Syntheses and Evaluation of Novel Isoliquiritigenin Derivatives as Potential Dual Inhibitors for Amyloid-beta Aggregation and 5-Lipoxygenase

Yi-Ping Chen, Zi-Ying Zhang, Yan-Ping Li, Ding Li, Shi-Liang Huang, Lian-Quan Gu, Jun Xu, Zhi-Shu Huang

PII: S0223-5234(13)00315-2

DOI: 10.1016/j.ejmech.2013.05.015

Reference: EJMECH 6193

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 6 March 2013

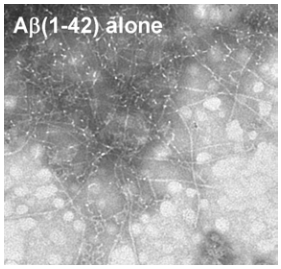
Revised Date: 12 May 2013

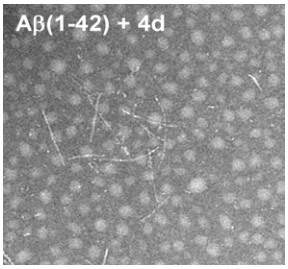
Accepted Date: 13 May 2013

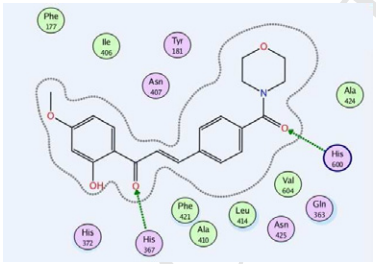
Please cite this article as: Y.-P. Chen, Z.-Y. Zhang, Y.-P. Li, D. Li, S.-L. Huang, L.-Q. Gu, J. Xu, Z.-S. Huang, Syntheses and Evaluation of Novel Isoliquiritigenin Derivatives as Potential Dual Inhibitors for Amyloid-beta Aggregation and 5-Lipoxygenase, *European Journal of Medicinal Chemistry* (2013), doi: 10.1016/j.ejmech.2013.05.015.

Syntheses and Evaluation of Novel Isoliquiritigenin Derivatives as Potential Dual Inhibitors for Amyloid-beta Aggregation and 5-Lipoxygenase

Yi-Ping Chen[a,b], Zi-Ying Zhang[a], Yan-Ping Li[a], Ding Li[a], Shi-Liang Huang[a,*], Lian-Quan Gu[a], Jun Xu[a], Zhi-Shu Huang[a,*]







IC_{50} = 3.2±1.2 μM for Aβ (1-42) aggregation

IC_{50} = 6.1±0.1 μM for 5-LO

A series of new isoliquiritigenin derivaives were synthesized and evaluated as a dual inhibitor of Aβ self-induced aggregation and 5-lipoxygenase (5-LO). Compound **4d** exhibited strong inhibitory potency against both targets.

Reseach highlights

► A series of novel Isoliquiritigenin (ISL) derivatives were synthesized.

► Most of synthesized compounds had better inhibition on Aβ aggregation and 5-LO.

►The amide derivatives(**4b**-**4d**) exhibit strong inhibitory potency against both targets .

Title page

Syntheses and Evaluation of Novel Isoliquiritigenin Derivatives as Potential Dual Inhibitors for Amyloid-beta Aggregation and 5-Lipoxygenase

Yi-Ping Chen[a,b], Zi-Ying Zhang[a], Yan-Ping Li[a], Ding Li[a], Shi-Liang Huang[a,*], Lian-Quan Gu[a], Jun Xu[a], Zhi-Shu Huang[a,*]

[a]*School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, People's Republic of China*
[b]*School of Pharmaceutical Sciences, Guangxi University of Chinese Medicine, Nanning 530001, People's Republic of China*

* To whom correspondence should be addressed. Tel.: 8620-39949052, Fax: 8620 39943056, E-mail addresses: lsshsl@mail.sysu.edu.cn (S.-L. Huang), ceshzs@mail.sysu.edu.cn (Z.-S. Huang).

Abstract

A series of new isoliquiritigenin (ISL) derivatives were synthesized and evaluated as dual inhibitors for amyloid-beta (Aβ) aggregation and 5-lipoxygenase (5-LO). It was found that all these synthetic compounds inhibited Aβ (1-42) aggregation effectively with their IC_{50} values ranged from 2.2 ± 1.5 μM to 23.8 ± 2.0 μM. These derivatives also showed inhibitory activity to 5-LO with their IC_{50} values ranged from 6.1 ± 0.1 μM to 35.9 ± 0.3 μM. Their structure-activity relationships (SAR) and mechanisms of inhibitions were studied. This study provided potentially important information for further development of ISL derivatives as multifunctional agents for Alzheimer's disease (AD) treatment.

***Keywords*:**
Isoliquiritigenin derivatives; Anti-Alzheimer agent; Amyloid-beta aggregation; 5-lipoxygenase; Inhibitors

Abbreviations:

AD: Alzheimer's disease
Aβ: amyloid beta protein
APP: amyloid precursor protein
ADAM10: a disintegrin and metalloproteinase domain-containing protein 10
BACE1: beta-site APP cleaving enzyme 1
CD: circular dichroism
CNS: Central Nervous System
CREB: cAMP-response element binding protein
EDC: 1-ethyl-3-(3-dimethylaminoprpyl) carbodiide
EM: electron microscopy
FLAP: 5-lipoxygenase activating protein
HOBt: N-hydroxybenzotriazole
ISL: isoliquiritigenin
5-LO: 5-lipoxygenase
MTT: methyl thiazolyl tetrazolium
NDGA: nordihydro-guaiaretic acid
Resv: resveratrol
SAR: structure-activity relationships

1. Introduction

Alzheimer's disease (AD), the most common form of dementia in the elderly, is a progressive neurodegenerative brain disorder. It is characterized by dementia, cognitive impairment, and memory loss [1-3]. The pathology of AD includes Tau protein hyperphosphorylation, aggregated amyloid beta protein (Aβ) deposits, cholinergic system dysfunction and so on [4]. Although many influencing factors have been found to be implicated in AD, its etiology and pathogenesis remain unclear. Amyloid plaques, widely accepted as the key pathological feature of AD, are mainly constituted by aggregation of the Aβ peptide which derived from the amyloid precursor protein (APP) and consists of 39–43 amino acid residues [5]. Based on the amyloid cascade hypothesis, the aggregation of Aβ has been considered to be a crucial step in the etiology of AD. The current therapy strategies have focused on modifying the amyloid plaques formation, and clearing the accumulation of this potentially neuron-toxic peptide. Therefore, the inhibition of amyloid plaques formation and clearance of potential neurotoxic peptide by small molecular compounds are promising strategies for the prevention and treatment of AD. Various compounds including anti-Aβ aggregation agents, Aβ peptide production blockage agents (β- and γ-secretase inhibitors), Aβ clearance agents (Aβ immunization and drugs modulating micro RNAs) [6], and anti-inflammatory drugs [7-9] have been reported to show these properties and prevent Aβ neurotoxicity.

The 5-lipoxygenase enzyme (5-LO) is widely distributed within the central nervous system (CNS) and its activity is regulated by 5-lipoxygenase activating protein (FLAP) [10]. A peculiar aspect of the 5-LO/FLAP pathway is the fact that its expression levels are significantly increased in CNS with aging, which is also region-specific in the hippocampus [11]. It has been confirmed that 5-LO activity was up-regulated in AD and the 5-LO protein levels in AD brains was higher than healthy ones [12, 13]. Firuzi *et al*. studies showed that 5-LO targeted gene disruption or its *in vivo*

selective pharmacological inhibition might lead to a significant reduction of Aβ deposition in the brains [14]. Furthermore, their study also revealed that 5-LO and its biologically active metabolites could affect CNS and AD via a possible mechanism involving the modulation of the γ-secretase activity. In addition, Chu *et al.* studies [15-17] showed that 5-LO regulated the formation of Aβ by activating the cAMP-response element binding protein (CREB) and preventing CREB activation by pharmacologic inhibition or dominant negative mutants, which blocks the 5-LO-dependent elevation of Aβ formation, γ-secretase mRNAs and protein levels. Chu *et al*. also provided evidences that FLAP was involved in the same AD-like amyloidotic phenotype *in vivo*, and they found that treatment with MK-591, a FLAP inhibitor, or Zileuton, a specific 5-LO inhibitor, both had similar effects in the 5-LO enzymatic pathway of the pathogenesis of AD-like amyloidosis and could result in significant reduction of the γ-secretase complex, but did not induce any change in the steady state levels of APP, beta-site APP cleaving enzyme 1 (BACE1) or a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) [16-17]. Therefore, 5-LO/FLAP pharmacological inhibition may be beneficial for the treatment and prevention of AD.

Because of the importance and connection in multiple mechanism of 5-LO/FLAP and Aβ in AD pathogenesis. In this study, we present the idea of designing novel dual-target agents which target both 5-LO and Aβ aggregation.

It has been reported that many flavonoids and polyphenols including quercetin, curcumin, and nordihydroguaiaretic acid (NDGA), inhibit not only Aβ aggregation [18-20], but 5-LO activity [21] as well. These compounds have also shown beneficial effects in animal models of neurodisease. Their structures are shown in Fig.1. Thereinto, chalcone is a member of flavonoids family, containing a structurally similar moiety to curcumin (Fig.1). Recently, it has been reported that radiolabeled chalcone derivatives can be used as amyloid imaging probes with high binding affinity

to Aβ aggregates, high uptake into the brain, and rapid clearance from the brain [22]. Some recent studies have indicated that some electron-donating groups such as amino, methylamino, dimethylamino, methoxyl or hydroxyl groups play critical roles for the binding of chalcone to Aβ aggregates [23-25].

Isoliquiritigenin (4, 2′, 4’-trihydroxychalcone, ISL, Fig.1), with the simple chalcone structure, one of the components of Licorice (*Radix Glycyrrhizae*), has exhibited a variety of pharmacological properties including antioxidative [26], anti-inflammatory [27], estrogenic [28], chemopreventive [29] and antitumor activities [30]. Licorice is widely used in medical formulations including anti-AD with a long application history in traditional Chinese medicine.

In the present study, a series of new ISL derivatives were designed and its dual inhibitors for amyloid-beta aggregation and 5-LO (Scheme 1) were both investigated. Their *in vitro* biological evaluations including inhibition of Aβ aggregation, cytotoxicity and anti-oxidant activity were carried out. Their structure-activity relationships were studied. The mechanisms for inhibiting β-sheet aggregation of Aβ (1–42) were further investigated by using circular dichroism (CD) and electron microscopy (EM) experiments. Molecular docking study with MOE was also carried out to understand the binding mode. Our studies provide potentially important information for further development of ISL derivatives as novel multifunctional agents for AD treatment.

2. Chemistry

A series of ISL derivatives were prepared by using Claisen–Schmidt condensation. As shown in Scheme 1, compounds **4** were prepared through condensation, hydrolysis, and amidation reactions [31]. The key step was the conversion of compound **3** to **4** through reacting with various corresponding amines in the presence of *N*-hydroxybenzotriazole (HOBt) and 1-ethyl-3-

(3-dimethylaminoprpyl)carbodiide (EDC) producing various amides **4** with yields ranged from 30% to 79%.

Compounds **5** were prepared through reactions of compound **1** with various aryl aldehydes **7** (as shown in Scheme 1). Most of these reactions gave ordinary condensation products, but some reactions only produced their further Williamson etherification products. For example, compounds **5a**, **5c**, **5f** were generated from corresponding aldehydes **7a$_1$**, **7b**, **7c$_2$**, respectively. Some aryl aldehydes **7** (**7a$_1$**, **7a$_2$**, **7b**, **7c$_1$**, **7c$_2$** and **7d**) were prepared through the reaction of the starting material aldehydes **6** with various alkyl bromides and K_2CO_3/DMF. The structures of all these target compounds were determined by using ^{1}H NMR, ^{13}C NMR, HRMS, and their purity was measured by using HPLC.

3. Results and Discussion

3.1. Aβ(1-42) self-induced aggregation inhibitory activity

The inhibitory activity of all compounds to Aβ(1-42) were tested following the method of Thio flavinT fluorescence (ThT) assay *in vitro* [32] using resveratrol (Resv, Fig.1) as a reference. As shown in Table 1, most of these compounds presented better inhibitory activity (with a range of IC_{50} value of 2.2 ± 1.5~ 23.8 ± 2.0 μM) than the original compound ISL (IC_{50} 19.7± 0.8 μM) except for **2**, **4f** and **4j**, and part of derivatives (**4a**-**4d**, **4k**-**4n**, **5d**, **5e**, and **5g**-**5i**) showed better inhibitory activity compareing with Resv (IC_{50} 15.9 ± 0.50 μM). Compounds **4b** and **4d**, which respectively had 4-substituted side chain of *N*-methylpiperazin-1-yl and morpholine group in A ring, displayed the most potent Aβ(1-42) aggregation inhibitory effects with IC_{50} values of 2.2 ± 1.5 μM and 3.2 ± 0.5 μM, respectively.

Analyzing the structure-activity relationships (SAR), it was found that the introduction of 4-substituted side chain in A ring could increase inhibitory activity of compounds, and the structure of side chain had an significant effects on their activity. It demonstrated the activity trend following: compounds **4b**-**4d** (R = six-member cyclic amines) > **4k**-**4n** (R = aryl amines) ≈ **5a**-**5i** (R′ = ethers) > **4e**-**4i** (R = alkyl amines) ≈ **3** (R = OH) > **2** (R = OMe).

3.2. In vitro 5-LO inhibition

Inhibitory activity of all targeted compounds for 5-LO were determined by using potato 5-LO (Cayman Chemical, USA) and an enzyme immuno assay (EIA) kit (Cayman Chemical, USA) according to manufacturer's instructions with NDGA (Fig. 1) as a reference. As shown in Table 1, most of these compounds presented similar or better inhibitory activity to 5-LO (with a range of IC_{50} value of 6.1 ± 0.1~ 35.8 ± 0.3 μM) than the original compound ISL (IC_{50} 18.6 ± 0.2 μM) except for **2**, **3**, **4e**, **4j** and **4l**. And part of derivatives (**4a**-**4d**, **5d**, and **5f**) showed better and others showed similar or weak inhibitory activity comparing with positive reference compound NDGA (IC_{50} 12.4 ± 1.3 μM). Among these compounds, **4b**-**4d**, with a 4-substituted side chain of *N*-methylpiperazin-1-yl, and piperidine and morpholine group in A ring, displayed the best inhibitory activity to 5-LO with IC_{50} values ranged from 6.1 ± 0.1 to 7.9 ± 0.1 μM.

The SAR demonstrated that their activity follow the trend of compounds **4a**-**4d** (R = cyclic amines , IC_{50} = 6.1~10.5 μM)> **5a**-**5i**(R′ = ethers, IC_{50} = 9.7 ~ 16.8μM)> **4j**-**4n**(R = aryl amines , IC_{50} =11.4 ~ 24.9 μM) ≈ **4e**-**4i** (R = alkyl amines , IC_{50} =12.9 ~ 22.1 μM) > **3** (R = OH) > **2** (R = OMe).

3.3. Molecular docking studies

To further study the interaction mode and analysis the SAR profile of these derivatives for

Aβ(1-42) and 5-LO, molecular docking study was performed using software package MOE2010. The X-ray crystal structure of the human 5-LO complex (PDB code 3O8Y) [33] and protein Aβ(1-42) structure (PDB code 1IYT) [34] used in the docking study were obtained from the Protein Data Bank (www.rcsb.org). Our docking studies indicated that most of these compounds exhibited mainly σ-π interaction and hydrogen bonding with 5-LO or Aβ(1-42).

A binding mode study of compound **4d** with 5-LO (Fig. 2) showed that there were two intermolecular hydrogen bond interactions. One is hydrogen bond between His600 residue and the carbonyl oxygen in the side-chain of A ring of compound **4d** (N-O distance: 2.82 Å), and the other one is between His367 residue and the carbonyl oxygen in the chalcone structure of **4d** (N-O distance: 2.87 Å). This indicates that hydrogen bond interactions play an important role in their binding. Two reasons are concluded to address the strong inhibitory activity of **4d** to 5-LO. Firstly, the length and flexibility of substituents affect the formation and stability of hydrogen bonding and thus influence the activities. Hydrogen bondings between **4d** and His600, His367 residues are contributed to the binding affinity of this compound to 5-LO. Impairment or destruction of the hydrogen bonds could fundamentally lead to a descent inhibition. Secondly, the side chains of compounds wedge in the deep cavity of the pocket, the size fitness of which directly influences their activities. **4d** with a 6-member ring fits perfectly to the pocket, which may lead to its high activity.

The interactions were also observed in the complex of compound **4d** /Aβ(1–42) (PDB code: 1IYT). B ring of compound **4d** was combined with the central hydrophobic region Leu17-Ala21 (LVFFA), while the side-chain morpholine ring resided in the ring area Asp23-Lys28 (DVGSNK). A σ-π interaction was found between B ring of **4d** and Leu17 residue (Fig. 3), and a hydrogen bond was found between His13 residue and the oxygen of hydroxyl group in the B ring (N-O distance:

3.14Å). The result indicated that van der waals force together with hydrogen bond play important roles in the stability of the **4d** /Aβ(1–42) complex.

*3.4. Effect of compound **4d** on Aβ β-sheet formation*

It has been reported that Aβ(1–42) adopted a conformational mixture of σ-helix, β-sheet, and random coil in the aqueous solution and formed a intramolecular β-sheet structure in the fibrillation through a conformational change [35]. In order to investigate the effect of compound **4d** on the structural transition of Aβ(1–42), Circular dichroism (CD) spectroscopy was employed to monitor the changes of the secondary structure of Aβ(1–42). It has been showed that the contents of β-sheet structure (occurrence of a peak around 195 nm) and σ-helix structure (occurrence of a broad minimum around 217 nm) changed after the 48 h incubation (Fig. 4).

As shown in Fig. 4A, there was no aggregation of Aβ(1–42) before incubation. During 2 days incubation (Fig. 4B and 4C), the CD spectrum of Aβ(1–42) with (red line) and without **4d** (black line) were both found to have negative bands around 217 nm and positive bands around 195 nm. However, the presence of **4d** in Aβ(1–42) sample resulted in a significant decrease of β-sheet structure at all tested time, with slight change to the intensity of the band around 217 nm. These results revealed that compound **4d** could reduce or retard the β-sheet structure formation, without significant effect on the content of σ-helix structure of peptide.

*3.5. Effect of compound **4d** on abundance of Aβ fibrils*

In order to complement the ThT binding assay and CD assay, electron microscopy (EM) assay was employed to monitor and clarify the effect of compound **4d** on Aβ aggregation. As shown in Fig. 5, After 4 days incubation, a large number of fibrils were observed in the control sample of Aβ(1-42) alone (Fig. 5A). In contrast, only a few fibrils were found in the Aβ(1-42) sample

incubated with compound **4d** (Fig. 5B). The EM results were in agreement with the results of ThT and CD studies, which were strongly further proving that compound **4d** can inhibit and slow down the Aβ(1–42) fibrils formation.

3.6. Cytotoxicity and antioxidation assay

The cytotoxicity of the ISL derivatives was examined in human neuro-blastoma SH-SY5Y cells. In methyl thiazolyl tetrazolium (MTT) assays, the cells were treated with different concentrations of compounds (0-100 μM), and our data indicated that all the compounds have their IC_{50} values above 100 μM. It is implied that all the target compounds have lower cytotoxicity. Their *in vitro* anti-oxidative activity was also examined, but all exhibited lower anti-oxidative activity than Trolox (data no shown).

Finally, it should be pointed out that all the data we received were obtained in *vitro*, and the necessary IC_{50} values may be extremely difficult to meet with in *vivo* testing. We will continue to explore this series of compounds in order to optimize the structural features and to discover more potent derivatives with high efficacy.

4. Conclusion

In the present study, a series of new ISL derivatives have been synthesized and their biological activities were evaluated. The SAR indicated that most of compounds had more effective and similar inhibitory potencies against self-induced Aβ(1-42) aggregation and 5-LO than Resv and the parental compound ISL except for compounds **2** and **3**. Additionally, it was interesting that **4b**-4**d** present strong inhibitory potency against both targets mentioned above, they are amides with 4-substituted side chain of cyclic amine group in A ring . Further investigations of compound **4d** by

using CD and EM experiments confirmed that compound **4d** could inhibit β-sheet aggregation and fibril formation. In summary, our study provided potentially important information for further development of ISL derivatives as new multifunctional agents for AD treatment.

5. Experimental Section

5.1. Chemistry

General procedures:

Commercially available solvents and reagents (chemicals) were used without further purification, unless otherwise stated. Analytical thin-layer chromatography (TLC) was done with HSGF 254. All target products were characterized by using NMR and High resolution mass spectra (HRMS). NMR spectra were recorded using TMS as the internal standard in $CDCl_3$ with a Bruker Avance III 400 spectrometer. The chemical shifts are reported in parts permillion (ppm), the coupling constants (*J*) are expressed in hertz (Hz) and signals are described as singlet (s), doublet (d), triplet (t), quartet (q), quint, broad (br), as well as multiplet (m).

The purities of synthesized compounds were confirmed to be higher than 95% by using analytical HPLC performed with a LC-20A system equipped with a Ultimate QB-C18 column (4.6×250 mm, 5 μm particle size) and eluted with acetonitrile/water (65:35-80:20) containing 0.1% TFA at a flow rate of 0.5 mL/min, with calculation of the relative purity of each compound based on its absorption at 254 nm.

*5.1.1. Synthesis of ISL derivatives **2** and **3***

*5.1.1.1. (E)-methyl-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)benzoate (**2**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) (16.6 g, 100 mmol) and methyl 4-formylbenzoate (16.4 g, 100 mmol) were dissolved in MeOH (500 mL), and 8% KOH in H_2O (100 mL) was added. The reaction mixture was stirred at room temperature for 48 h, and large amount of yellow solid was precipitated. The yellow precipitate was filtered and washed with appropriate amount of water, then recrystallized with MeOH to afford pure product **2** (24.5 g, 78%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.33 (s, 1H), 8.10 (d, *J* = 8.2 Hz, 2H), 7.90 (d, *J* = 15.5 Hz, 1H), 7.85 (d, *J* = 8.7 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 2H), 7.66 (d, *J* = 15.5 Hz, 1H), 6.52 (dd, *J* = 8.4, 2 Hz, 1H), 6.50 (d, *J* = 2 Hz, 1H), 3.96 (s, 3H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.4, 166.8, 166.5, 166.43, 142.7, 139.0, 131.6, 131.3, 130.2 (2C), 128.3 (2C), 122.6, 114.1, 107.9, 101.1, 55.6, 52.3. ESI-HRMS m/z: calcd for $C_{18}H_{16}O_5$ $[M+Na]^+$ 335.0886, found 335.0890. Purity: 95.5% by HPLC.

*5.1.1.2. (E)-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-enyl) benzoic acid (**3**)*

Compound **2** (2.84 g, 10 mmol) was mixed with 10% KOH in H_2O (20 mL), and heated on a water bath at 50-60 °C until compound **2** completely dissolved. Then 10% HCl solution was added to neutralize the solution to generate yellow precipitate. The yellow precipitate was filtered and washed with appropriate amount of water, and recrystallized with MeOH to afford pure product **3** (2.6 g, 87%) as a yellow solid. ^{1}H NMR (400 MHz, *d6*-DMSO) δ 13.34 (s, 1H), 13.13 (s, 1H), 8.30 (d, *J* = 9.1 Hz, 1H), 8.12 (d, *J* = 15.5 Hz, 1H), 8.03 (d, *J* = 8.9 Hz, 2H), 8.01 (d, *J* = 8.8 Hz, 2H), 7.85 (d, *J* = 15.5 Hz, 1H), 6.59 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.54 (d, *J* = 2.4 Hz, 1H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, *d6*-DMSO) δ 191.5, 166.7, 166.1, 165.7, 142.4, 138.5, 132.7, 132.1, 129.6 (2C), 128.9 (2C), 123.3, 113.8, 107.4, 100.8, 55.7. ESI-HRMS m/z: calcd for $C_{17}H_{14}O_5$ $[M-H]^-$ 297.0754, found 297.0763. Purity: 96.5% by HPLC.

*5.1.2. General procedure for synthesis of amide compounds **4a-4n***

A mixture of compound **3** (298 mg, 1.0 mmol), HOBt (270.2 mg, 2.0 mmol), and EDCI (383.4 mg, 2.0 mmol) was dissolved with CH_2Cl_2, and stirred for 10 min. The mixture was added with amine (2.0 mmol), and stirred at the room temperature for 2h. The reaction mixture was concentrated in vacuo to give the crude product. The crude product was purified by chromatography with EtOAc/PE, and crystallized with EtOAc to afford pure products.

5.1.2.1.(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(4-(pyrrolidine-1-carbonyl)phenyl)-prop-2-en-1-one ***(4a)***

Compound **3** was reacted with pyrrolidine following the general procedure to give the desired product **4a** (159 mg, 52%) as a yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.38 (s, 1H), 7.88 (d, *J* = 15.5 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 1H), 7.69 (d, *J* = 8.2 Hz, 2H), 7.62 (d, *J* = 15.0 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 6.51 (dd, *J* = 8.0, 2.5 Hz, 1H), 6.49 (d, *J* = 2.5 Hz, 1H), 3.88 (s, 3H), 3.67 (t, *J* = 6.9 Hz, 2H), 3.46 (t, *J* = 6.6 Hz, 2H), 2.04 – 1.95 (m, 2H), 1.95 – 1.86 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.5, 167.8, 165.7, 165.3, 142.2, 137.9, 135.1, 130.3, 127.3 (2C), 126.8 (2C), 120.5, 113.0, 106.8, 100.1, 54.6, 48.5, 45.3, 25.4, 23.4. ESI-HRMS m/z: calcd for $C_{21}H_{21}NO_4$ $[M-H]^-$ 350.1389, found 350.1392. Purity: 96.7% by HPLC.

5.1.2.2.(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(4-(4-methylpiperazine-1-carbonyl)phenyl)prop-2-en-1-one ***(4b)***

Compound **3** was reacted with 1-methylpiperazine following the general procedure to give the desired product **4b** (228 mg, 60%) as a yellow solid. ^{1}H NMR (400 MHz, *d6*-DMSO) δ 13.37 (s, 1H), 7.88 (d, *J* = 15.5 Hz, 1H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 15.5 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 6.51 (dd, *J* = 8.0, 2.5 Hz, 1H), 6.48 (d, *J* = 2.5 Hz, 1H), 3.88 (s, 3H), 3.75-3.82 (br, 2H), 3.54–3.40 (br, 2H), 2.55–2.36 (br, 4H), 2.34 (s, 3H). ^{13}C NMR (101 MHz,

d6-DMSO) δ 191.7, 168.3, 166.1, 165.7, 143.0, 137.8, 135.5, 132.7, 129.03 (2C), 127.4 (2C), 122.3, 113.9, 107.4, 100.9, 55.7, 54.6, 54.3, 46.9, 45.5, 41.4. ESI-HRMS m/z: calcd for $C_{22}H_{24}N_2O_4$ $[M-H]^-$ 379.1652, found 379.1658. Purity: 95.8% by HPLC.

5.1.2.3.(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(4-(piperidine-1-carbonyl)phenyl)prop-2-en-1-one ***(4c)***

Compound **3** was reacted with piperidine following the general procedure to give the desired product **4c** (129 mg, 35%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.37 (s, 1H), 7.88 (d, *J* = 15.5 Hz, 1H), 7.84 (d, *J* = 8.5 Hz, 1H), 7.69 (d, *J* = 8.1 Hz, 2H), 7.61 (d, *J* = 15.5 Hz, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 6.51 (dd, *J* = 8.0, 2.5 Hz, 1H), 6.48 (d, *J* = 2.5 Hz, 1H), 3.88 (s, 3H), 3.73 (br, 2H), 3.37 (br, 2H), 1.71 (br, 4H), 1.55 (br, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.5, 168.4, 165.7, 165.4, 142.2, 137.3, 134.8, 130.3, 127.5 (2C), 126.5 (2C), 120.3, 113.0, 106.8, 100.1, 54.6, 47.7, 42.2, 25.5, 24.6, 23.5. ESI-HRMS m/z: calcd for $C_{22}H_{23}NO_4$ $[M-H]^-$ 364.1544, found 364.1549. Purity: 99.7% by HPLC.

5.1.2.4.(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(4-(morpholine-4-carbonyl)phenyl)prop-2-en-1-one ***(4d)***

Compound **3** was reacted with morpholine following the general procedure to give the desired product **4d** (238 mg, 65%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.35 (s, 1H), 7.87 (d, *J* = 15.5 Hz, 1H), 7.82 (d, *J* = 8.7 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.61 (d, *J* = 15.5 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 2H), 6.50 (dd, *J* = 8.0, 2.5 Hz, 1H), 6.47 (d, *J* = 2.5 Hz, 1H), 3.87 (s, 3H), 3.78 (s, 4H), 3.66 (s, 2H), 3.47 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.40, 169.49, 166.68, 166.34, 142.87, 137.02, 136.20, 131.31, 128.56 (2C), 127.79 (2C), 121.62, 113.96, 107.77, 101.06, 66.77 (2C),

55.58, 48.05, 42.67. ESI-HRMS m/z: calcd for $C_{21}H_{21}NO_5$ $[M-H]^-$ 366.1345, found 366.1341. Purity: 95.2% by HPLC.

*5.1.2.5.(E)-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-N-isopropylbenzamide (**4e**)*

Compound **3** was reacted with propan-2-amine following the general procedure to give the desired product **4e** (122 mg, 36%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.36 (s,1H), 7.88 (d, *J* = 15.4 Hz, 1H), 7.84 (d, *J* = 8.9 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 2H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.63 (d, *J* = 15.5 Hz, 1H), 6.51 (dd, *J* = 8.0, 2.5 Hz, 1H), 6.48 (d, *J* = 2.5 Hz, 1H), 5.98 (d, *J* = 8.5 Hz, 1H), 4.30 (m, 1H), 3.88 (s, 3H), 1.30 (d, *J* = 6.5 Hz, 6H).^{13}C NMR (101 MHz, $CDCl_3$) δ 191.5, 166.7, 166.4, 165.89, 142.9, 137.4, 136.5, 131.3, 128.5 (2C), 127.5 (2C), 121.9, 114.0, 107.9, 101.1, 55.6, 42.1, 22.8. ESI- HRMS m/z: calcd for $C_{20}H_{21}NO_4$. $[M-H]^-$ 338.1396, Found 338.1392. Purity: 95.9% by HPLC.

*5.1.2.6.(E)-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-N-propylbenzamide (**4f**)*

Compound **3** was reacted with propan-1-amine following the general procedure to give the desired product **4f** (102 mg, 30%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.33 (s, 1H), 7.87 (d, *J* = 15.7 Hz, 1H), 7.83 (d, *J* = 7.4 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.69 (d, *J* = 7.8 Hz, 2H), 7.62 (d, *J* = 15.5 Hz, 1H), 6.51 (dd, *J* = 8.0, 2.5 Hz, 1H), 6.48 (d, *J* = 2.5 Hz, 1H), 6.20 (br, 1H), 3.87 (s, 3H), 3.44 (q, *J* = 8.0 Hz, 2H), 2.27–1.51 (m, 2H), 1.01 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.5, 166.8, 166.7, 166.4, 142.8, 137.5, 136.4, 131.3, 128.5 (2C), 127.5 (2C), 121.9, 114.0, 107.9, 101.1, 55.6, 41.9, 22.9, 11.4. ESI-HRMS m/z: calcd for $C_{20}H_{21}NO_4$. $[M-H]^-$ 338.1394, Found 338.1392. Purity: 99.2% by HPLC.

*5.1.2.7.(E)-N-dodecyl-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)benzamide (**4g**)*

Compound **3** was reacted with dodecan-1-amine following the general procedure A to give the desired product **4g** (272 mg, 58%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.35 (s, 1H), 7.88 (d, *J* = 15.5 Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.62 (d, *J* = 15.5 Hz, 1H), 6.51 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 3.87 (s, 3H), 3.47 (q, *J* = 7.1 Hz, 2H), 1.49–1.21 (m, 21H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.5, 166.8, 166.7, 166.5, 142.9, 137.5, 136.4, 131.3, 128.5 (2C), 127.5 (2C), 121.9, 114.0, 107.9, 101.1, 55.6, 40.3, 31.9, 29.6 (2C), 29.6 (2C), 29.6, 29.5, 29.3, 27.0, 22.7, 14.1. ESI-HRMS m/z: calcd for $C_{29}H_{39}NO_4$ $[M-H]^-$ 464.2805, found 464.2801. Purity: 97.5% by HPLC.

*5.1.2.8.(E)-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-N-phenethylbenzamide (**4h**)*

Compound **3** was reacted with 2-phenylethanamine following the general procedure to give the desired product **4h** (320 mg, 79%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.34 (s, 1H), 7.87 (d, *J* = 15.6 Hz, 1H), 7.83 (d, *J* = 8.7 Hz, 1H), 7.75 (d, *J* = 8.3 Hz, 2H), 7.69 (d, *J* = 8.3 Hz, 2H), 7.63 (d, *J* = 15.5 Hz, 1H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.32–7.22 (m, 3H), 6.52 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.48 (d, *J* = 2.5 Hz, 1H), 6.18 (t, *J* = 4.9 Hz, 1H), 3.88 (s, 3H), 3.76 (q, *J* = 6.7 Hz, 2H), 2.97 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.5, 166.8, 166.7, 166.5, 142.8, 138.8, 137.6, 136.1, 131.3, 128.8 (2C), 128.7 (2C), 128.6 (2C), 127.5 (2C), 126.7, 122.0, 114.0, 107.9, 101.1, 55.6, 41.2, 35.6. ESI-HRMS m/z: calcd for $C_{25}H_{23}NO_4$ $[M-H]^-$ 400.1582, Found 400.1554. Purity: 96.3% by HPLC.

*5.1.2.9.(E)-N-benzyl-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)benzamide (**4i**)*

Compound **3** was reacted with phenylmethanamine following the general procedure to give the desired product **4i** (265 mg, 68%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.33 (s, 1H),

7.88 (d, *J* = 13.4 Hz, 1H), 7.85 (d, *J* = 6.1 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.63 (d, *J* = 15.5 Hz, 1H), 7.38 (m, 5H), 6.51 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.48 (d, *J* = 2.8 Hz, 1H), 6.44 (t, *J* = 5.1 Hz, 1H), 4.67 (d, *J* = 5.6 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, *d6*-DMSO) δ 191.7, 166.1, 165.7, 165.5, 142.8, 139.5, 137.1, 135.7, 132.8, 128.9, 128.25 (2C), 127.7, 127.2 (4C), 126.7, 122.7, 113.9, 107.5, 100.9, 55.7, 42.7. ESI-HRMS m/z: calcd for $C_{24}H_{21}NO_4$ $[M-H]^-$ 386.1386, Found 386.1392. Purity: 98.7% by HPLC.

5.1.2.10.(E)-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-N-methyl-N-phenylbenzamide ***(4j)***

Compound **3** was reacted with *N*-methylaniline following the general procedure to give the desired product **4j** (183 mg, 47%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$). δ 13.33 (s, 1H), 7.77 (d, *J* = 6.2 Hz, 1H), 7.74 (d, *J* = 11.8 Hz, 1H), 7.50 (d, *J* = 15.5 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 7.28–7.22 (m, 2H), 7.17 (tt, *J* = 7.2, 2 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 6.47 (dd, *J* = 7.0, 2.5 Hz, 1H), 6.45 (d, *J* = 2.5 Hz, 1H), 3.85 (s, 3H), 3.52 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.5, 169.7, 166.7, 166.3, 144.6, 143.1, 137.8, 135.8, 131.3, 129.4 (2C), 129.3 (2C), 127.8 (2C), 126.9 (2C), 126.7, 121.4, 113.9, 107.7, 101.1, 55.6, 38.4. ESI-HRMS m/z: calcd for $C_{24}H_{21}NO_4$ $[M-H]^-$ 386.1385, Found 386.1392. Purity: 97.9% by HPLC.

*5.1.2.11.(E)-N-(2,4-dimethylphenyl)-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)benza mide (**4k**)*

Compound **3** was reacted with 2,4-dimethylaniline (2.0 mmol) following the general procedure to afford **4k** (253 mg, 63%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$). δ 13.33 (s, 1H), 7.93 (d, *J* = 8.3 Hz, 2H), 7.90 (d, *J* = 16.4 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 1H), 7.76 (d, *J* = 7.8 Hz, 3H), 7.65

(d, *J* = 15.5 Hz, 2H), 7.07 (m, 2H), 6.51 (m, 2H), 3.87 (s, 3H), 2.33 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.5, 166.9, 166.5, 164.8, 142.7, 138.0, 136.5, 135.5, 132.9, 131.3, 131.3, 129.7, 128.7 (2C), 127.8 (2C), 127.5, 123.5, 122.3, 114.1, 107.9, 101.2, 55.7, 20.9, 17.8. ESI-HRMS m/z: calcd for $C_{25}H_{23}NO_4$ $[M-H]^-$ 400.1543, Found 400.1549. Purity: 95.7% by HPLC.

*5.1.2.12.(E)-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-N-(2-methoxyphenyl)benzamide (**4l**)*

Compound **3** was reacted with 2-methoxyaniline following the general procedure to give the desired product **4l** (285 mg, 71%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.36 (s, 1H), 8.59 (s, 1H), 8.53 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.94 (d, *J* = 8.3 Hz, 2H), 7.89 (d, *J* = 15.5 Hz, 1H), 7.83 (d, *J* = 8.9 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.64 (d, *J* = 15.5 Hz, 1H), 7.11 (td, *J* = 7.6, 2 Hz, 1H), 7.04 (td, *J* = 7.6, 2 Hz, 1H), 6.94 (dd, *J* = 8.1, 1.2 Hz, 1H), 6.51 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 3.95 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (101 MHz,) δ 190.3, 165.7, 165.4, 163.2, 147.1, 141.6, 136.8, 135.6, 130.3, 127.6 (2C), 126.6 (2C), 126.5, 123.1, 121.1, 120.1, 118.8, 112.9, 108.9, 106.8, 100.1, 54.8, 54.6. ESI-HRMS m/z: calcd for $C_{24}H_{21}NO_5$ $[M-H]^-$ 402.1335, Found 402.1341. Purity: 98.5% by HPLC.

*5.1.2.13.(E)-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-N-(2-hydroxyphenyl)benzamide (**4m**)*

Compound **3** was reacted with 2-aminophenol following the general procedure to give the desired product **4m** (183 mg, 47%) as a yellow solid. ^{1}H NMR (400 MHz, d_6-DMSO) δ 13.34 (s, 1H), 9.70 (br, 1H), 9.63 (s, 1H), 8.32 (d, *J* = 9.1 Hz, 1H), 8.13 (d, *J* = 15.5 Hz, 1H), 8.09–8.02 (m, 4H), 7.87 (d, *J* = 15.5 Hz, 1H), 7.64 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.05 (td, *J* = 8.0, 1.4Hz, 1H), 6.93 (dd,

J = 8.1, 1.4 Hz, 1H), 6.83 (td, *J* = 7.8, 1.4 Hz, 1H), 6.59 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 3.85 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, *d6*-DMSO) δ 191.6, 166.8, 166.2, 165.7, 142.5, 138.6, 132.8, 132.2, 129.7 (2C), 129.0 (2C), 127.8, 127.3, 124.4, 123.5 (2C), 119.1, 113.9, 109.5, 107.5, 100.9, 55.8. ESI-HRMS m/z: calcd for $C_{23}H_{19}NO_5$ $[M-H]^-$ 388.1179, Found 388.1185. Purity: 99.1% by HPLC.

*5.1.2.14.(E)-4-(3-(2-hydroxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-N-(2-hydroxy-5-nitrophenyl)benzamide (**4n**)*

Compound **3** was reacted with 2-amino-4-nitrophenol following the general procedure to give the desired product **4n** (210 mg, 48%) as a yellow solid. ^{1}H NMR (400 MHz, d_6-DMSO) δ 13.30 (s, 1H), 8.33 (d, *J* = 9.0 Hz, 1H), 8.24 – 8.17 (m, 3H), 8.13 (d, *J* = 8.4 Hz, 2H), 8.03 (d, *J* = 2.4 Hz, 1H), 7.96 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.90 (d, *J* = 15.5 Hz, 1H), 6.85 (d, *J* = 9.1 Hz, 1H), 6.78 (s, 2H), 6.60 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.55 (d, *J* = 2.3 Hz, 1H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, *d6*-DMSO) δ 191.6, 166.2, 165.7, 163.8, 148.3, 142.3, 139.5, 135.1, 134.5, 132.9, 130.6 (2C), 130.2, 128.9 (2C), 124.0, 123.7, 119.7, 113.9 (2C), 107.6, 100.9, 55.8. ESI-HRMS m/z: calcd for $C_{23}H_{19}N_2O_7$ [M-H]- 433.1038, Found 433.1036. Purity: 97.6% by HPLC.

*5.1.3. Syntheses of various aryl aldehydes **7a$_1$–7a$_2$**, **7b**, **7c$_1$-7c$_2$** and **7d***

General procedure for the syntheses of various aryl aldehydes **7a$_1$–7a$_2$**, **7b**, **7c$_1$-7c$_2$** and **7d**.

To an aryl aldehyde **6** (10 mmol) and powdered K_2CO_3 (1.6 g) in DMF (15 mL), an appropriate amount of dibromoalkane derivative (40 mmol) or mono bromoalkane derivative (15 mmol) was added. After stirring at 80 °C for 4 h, white solid was precipitated. The precipitate was filtered and washed with appropriate amount of water to afford products **7a$_1$–7a$_2$**, **7b**, **7c$_1$-7c$_2$** and **7d**, and the products were pure enough for the next step of the reaction without further purification.

*5.1.4. Synthesis of ISL derivatives **5a-5i***

General procedures for the preparation of compounds **5a**-**5i**.

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) (166 mg, 1 mmol) and various aryl aldehyde **7** or commercially available aldehyde (1 mmol) were dissolved in EtOH (50 mL), in which was added 10% NaOH solution(10 mL). The reaction mixture was stirred at room temperature for 24 h, and yellow solid was precipitated. The yellow precipitate was filtered and washed with appropriate amount of water, the crude products recrystallized with MeOH to afford pure products.

*5.1.4.1.(E)-3-(4-(3-ethoxypropoxy)-3-methoxyphenyl)-1-(2-hydroxy-4-Methoxyphenyl)prop-2-en-1-one (**5a**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) was reacted with **$7a_1$** following the general procedure to give the desired product **5a** as a yellow solid (280 mg, 65%). ^{1}H NMR (400 MHz, d_6-DMSO). δ 13.53 (s, 1H), 7.85 (d, *J* = 5.7 Hz, 1H), 7.82 (s, 1H), 7.43 (d, *J* = 15.4 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.16 (d, *J* = 1.6 Hz, 1H), 6.94 (d, *J* = 8.3 Hz, 1H), 6.51–6.47 (m, 2H), 4.19 (t, *J* = 6.4 Hz, 2H), 3.94 (s, 3H), 3.86 (s, 3H), 3.61 (t, *J* = 6.1 Hz, 2H), 3.50 (q, *J* = 7.0 Hz, 2H), 2.13 (quint, *J* = 6.3 Hz, 2H), 1.20 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.8, 166.6, 166.1, 151.3, 149.6, 144.6, 131.1, 127.7, 123.3, 117.9, 114.2, 112.7, 111.0, 107.6, 101.1, 66.9, 66.3, 66.2, 56.2, 55.5, 29.6, 15.2. ESI-HRMS m/z: calcd for $C_{22}H_{26}O_6$ $[M-H]^-$ 385.1654, found 385.1657. Purity: 98.6% by HPLC.

*5.1.4.2.(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-methoxy-4-(3-phenoxypropoxy)phenyl)prop-2-en-1-one (**5b**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) was reacted with **$7a_2$** following the general procedure to give the desired product **5b** as a yellow solid (290 mg, 67%). ^{1}HNMR (400 MHz,

$CDCl_3$) δ 13.53 (s, 1H), 7.85 (d, *J* = 4.4 Hz, 1H), 7.82 (d, *J* = 14.8 Hz, 1H), 7.43 (d, *J* = 15.4 Hz, 1H), 7.30 (d, *J* = 7.9 Hz, 2H), 7.23 (d, *J* = 8.7 Hz, 1H), 7.16 (s, 1H), 6.94 (m, 4H), 6.49 (m, 2H), 4.28 (t, *J* = 6.3 Hz, 2H), 4.19 (t, *J* = 6.0 Hz, 2H), 3.93 (s, 3H), 3.86 (s, 3H), 2.34 (quint, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.8, 166.7, 166.1, 158.8, 151.0, 149.6, 144.6, 131.1, 129.5 (2C), 127.9, 123.2, 120.8, 118.1, 114.5 (2C), 114.2, 112.7, 110.9, 107.6, 101.1, 65.7, 64.2, 56.09, 55.57, 29.20. ESI-HRMS m/z: calcd for $C_{26}H_{26}O_6$ $[M-H]^-$ 433.1648, found 433.1651. Purity: 95.9% by HPLC.

*5.1.4.3.(E)-3-(3-(2-ethoxyethoxy)phenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en -1-one (**5c**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) was reacted with **7b** following the general procedure to give the desired product **5c** as a yellow solid (185 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.41 (s, 1H), 7.85 (d, *J* = 15.5 Hz, 1H), 7.83 (d, *J* = 8.8 Hz, 1H), 7.56 (d, *J* = 15.5 Hz, 1H), 7.34 (t, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 7.7 Hz, 1H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.01 (dd, *J* = 8, 2.4 Hz, 1H), 6.51 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.48 (d, *J* = 2.5 Hz, 1H), 4.19 (t, *J* = 5.2 Hz, 2H), 3.88 (s, 3H), 3.83 (t, *J* = 5.2 Hz, 2H), 3.64 (q, *J* = 7.0 Hz, 2H), 1.27 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.8, 166.7, 166.3, 159.3, 144.3, 136.2, 131.3, 129.9, 121.5, 120.6, 116.9, 114.4, 114.1, 107.7, 101.1, 68.9, 67.7, 66.9, 55.6, 15.2. ESI-HRMS m/z: calcd for $C_{20}H_{22}O_5$ $[M-H]^-$ 341.1388, found 341.1389. Purity: 95.4% by HPLC.

*5.1.4.4.(E)-3-(4-(benzyloxy)phenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (**5d**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) was reacted with **7c$_1$** following the general procedure to give the desired product **5d** as a yellow solid (180mg, 50%).^{1}H NMR (400 MHz) δ 13.54 (s, 1H), 7.86 (d, *J* = 15.4 Hz, 1H), 7.82 (d, *J* = 9.6 Hz, 1H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.49–7.34 (m, 6H), 7.02 (d, *J* = 8.7 Hz, 2H), 6.53–6.45 (m, 2H), 5.12 (s, 2H), 3.86 (s, 3H). ^{13}C

NMR (101 MHz, $CDCl_3$) δ 191.9, 166.6, 166.1, 160.9, 144.2, 136.4, 131.1, 130.4 (2C), 128.7 (2C), 128.2, 127.8, 127.5 (2C), 118.0, 115.4 (2C), 114.2, 107.6, 101.2, 70.2, 55.6. ESI-HRMS m/z: calcd for $C_{23}H_{20}O_4$ $[M-H]^-$ 359.1275, found 359.1283. Purity: 97.6% by HPLC.

*5.1.4.5.(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**5e**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) was reacted with 4-methoxybenzaldehyde following the general procedure to give the desired product **5e** as a yellow solid (174 mg, 32%). ^1H NMR (400 MHz, $CDCl_3$) δ 13.55 (s, 1H), 7.84 (d, *J* = 15.5 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 15.4 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 6.55–6.37 (m, 2H), 3.84 (s, 3H), 3.83 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 191.9, 166.6, 166.0, 161.8, 144.2, 131.1, 130.4 (2C), 127.5, 117.8, 114.5 (2C), 114.2, 107.6, 101.1, 55.5, 55.4. ESI-HRMS m/z: calcd for $C_{17}H_{16}O_4$ $[M-H]^-$ 283.0966, found 283.0970. Purity: 97.3% by HPLC.

*5.1.4.6.(E)-3-(4-(2-ethoxylethoxy)phenyl)-1-(2-hydroxyl-4-Methoxyphenyl)prop-2-en-1-one (**5f**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) was reacted with **$7c_2$** following the general procedure to give the desired product **5f** as a yellow solid (170 mg, 50%). ^1H NMR (400 MHz, $CDCl_3$) δ 13.54 (s, 1H), 7.86 (d, *J* = 15.4 Hz, 1H), 7.83 (d, *J* = 9.4 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 15.4 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 2H), 6.55–6.41 (m, 2H), 4.18 (t, *J* = 5.2 Hz, 2H), 3.86 (s, 3H), 3.82 (t, *J* = 5.2 Hz, 2H), 3.61 (q, *J* = 7.0 Hz, 2H), 1.25 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.9, 166.6, 166.0, 161.1, 144.2, 131.1, 130.3 (2C), 127.6, 117.9, 115.1 (2C), 114.2, 107.6, 101.1, 68.8, 67.6, 66.9, 55.6, 15.1. ESI-HRMS m/z: calcd for $C_{20}H_{22}O_5$ $[M-H]^-$ 341.1381, found 341.1389. Purity: 96.2% by HPLC.

*5.1.4.7.(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**5g**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) was reacted with 2,4,5- trimethoxybenzaldehyde following the general procedure C to give the desired product **5g** as a yellow solid (179 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.69 (s, 1H), 8.16 (d, *J* = 15.5 Hz, 1H), 7.82 (d, *J* = 8.7 Hz, 1H), 7.52 (d, *J* = 15.5 Hz, 1H), 7.11 (s, 1H), 6.52 (s, 1H), 6.49–6.45 (m, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.3, 166.6, 165.8, 154.9, 152.7, 143.3, 139.8, 131.1, 118.1, 115.4, 114.3, 111.8, 107.4, 101.0, 96.8, 56.62, 56.3, 56.1, 55.5. ESI-HRMS m/z: calcd for $C_{19}H_{20}O_6$ $[M-H]^-$ 343, found 343.1182. Purity: 97.3% by HPLC.

*5.1.4.8. (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**5h**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) was reacted with 3,4,5-trimethoxybenzaldehyde following the general procedure to give the desired product **5h** as a yellow solid (190 mg, 55.4%). ^{1}H NMR (400 MHz, $CDCl_3$). 13.46 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 1H), 7.81 (d, *J* = 15.5 Hz, 1H), 7.45 (d, *J* = 15.4 Hz, 1H), 6.87 (s, 2H), 6.58–6.41 (m, 2H), 3.93 (s, 6H), 3.91 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.6, 166.7, 166.2, 153.5 (2C), 144.5, 140.6, 131.2, 130.3, 119.5, 114.1, 107.7, 105.8 (2C), 101.0, 61.0, 56.2 (2C), 55.6. ESI-HRMS m/z: calcd for $C_{19}H_{20}O_6$ $[M-H]^-$ 343.1181, found 343.1182. Purity: 97.5% by HPLC.

*5.1.4.9.(E)-3-(4-(benzyloxy)-3-bromo-5-methoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (**5i**)*

1-(2-Hydroxy-4-methoxyphenyl)ethanone (**1**) was reacted with **7d** following the general procedure to give the desired product **5i** as a yellow solid (150 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.39 (s, 1H), 7.82 (d, *J* = 8.8 Hz, 1H), 7.74 (d, *J* = 15.4 Hz, 1H), 7.55 (d, *J* = 6.7 Hz, 2H), 7.49 (d, *J* = 1.8 Hz, 1H), 7.46 (d, *J* = 15.4 Hz, 1H), 7.42–7.33 (m, 3H), 7.08 (d, *J* = 1.8 Hz, 1H),

6.50 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.48 (d, *J* = 2.3 Hz, 1H), 5.12 (s, 2H), 3.94 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.3, 165.7, 165.3, 152.9, 146.2, 141.6, 135.8, 130.9, 130.2, 127.5 (2C), 127.3 (2C), 127.2, 123.9, 119.6, 117.7, 112.9, 110.8, 106.8, 100.1, 73.9, 55.2, 54.6. ESI-HRMS m/z: calcd for $C_{24}H_{21}O_5Br$ $[M-H]^-$ 467.0501, found 467.0494. Purity: 98.6% by HPLC.

5.2. Biological assays

5.2.1. Inhibition of Aβ (1-42) self-induced aggregation

The thioflavin-T fluorescence method was used as described by LeVine [32] with minor modification. Experiments were performed by incubating the peptides in 50 mM phosphate buffer (pH 7.4) at 37 °C for 5 days (120 h, Aβ(1-42) 40 μM) with the tested compounds at different concentrations (0, 2.5, 5, 10, 20, 40, 80 μM). After incubation, the samples were diluted to a final volume of 180 μL with 50 μM glycine-NaOH buffer (pH 8.5) containing 5 μM Thioflavin-T. Fluorescence signal was measured (excitation wavelength 450 nm, emission wavelength 485 nm and slit widths set to 5 nm) on a monochromators based multimode microplate reader (INFINITE M1000), adapted for 96-well microtiter plates. Each inhibitor was examined in triplicate. The fluorescence intensities were recorded, and the percentage of inhibition on aggregation was calculated by using the following equation: (1 – IFi / IFc) * 100% in which IFi and IFc were the fluorescence intensities obtained for absorbance in the presence and absence of inhibitors, respectively, after subtracting the background fluorescence of the 5 μM Thioflavin-T solution.

5.2.2. In vitro 5-lipoxygenase inhibition assay

The IC_{50} values of all compounds were determined using potato 5-LO (Catalog No. 60401, Cayman Chemical, Ann Arbor, MI, USA) and an enzyme immuno assay (EIA) kit (Catalog No. 760700, Cayman Chemical, Ann Arbor, MI, USA) according to manufacturer's instructions and Li

et al. [36] with minor modification.

The lipoxygenase inhibitor screening assay measured the hydroperoxides produced in the lipoxygenation reaction. Pre-assay preparation was carefully performed according to the instructions. To a 90 µL solution of 5-LO enzyme in 0.1 M Tris-HCl buffer, pH 7.4, 10 µL of various concentrations of testing drug solutions (0, 2.5, 5, 10, 25, 40, 80 µM in a final volume of 210 µL) was added, respectively. The 5-LO reaction was initiated by the addition of 10 µL substrate of linoleic acid (the final concentrations of 100 µM in the well). After putting the 96-well plate on a shaker for 5 min, 100 µL of chromogen was added, and the plate was put on the shaker for another 5 min. The 5-LO activity was determined by measuring absorbance at a wavelength of 490 nm. The percentage inhibition was calculated through the comparison of enzymatic activity with or without inhibitor. The concentration required for the tested compound to cause 50% inhibition (IC_{50}, µM) was calculated from the concentration–inhibition response curve. Each assay was repeated at least twice, and the average of measured data was reported.

5.2.3. Molecular modeling

All calculations and analysis were carried out with Molecular Operating Environment (MOE) program (Chemical Computing Group, Montreal, Canada). The X-ray crystal structure of Aβ (1-42) (PDB code 1IYT) and the human 5-LO complex (PDB code 3O8Y**)** used in the docking study were obtained from the Protein Data Bank (www.rcsb.org).

Heteroatoms and water molecules in the PDB files were removed at the beginning, and all hydrogen atoms were subsequently added to the proteins. Amber99 force field was assigned to the enzymes and the partial charges were calculated with the same force field. Protonation states of both enzymes at pH 7 were obtained by following the Protonate 3D protocol in which all

configurations were set as default. Compounds were drawn in MOE with all hydrogen atoms added. During the docking procedure, poses of compounds were initially generated by Triangle Matcher method, and scored with Affinity dG and london dG function for 5-LO and Aβ(1-42), respectively. 30 Poses of each compound were dedicated to the next refinement procedure. All poses were fine tuned with the Forcefield Refinement scheme with MMFF94x force field, and rescored with the same function. A maximum of 5 poses for each compound were preserved eventually.

Finally, some poses were separately delivered to a further restrained minimization processing in that potential hydrogen bondings might be overlooked in the rigid docking procedure.

5.2.4. CD assay

The CD assay was used as described by Chen *et al.* [37] and Yan *et al.* [38] with minor modification, and 20 μM Aβ(1-42) (Anaspec Inc) was mixed with and without 10 μM **4d** in 50 mM sodium phosphate buffer (pH 7.4). All solutions were incubated at 37 °C for 2 days. CD spectra were obtained using a Jasco-810-150S spectropolarimeter (Jasco, Japan). A quartz cell with 1 mm optical path was used, and spectra were recorded at 25 °C between 190 and 260 nm with a bandwidth of 0.5 nm, a 3 s response time, and scan speed of 10 nm/min. Background spectra and when applicable, spectra of compound **4d** were subtracted.

5.2.5. EM assay

Aβ (1-42) peptide was dissolved in 50 mM phosphate buffer (pH 7.4), which was incubated in the presence and absence of **4d** at 37 °C. The final concentrations of Aβ(1-42) and **4d** were 40 μM and 20 μM, respectively. After 4 days incubation, aliquots of 10 μL samples were placed on carbon-coated copper/rhodium grid. After 1 min, the grid was washed with water and negatively stained with 2% uranyl acetate solution for 1 min. After draining off the excess of staining solution

by means of a filter paper, the specimen was transferred for examination in a transmission electron microscope (JEOL JEM-1400).

5.2.6. Cell culture and MTT assay

SH-SY5Y cells were purchased from American Type Culture Collection (ATCC TIB71, Manassas, VA) and cultured as described by Li *et al.* [39] with minor modification. Cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum (GIBCO), 1 mM glutamine, 100 U /mL penicillin, and 100 µg/mL streptomycin under 5% CO_2 at 37 ℃ in humidified atmosphere. To measure cell viability, MTT assay was performed as described previously. SH-SY5Y cells were sub-cultured in 96-well plates at a seeding density of 10,000 cells per well. After 24 h, they were treated with different concentrations of compounds (0-100 µM). After 48 h, the survival of cells was determined with MTT assay. Briefly, 20 µl of MTT (5 mg/mL) was added to each well and incubated for 4 h. The MTT medium in each well was carefully removed and 100 µL DMSO was added into each well, followed by incubation at 37 ℃ for 10 min with horizontal shaking. The absorbance of each well was measured with a micro culture plate reader at the wavelength of 570 nm.

Acknowledgments

We thank the Natural Science Foundation of China (81273433) and Specialized Research Fund for the Doctoral Program of Higher Education of China (20110171110051) and for financial support.

Reference

[1] R. Brookmeyer, E. Johnson, K. Ziegler-Graham, H.M. Arrighi, Alzheimers Dement. 3 (2007) 186–191.

[2] C. Haass, B. De Strooper, Science. 286 (1999) 916-919.

[3] T. Hasegawa, W. Ukai, D.G. Jo, X. Xu, M.P. Mattson, M. Nakagawa, W. Araki, T. Saito, T. Yamada, J. Neurosci. Res. 80 (2005) 869-876.

[4] M. Citron, Nat. Rev. Drug. Discov. 9 (2010) 387-398.

[5] B. Sommer, Curr. Opin. Pharmacol. 2 (2002) 87-92.

[6] S. Ling, J. Zhou, J.A. Rudd, Z. Hu, M. Fang, Anat. Rec. (Hoboken). 294 (2011) 1307-1318.

[7] T. Thomas, T.G. Nadackal, K. Thomas, Neuroreport. 12 (2001) 3263-3267.

[8] J.R. Harris, Micron. 33 (2002) 609-626.

[9] M. Hirohata, K. Ono, H. Naiki, M. Yamada, Neuropharmacology. 49 (2005) 1088-1099.

[10] A.K. Mandal, J. Skoch, B.J. Bacskai, B.T. Hyman, P. Christmas, D. Miller, T.T. Yamin, S. Xu, D. Wisniewski, J.F. Evans, R.J. Soberman, P. Natl. Acad. Sci. USA. 101 (2004) 6587–6592.

[11] T. Uz, C. Pesold, P. Longone, H. Manev, FASEB J. 123 (1998) 439–449.

[12] M.D. Ikonomovic, E.E. Abrahamson, T. Uz, H. Manev, S.T. Dekosky, J. Histochem. Cytochem. 56 (2008) 1065–1073.

[13] C.M. Chinnici, Y. Yao, D. Praticò, Neurobiol. Aging. 28 (2007) 1457–1462.

[14] O. Firuzi, J. Zhuo, C.M. Chinnici, T. Wisniewski, D. Praticò, FASEB J. 22 (2008) 1169–1178.

[15] J. Chu, D. Praticò, Ann. Neurol. 69 (2011) 34–46.

[16] J. Chu, D. Praticò, J. Neuroinflamm. 9 (2012) 127.

[17] J. Chu, D. Praticò, Am. J. Pathol. 178 (2011) 1762-1769.

[18] K. Ono, T. Hamaguchi, H. Naiki, M. Yamada, BBA-Mol. Basis. Dis. 1762 (2006) 575-586.

[19] K. Matsuzaki, T. Noguch, M. Wakabayashi, K. Ikeda, T. Okada, Y. Ohashi, M. Hoshino, H. Naiki, BBA-Biomembranes. 1768 (2007) 122-130.

[20] F. Yang, G.P. Lim, A.N. Begum, O.J. Ubeda, M.R. Simmons, S.S. Ambegaokar, P.P. Chen, R. Kayed, C.G. Glabe, S.A. Frautschy, G.M. Cole, J. Biol. Chem. 280 (2005) 5892-5901.

[21] O. Werz, Planta. Med. 73 (2007) 1331-1357.

[22] M. Ono, M. Hori, M. Haratake, T. Tomiyama, H. Mori, M. Nakayama, Bioorg. Med. Chem. 15 (2007) 6388-6396.

[23] Z.P. Zhuang, M.P. Kung, C. Hou, D.M. Skovronsky, T.L. Gur, K. Plossl, J.Q. Trojanowski, V.M. Lee, H.F. Kung, J. Med. Chem. 44 (2001) 1905-1914.

[24] M. Ono, M.P. Kung, C. Hou, H.F. Kung, Nucl. Med. Biol. 29 (2002) 633-642.

[25] H.F. Kung, C.W. Lee, Z.P. Zhuang, M.P. Kung, C. Hou, K. Plossl, J. Am. Chem. Soc. 123 (2001) 12740-12741.

[26] Y.W. Chin, H.A. Jung, Y. Liu, B.N. Su, J.A. Castoro, W.J. Keller, M.A. Pereira, A.D. Kinghorn, J. Agr. Food Chem. 55 (2007) 4691-4697.

[27] J.Y. Kim, S.J. Park, K.J. Yun, Y.W. Cho, H.J. Park, K.T. Lee, Eur. J. Pharmacol. 584 (2008) 175-184.

[28] S. Tamir, M. Eizenberg, D. Somjen, S. Izrael, J. Vaya, J. Steroid Biochem. 78 (2001) 291-298.

[29] M. Baba, R. Asano, I. Takigami, T. Takahashi, M. Ohmura, Y. Okada, H. Sugimoto, T. Arika, H. Nishino, T. Okuyama, Biol. Pharm. Bull. 25 (2002) 247-250.

[30] C.K. Lee, S.H. Son, K.K. Park, J.H. Park, S.S. Lim, W.Y. Chung, Pharmacol. Sci. 106 (2008) 444-451.

[31] H.Y. Tu, A.M. Huang, T.C. Hour, S.C. Yang, Y.S. Pu, C.N. Lin, Bioorg. Med. Chem. 18 (2010) 2089-2098.

[32] H. LeVine, 3rd, Protein Sci. 2 (1993) 404-410.

[33] N.C. Gilbert, S.G. Bartlett, M.T. Waight, D.B. Neau, W.E. Boeglin, A.R. Brash, M.E. Newcomer, Science. 331 (2011) 217-219.

[34] C. Yang, X. Zhu, J. Li, R. Shi, J. Mol. Model. 16 (2010) 813-821.

[35] P. P. Bose, U. Chatterjee, C. Nerelius, T. Govender, T. Norstrom, A. Gogoll, A. Sandegren, E. Gothelid, J. Johansson, P. I. Arvidsson, J. Med. Chem. 52 (2009) 8002-8009.

[36] Y. Li, S.H. Chen, T.M. Ou, J.H. Tan, D. Li, L.Q. Gu, Z.S. Huang, Bioorg. Med. Chem. 19 (2011) 2074-2083.

[37] S.Y. Chen, Y. Chen, Y.P. Li, S.H. Chen, J.H. Tan, T.M. Ou, L.Q. Gu, Z.S. Huang, Bioorg. Med. Chem. 19 (2011) 5596-5604.

[38] J.W. Yan, Y.P. Li, W.J. Ye, S.B. Chen, J. Q. Hou, J.H. Tan , T.M. Ou, D. Li, L.Q. Gu, Z.S. Huang, Bioorg. Med. Chem. 20 (2012) 2527-2534.

[39] Y.P. Li, F.X. Ning, M.B. Yang, Y.C. Li, M.H. Nie, T.M. Ou, J.H. Tan, S.L. Huang, D. Li, L.Q. Gu, Z.S. Huang, Eur. J. Med. Chem. 46 (2011) 1572-1581.

Figure and Scheme Captions

Figure 1. The structures of NDGA, curcurmin, resveratrol, quercetin and isoliquiritigenin.

Figure 2. Binding modes of compound **4d** with 5-LO generated with MOE.

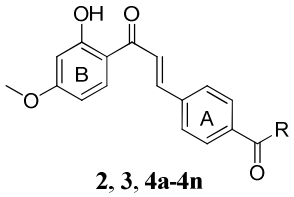
Figure 3. Binding modes of compound **4d** with Aβ(1-42) generated with MOE.

Figure 4. CD spectroscopy of Aβ(1-42) alone and with compound **4d** incubated for 0 h (A), 6 h (B), and 48 h (C).

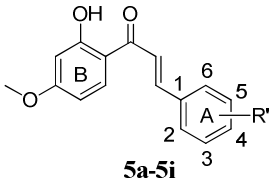
Figure 5. EM images of Aβ(1-42) (40 μM) in the absence (A) and presence (B) of 20 μM compound **4d**, after 4 days incubation at 37 ℃. Bar, 200 nm.

Scheme 1. Synthesis of intermediate aryl aldehyde **7** (**7a**-**7d**) and ISL derivatives of amides **4** (**4a**-**4n**) and ethers **5** (**5a**-**5i**). Reagents and conditions: (a) 8% KOH/MeOH, methyl 4-formylbenzoate; (b) various amines, anhydrous HOBt, EDCI; (c) 8% KOH/MeOH, various aryl aldehydes **7**: including **7a**-**7d**, 4-methoxybenzaldehyde, 2, 4, 5-trimethoxybenzaldehyde, 3, 4, 5-trimethoxybenzaldehyde; (d) K_2CO_3, DMF, 80 ℃, $PhO(CH_2)_3Br$, $PhCH_2Br$, $R_2Br = Br(CH_2)_nBr$, n =2 or 3.

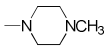
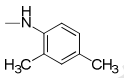
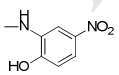
Table 1. *In vitro* inhibition of Aβ(1-42) self-aggregation and 5-LO by resveratrol (Resv), nordihydroguaiaretic acid (NDGA), isoliquiritigenin (ISL), **2**, **3**, amide compounds **4**(**4a**-**4n**), and ether compounds **5**(**5a**-**5i**).



2, **3**, **4a-4n**



5a-5i

Compds	R	IC_{50}[a] (μM)	IC_{50}[b] (μM)	Compds	R′	IC_{50}[a] (μM)	IC_{50}[b] (μM)
4a		14.6 ±1.5	10.5 ± 0.8	**5a**	3-OCH_3,4-$O(CH_2)_3OC_2H_5$	5.8 ± 0.6	12.4 ± 0.4
4b		2.2 ± 1.5	7.9 ± 0.1	**5b**	3-OCH_3, 4-$O(CH_2)_3OPh$	10.9 ±1.2	13.0 ± 0.2
4c		5.9 ± 0.3	6.8 ± 0.1	**5c**	3-$O(CH_2)_2OC_2H_5$	10.5 ± 0.8	16.8 ± 0.1
4d		3.2 ± 1.3	6.1 ± 0.1	**5d**	4-OCH_2Ph	7.8 ± 0.9	10.2 ± 0.1
4e	-$NHCH(CH_3)_2$	17.8 ± 0.6	22.1 ± 0.1	**5e**	4-OCH_3	8.4 ± 0.3	15.9 ± 0.1
4f	-NH-n-C_3H_7	19.9 ± 0.7	12.9 ±0.3	**5f**	4-$O(CH_2)_2OC_2H_5$	19.1 ± 0.1	9.7 ± 0.1
4g	-NH-n-$C_{12}H_{25}$	15.9 ± 0.5	15.7 ± 0.2	**5g**	2,4,5-tri-OCH_3	10.2 ± 1.1	14.1 ± 0.4
4h	-$NH(CH_2)_2Ph$	19.1 ± 0.9	14.2 ± 0.2	**5h**	3,4,5-tri-OCH_3	9.7 ± 0.1	10.3 ± 0.1
4i	-NHBz	18.9 ± 0.6	18.0 ± 0.1	**5i**	3-Br,4-OCH_2Ph, 5-OCH_3	12.5 ±1.7	11.6 ± 0.3
4j	-$N(CH_3)Ph$	20.1 ± 1.2	20.4 ± 0.1	**2**	R = -OCH_3	23.8 ± 2.0	35.8 ± 0.3
4k		8.3 ± 0.6	16.3 ± 0.1	**3**	R = -OH	18.5±2.6	30.3 ± 0.4
4l		7.3 ± 0.7	24.9 ± 0.4	**Resv**[c]		15.9 ± 0.5	
4m		10.1 ±1.3	16.5 ± 0.1	**NDGA**[d]			12.4 ± 1.3
4n		13.3 ± 0.8	11.4 ± 0.1	**ISL**		19.7 ± 0.8	18.6 ± 0.2

[a] Inhibitor concentration (mean ± SD of three experiments) required for 50% inhibition of self-induced Aβ(1-42) aggregation.

[b] Inhibitor concentration (mean ± SD of three experiments) required for 50% inhibition of potato 5-LO.

[c] Selectivity Positive control: Resv (Fig.1) for inhibition of self-induced Aβ(1-42) aggregation.

[d] Positive control: NDGA (Fig. 1) for 5-LO inhibition.

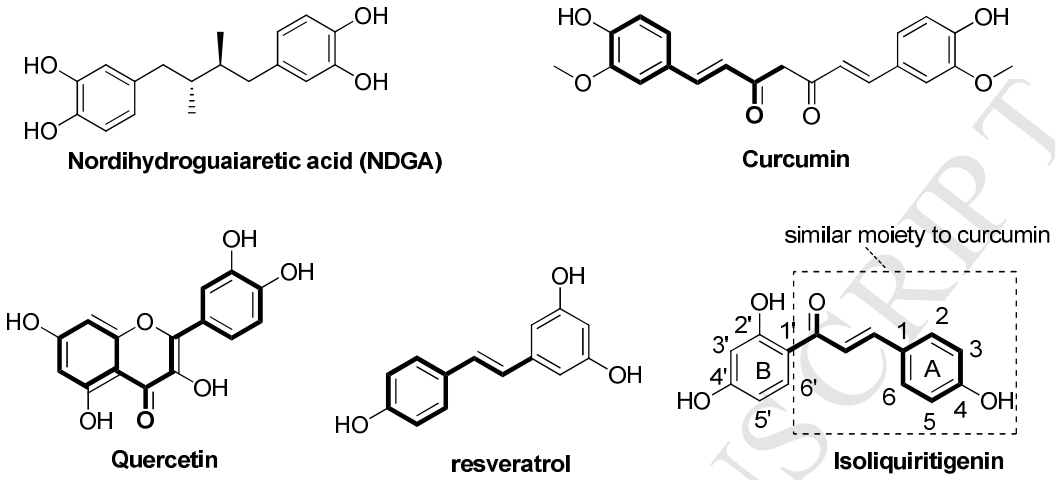


Figure 1

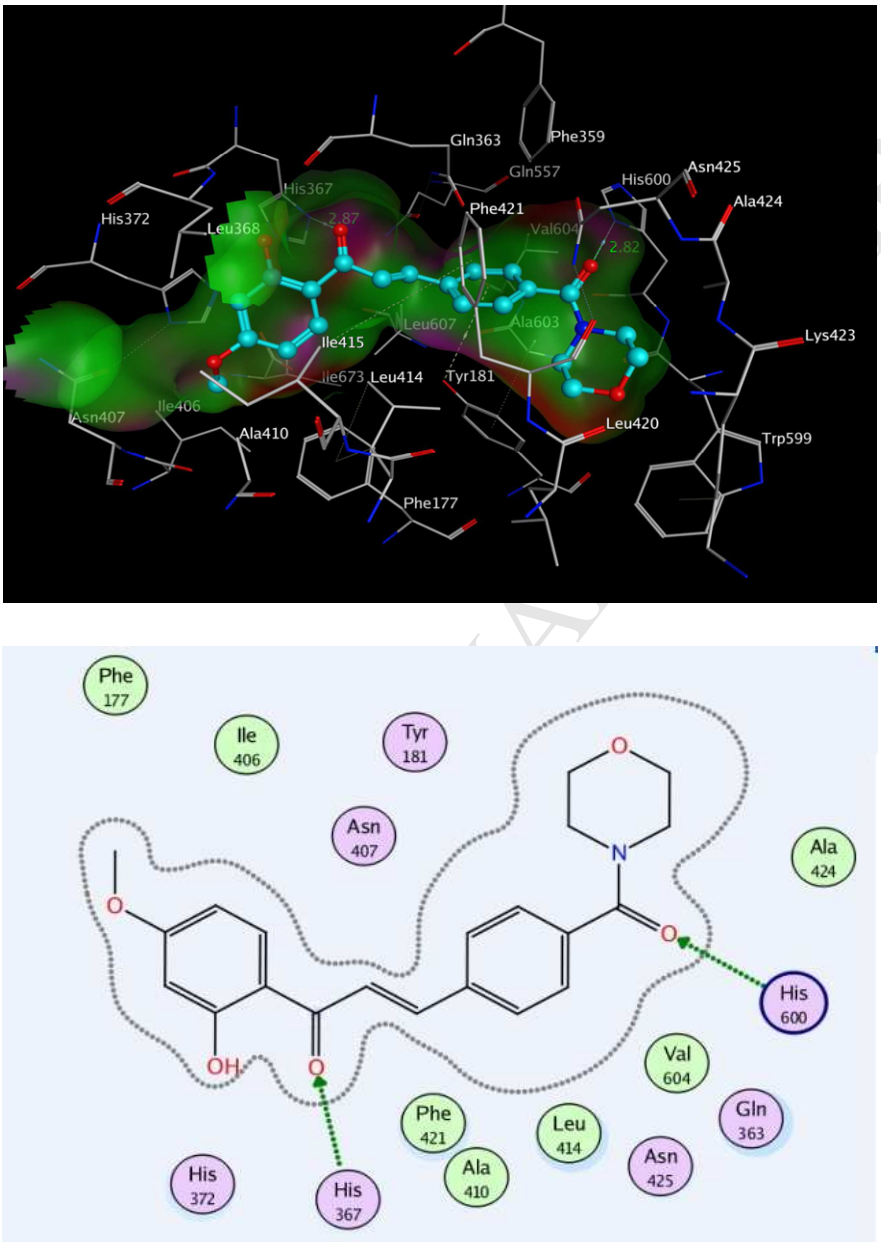
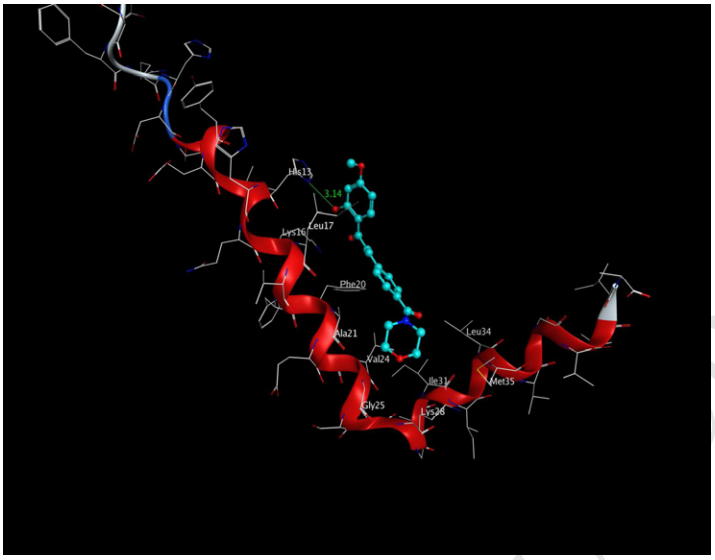

Gln363
Phe359
Gln557
His600
Asn425
His367
Ala424
His372
Leu368
2.87
Phe421
Val604
2.82
Leu607
Ala603
Lys423
Ile415
Ile673
Leu414
Tyr181
Asn407
Ile406
Ala410
Leu420
Trp599
Phe177
Phe 177
Ile 406
Tyr 181
Asn 407
Ala 424
O
N
O
O
His 600
OH
O
Val 604
Phe 421
Leu 414
Gln 363
Asn 425
His 372
His 367
Ala 410

Figure 2


His13
3.14
Leu17
Lys16
Phe20
Ala21
Val24
Leu34
Ile31
Met35
Gly25
Lys28

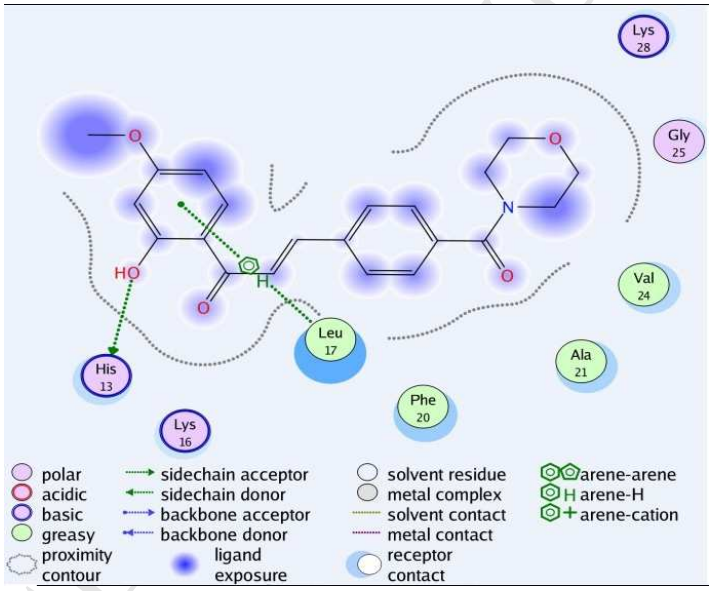

Lys 28
Gly 25
Val 24
Ala 21
Phe 20
Leu 17
His 13
Lys 16
HO
O
N
H
polar
acidic
basic
greasy
proximity contour
sidechain acceptor
sidechain donor
backbone acceptor
backbone donor
ligand exposure
solvent residue
metal complex
solvent contact
metal contact
receptor contact
arene-arene
arene-H
arene-cation

Figure 3

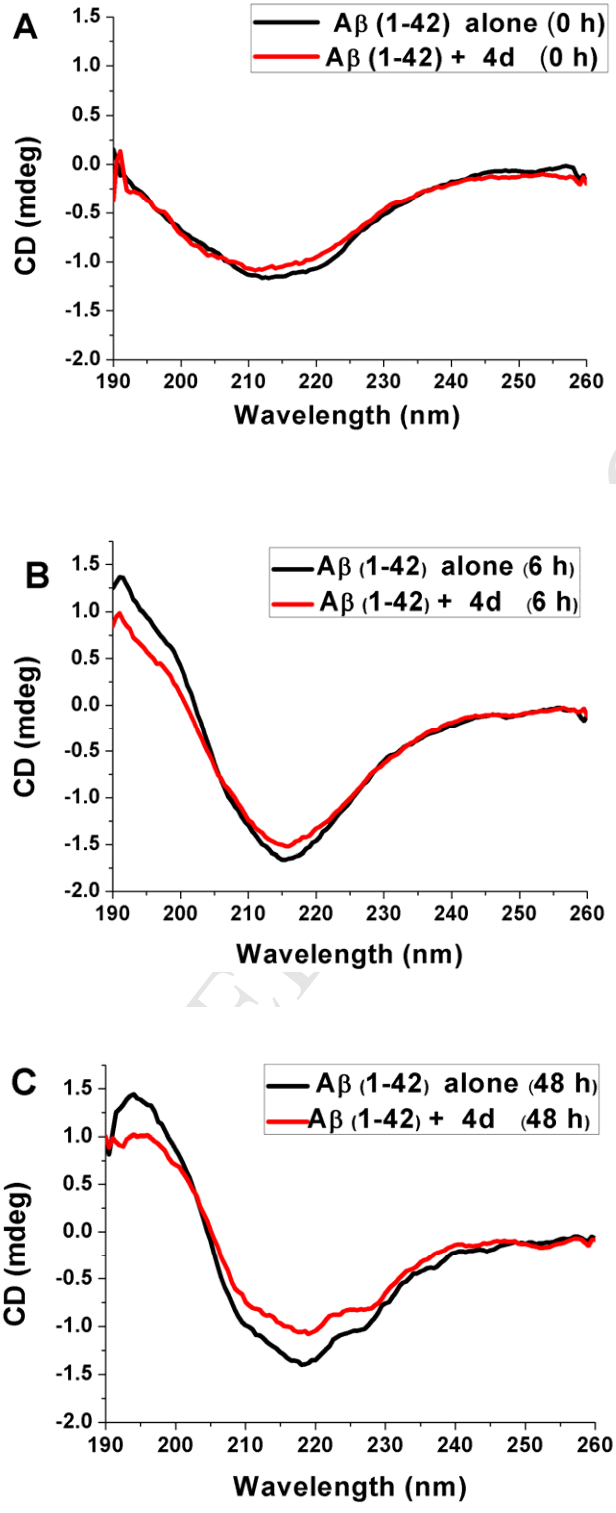

A
Aβ (1-42) alone (0 h)
Aβ (1-42) + 4d (0 h)
CD (mdeg)
Wavelength (nm)
B
Aβ (1-42) alone (6 h)
Aβ (1-42) + 4d (6 h)
CD (mdeg)
Wavelength (nm)
C
Aβ (1-42) alone (48 h)
Aβ (1-42) + 4d (48 h)
CD (mdeg)
Wavelength (nm)

Figure 4

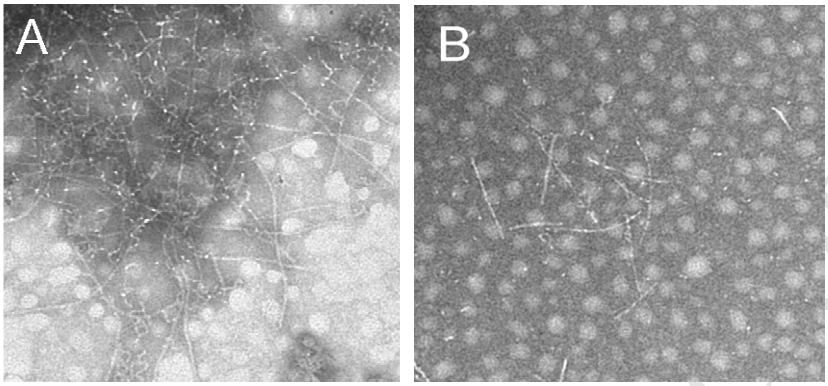
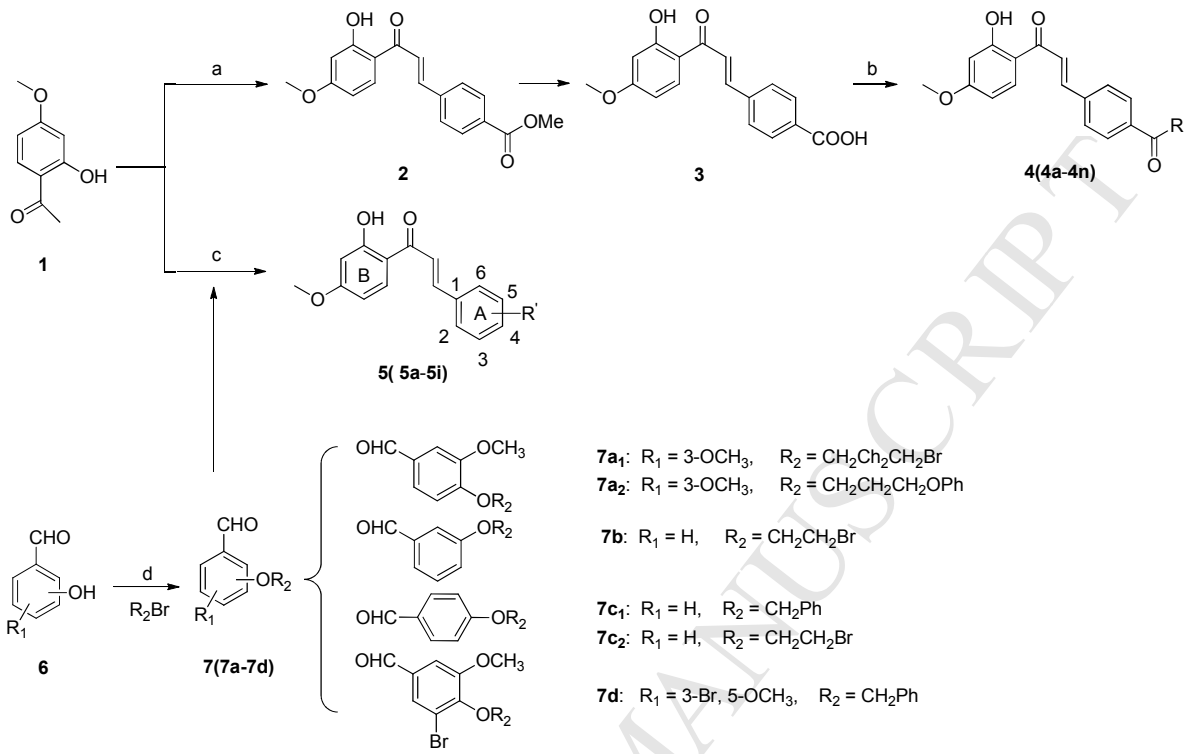


Figure 5



Scheme 1