested compound (10 μL, 50 μM, final concentration) was incubated at 37 °C for 24 h. Then 20 μL of the sample was diluted to a final volume of 200 μL with 50 mM glycine–NaOH buffer (pH 8.0) containing thioflavin T (5 μM). The detection method was the same as that of self-mediated $A\beta_{1-42}$ aggregation experiment.

For the disaggregation of self-induced Aβ fibrils experiment, the Aβ stock solution was diluted with 10 mM phosphate buffer (pH 7.4). The peptide (15μL, 50 μM) was incubated at 37 °C for 24 h. The tested compound (15μL, 50 μM) was then added and incubated at 37 °C for another 24 h. Then 20 μL of the sample was diluted to a final volume of 200 μL with 50 mM glycine–NaOH buffer (pH 8.0) containing thioflavin T (5 μM). The detection method was the same as above.

For the disaggregation of copper -induced Aβ fibrils experiment, the Aβ stock solution was diluted in 20 μM HEPES (pH 6.6) with 150 μM NaCl. The mixture of the peptide (10 μL, 25 μM, final concentration) with copper (10 μL, 25 μM, final concentration) was incubated at 37 °C for 24 h. The tested compound (10μL, 50 μM, final concentration) was then added and incubated at 37 °C for another 24 h.[50] Then 20 μL of the sample was diluted to a final volume of 200 μL with 50 mM glycine–NaOH buffer (pH 8.0) containing thioflavin T (5 μM). The detection method was the same as above.

Oxygen radical absorbance capacity (ORAC-FL) assay

The tested compound and fluorescein (FL) stock solution were diluted with 75 mM phosphate buffer (pH 7.4) to 10 μM and 0.117 μM, respectively. The solution of (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was diluted with the same buffer to 100, 80, 60, 50, 40, 20, and 10 μM. The solution of 2,2'-azobis-(amidinopropane)dihydrochloride (AAPH) was prepared before the experiment by dissolving 108.4 mg AAPH in 10 mL 75 mM phosphate buffer (pH 7.4) to a final concentration of 40 mM. The mixture of the tested compound (20 μL) and FL (120 μL; 70 nM, final concentration) was pre-incubated for 10 min at 37ºC, and then 60 μL of the AAPH solution was added. The fluorescence was recorded every minute for 120 min (excitation, 485 nm; emission, 520 nm). A blank using phosphate buffer instead of the tested compound was also carried out. All reaction mixtures were

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prepared triple and at least three independent runs were performed for each sample. The Antioxidant curves (fluorescence versus time) were normalized to the curve of the blank. The area under the fluorescence decay curve (AUC) was calculated as following equation:

$$\mathrm{AUC} = 1 + \sum_{i=1}^{i=120} (\mathrm{fi/f0})$$

Where f0 is the initial fluorescence reading at 0 min and fi is the fluorescence reading at time i. The net AUC was calculated by the expression: $AUC_{sample} - AUC_{blank}$. Regression equations between net AUC and Trolox concentrations were calculated. ORAC-FL value for each sample were calculated by using the standard curve which means the ORAC-FL value of tested compound expressed as Trolox equivalents.

Metal-chelating study

The chelating studies were performed with a UV-Vis spectrophotometer. The absorption spectra of each compound (20 μM, final concentration) alone or in the presence of $CuSO_4$, $FeSO_4$, or $ZnCl_2$ (40 μM, final concentration) for 30 min in 20% (v/v) ethanol/buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) were recorded at room temperature.

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Conflict of Interest

The author declare no conflict of interests.

Notes and references

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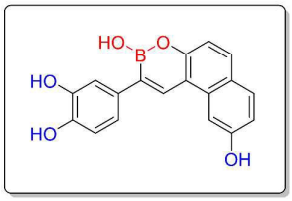

Inhibition of self-mediated $A\beta_{1-42}$ aggregation

- 82.8% inhibition rate at 20 μM
- IC50 = 3.4 μM

Anti-oxidant activity *in vitro*: ORAC = 4.55

Metal-chelating properties