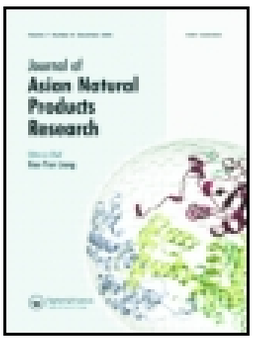


Taylor & Francis
Taylor & Francis Group

Journal of Asian Natural Products Research

ISSN: 1028-6020 (Print) 1477-2213 (Online) Journal homepage: http://www.tandfonline.com/loi/ganp20

Design, synthesis and biological evaluation of honokiol derivatives as influenza neuraminidase inhibitors

Ding Lin, Yang-Jie Yi, Meng-Wu Xiao, Jia Chen, Jiao Ye, Ai-Xi Hu, Wen-Wen Lian, Ai-Lin Liu & Guan-Hua Du

To cite this article: Ding Lin, Yang-Jie Yi, Meng-Wu Xiao, Jia Chen, Jiao Ye, Ai-Xi Hu, Wen-Wen Lian, Ai-Lin Liu & Guan-Hua Du (2018): Design, synthesis and biological evaluation of honokiol derivatives as influenza neuraminidase inhibitors, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2018.1509854

To link to this article: https://doi.org/10.1080/10286020.2018.1509854

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH
https://doi.org/10.1080/10286020.2018.1509854

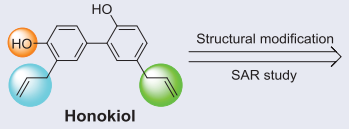
Design, synthesis and biological evaluation of honokiol derivatives as influenza neuraminidase inhibitors

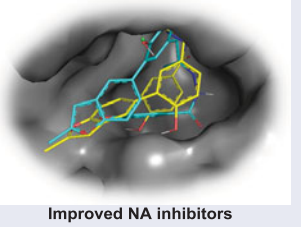
Ding Lin[a], Yang-Jie Yi[a], Meng-Wu Xiao[a], Jia Chen[a], Jiao Ye[a], Ai-Xi Hu[a], Wen-Wen Lian[b], Ai-Lin Liu[b] and Guan-Hua Du[b]

[a]College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China; [b]Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

ABSTRACT

Honokiol, a natural polyphenol, which was reported to have satisfactory influenza neuraminidase (NA) inhibitory activity, was structurally modified. Twenty-three compounds were synthesized and the *ortho*-effects in the epoxidation and hydrolyzation reactions were studied. The derivatives were evaluated for NA inhibitory activity and the benzoylhydrazone derivatives showed much better anti-NA activity than honokiol. Structure-activity relationship analysis suggested that the polyphenols exhibited better anti-NA activity than monophenols and biphenols. Furthermore, probable binding mode of drug with target revealed that the most active compound had much stronger interactions with the active site of NA than honokiol suggesting the potent anti-influenza virus activity.




Improved NA inhibitors

ARTICLE HISTORY
Received 20 May 2018
Revised 6 August 2018
Accepted 6 August 2018

KEYWORDS
Influenza; neuraminidase inhibitor; honokiol; polyphenol; structure-activity relationship

1. Introduction

Influenza is an acute viral infectious disease of the respiratory tract causing 0.25–0.5 million deaths worldwide annually [1]. Influenza virus neuraminidase (NA), an enzyme located on the viral surface, which plays a key role in the release of virions from infected host cells, has been established as a primary target for anti-influenza drugs [2]. Several NA inhibitors (NAIs), such as oseltamivir [3] and zanamivir [4], have been used in prophylaxis and clinical therapy for influenza. Nonetheless, the

CONTACT Ai-Xi Hu axhu@hnu.edu.cn; Ai-Lin Liu liuailin@imm.ac.cn

Supplemental data for this article can be accessed on the publisher's website at https://doi.org/10.1080/10286020.2018.1509854.

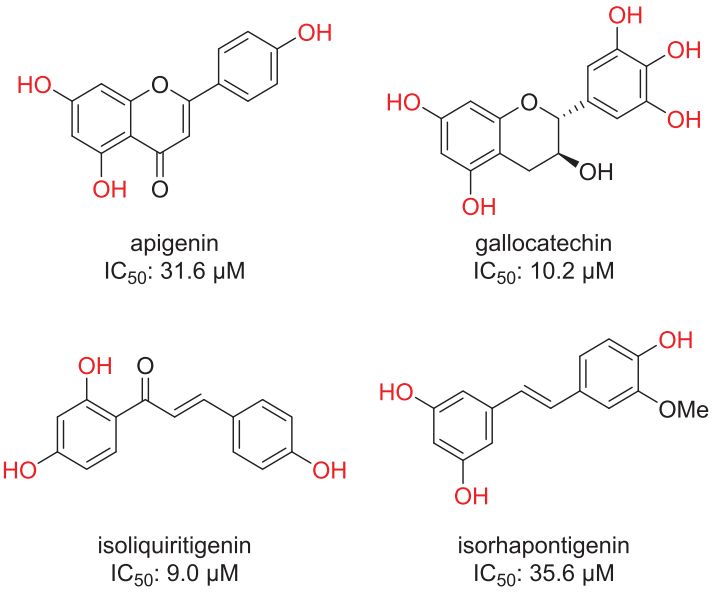


Figure 1. Natural polyphenolic NAIs.

drug resistance caused by the high mutability of influenza virus has become increasingly severe [5–7]. There is an immediate need to develop new anti-influenza agents with novel skeleton.

In recent years, a growing body of researches suggested NAIs from natural products were of great potential for the development of anti-influenza drugs. A variety of natural products have been found possessing favorable anti-NA activity, especially polyphenolic compounds (Figure 1) such as flavones [8,9], catechins [10,11], chalcones [12,13], stilbenoids [14,15], etc. However, the research of natural NAIs was blocked by the limitations of natural structure.

We hope to improve the biological activity of natural NAIs by chemical modification. Through analyzing the structure-activity relationship of natural NAIs, hydroxyl and other polar groups were considered as the essential functional groups for good NA inhibitory effects [16]. In this work, honokiol (Figure 2), one of the simplest natural polyphenol, which was reported to have satisfactory inhibitory activity against the NA of historic influenza A strain PR/8/34 and the oseltamivir-resistant strain B/55/08 [17], was chosen to be structurally modified. In order to enhance the anti-NA activity, hydroxyl and other polar groups were introduced into the structure of honokiol. The derivatives were evaluated for their anti-NA activity *in vitro* and the structure-activity relationship (SAR) was discussed.

2. Results and discussion

2.1. Chemistry

The epoxidation reaction was used to modify the allyl of honokiol. However, accidental phenomena were observed in which there were up to four new components in the

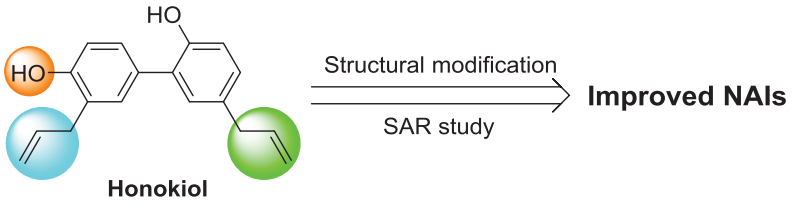


Figure 2. Structural modification of honokiol.

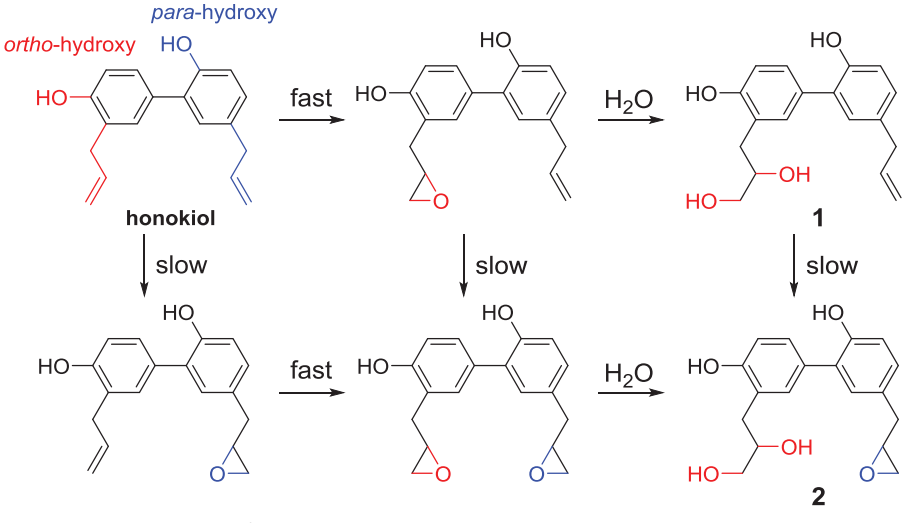


Figure 3. Reaction process of the epoxidation and hydrolyzation.

reaction mixture. By monitoring the reaction and characterization of every components, the reaction process was speculated as shown in Figure 3. Influenced by *ortho*-hydroxy, the epoxidation of allyl was much faster, and the hydrolyzation of epoxypropane was much easier which could even happen by absorbing moisture from air.

The mechanisms of *ortho*-effect in the epoxidation and hydrolyzation were assumed as described in Figure 4. The phenolic hydroxyl could form hydrogen bond with peroxy acid to promote the epoxidation occurring in the ortho-position. And the intramolecular hydrogen bond makes the epoxypropane in the ortho-position more likely to be attacked by nucleophile.

According to the above research, compounds **1–3** were prepared from honokiol via the tandem process of epoxidation and hydrolyzation by controlling the dosage of m-chloroperoxybenzoic acid and the temperature of hydrolyzation (Scheme 1). Compound **4**, a new found natural product extracted from *Magnolia officinalis* in 2013 [18] was synthesized starting from honokiol by Wacker-type intramolecular cyclization [19]. Compound **5** was obtained from **4** by Wacker oxidation [19]. And the condensation reaction of **5** with hydrazide afforded acethydrazone (**6**) and substituted benzoylhydrazones (**7–23**) containing E and Z configurations [20].

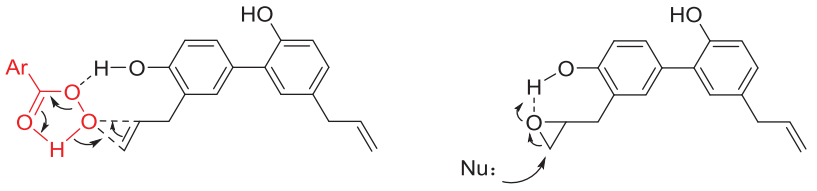
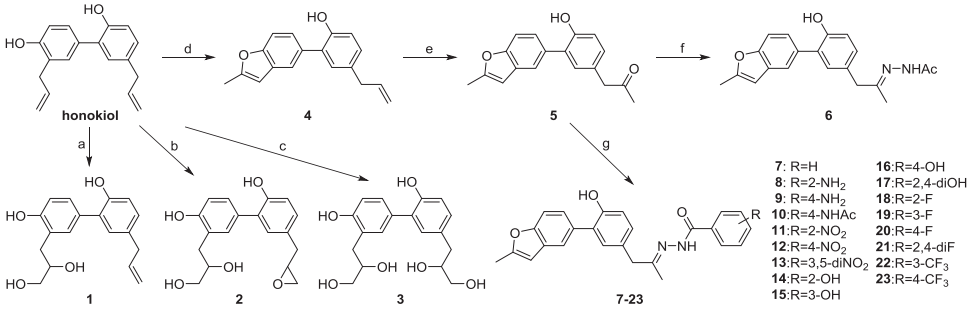


Figure 4. The mechanism of *ortho*-effect.



Scheme 1. Synthetic route of compounds **1**–**23**. (a) i: m-CPBA (2.0 eq), CH_2Cl_2, r.t.; ii: NaOH, THF/H_2O, r.t., 38%; (b) i: m-CPBA (3.5 eq), CH_2Cl_2, r.t.; ii: NaOH, THF/H_2O, r.t., 71%; (c) i: m-CPBA (3.5 eq), CH_2Cl_2, r.t.; ii: NaOH, THF/H_2O, reflux, 66%; (d) $PdCl_2$, NaOAc, O_2, DMA/H_2O, 60 °C, 86%; (e) $PdCl_2$, O_2, DMA/H_2O, 60 °C, 64%; (f) acethydrazide, HOAc, EtOH, 50 °C, 59%; (g) substituted benzoylhydrazide, HOAc, EtOH, reflux, 53–96%.

2.2. Anti-NA activity and SAR

The anti-NA efficacy of compounds **1**–**23** was preliminarily evaluated at the concentration of 40 μg/ml and the inhibition rates were presented in Figure 5. As a matter of experience, the introduction of polar groups in appropriate position may improve the anti-NA activity because it may enhance the interaction between drug and NA [21–23]. Therefore, compounds **1**–**3** were designed and synthesized, but the introduction of hydroxyl on the side chain made little difference to anti-NA activity compared with honokiol. In addition, to study the effect of phenolic hydroxyl, benzofuran derivatives **4**–**6** were synthesized and evaluated. Among them, compound **5** showed obviously better anti-NA activity than honokiol, and acetylhydrazone (**6**) exhibited the highest inhibition rate (40.87%). Acetylhydrazone (**6**) contain the structure of acylhydrazone which was reported to have potent anti-influenza and anti-NA efficiency [24–27]. Therefore, for further improving the NA inhibitory activity, benzoylhydrazones (**7**–**23**) were synthesized.

All of the benzoylhydrazones (**7**–**23**) showed better anti-NA efficiency than honokiol. Some of the benzoylhydrazones displayed more than 50% inhibitory rate against NA and they were further tested for IC_{50} values which are presented in Table 1. Among them, compound **16** exhibited the most potent anti-NA activity with an IC_{50} value of 14.87 μg/ml (35.88 μM). And it is shown that the anti-NA activity of benzoylhydrazones (**7**–**23**) was greatly influenced by the substituents. In a whole, the

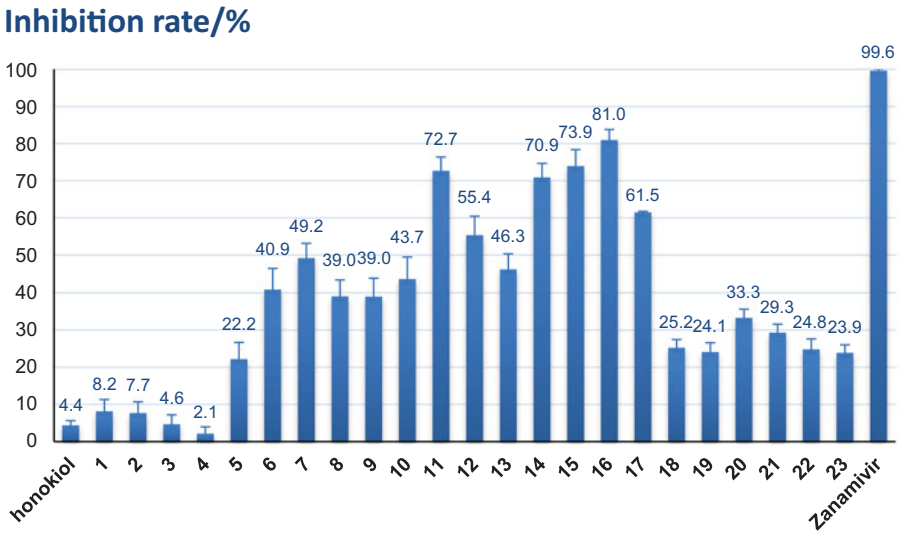
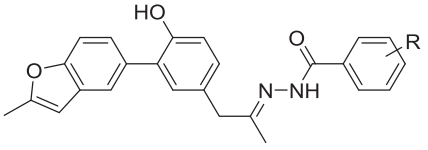


Figure 5. The inhibition rates of honokiol and compounds **7–23** against influenza NA at the concentration of 40 μg/ml using Zanamivir as positive control.

Table 1. The IC_{50} of compounds **11, 12**, and **14–17** against influenza NA.



Compd.	R	IC_{50} /μg·ml^{-1}	IC_{50} /μM
11	2-NO_2	32.97	73.35
12	4-NO_2	39.39	88.82
14	2-OH	22.56	54.43
15	3-OH	17.74	42.80
16	4-OH	14.87	35.88
17	2,4-diOH	31.28	72.67

improvement of anti-NA activity by substituents could be ranked as: $OH > NO_2 > NH_2 \approx NHAc > F \approx CF_3$.

Just like the phenomenon occurring in natural NAIs [16], the compounds with polyphenol (**13–16**) exhibited better anti-NA activity than those with monophenol (**3–12** and **17–22**). This suggested that we could introduce additional phenol unit into NAIs to enhance the activity. Furthermore, it is worth noting that unlike the low-active polyphenols **1**, **2** and honokiol, the phenols of high-active polyphenols **13–16** and those natural NAIs [28] with excellent anti-NA activity are not directly linked. It is suggested that the phenols of polyphenolic NAIs should be apart at a certain distance.

2.3. Molecular docking

To better understand the potency of active compounds and guide further structure–activity relationship studies, honokiol, the natural product without modification,

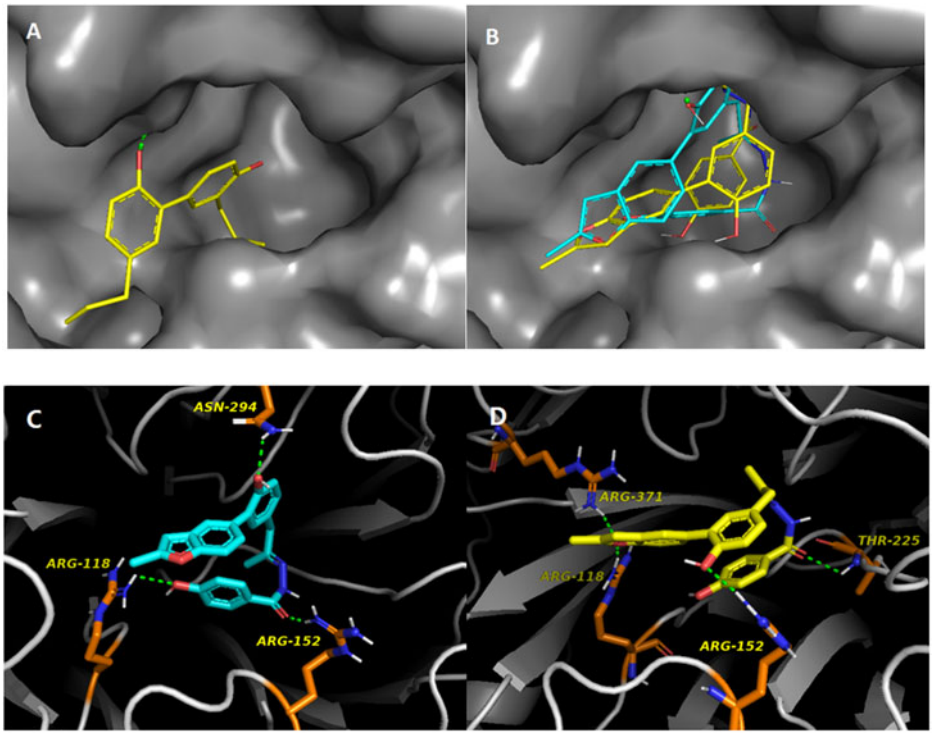


Figure 6. Comparison of the interaction models of honokiol and **16**(E/Z) with A/H1N1-NA (3TI6). A. Binding models of honokiol with NA. B–D. Binding models of **16**(E/Z) with NA.

and the most potent compound **16** were docked into the active site of NA. The predicted binding mode showed honokiol could only be attached to the entrance of the enzyme active site with a week interaction (Figure 6(A)). The possible cause for this is that the structure of honokiol is too small to occupy the active cavity or form hydrogen bonds with other amino acid residues. Compound **16** is a mixture of cis-trans isomers. It is displayed that both E and Z configurations of compound **16** are well accommodated in the catalytic cavity of NA (Figure 6(B)) and could be stabilized via interacting with NA active site by forming hydrogen bonds with several key residues (Arg118, Arg371, Arg152, Asn294, Thr-225) (Figure 3(C, D)).

3. Conclusions

In conclusion, honokiol, a natural polyphenol NAI was structurally modified and evaluated for NA inhibitory activity *in vitro*. As a result, compounds **1–23** were synthesized and the *ortho*-effects in the epoxidation and hydrolyzation were studied in detail. The derivatives containing benzoylhydrazone (**7–23**) showed much better anti-NA activity than honokiol. The polyphenolic derivatives (**14–17**) showed better anti-NA inhibitory activity than that of the monophenols (**4–13** and **18–23**) and biphenols (**1–3** and honokiol). The structure-activity relationship was discussed and the

amelioration of anti-NA activity by substituents could be ranked as: $OH > NO_2 > NH_2 \approx NHAc > F \approx CF_3$. Furthermore, molecular docking indicated that the most active compound **16** has much stronger interactions with the NA active site than that of honokiol. All of the above results gave suggestion for the future study that polyphenols are a kind of active structure of NAIs. The phenols of polyphenolic NAIs should be apart at a certain distance and we could introduce additional phenol unit into NAIs to enhance the anti-NA activity.

4. Experimental

4.1. Chemistry

All the reagents were commercially available and used directly without further purification. Melting points were determined on an X-4 binocular microscope melting point apparatus (Beijing Taike Instruments Co., Ltd., Beijing, China). ^1H and ^{13}C NMR spectra were recorded on a Bruker-400 (Bruker, Karlsruhe, Switzerland), using tetramethylsilane (TMS) as the internal standard and chemical shifts (δ) were expressed in ppm. Mass spectra were obtained by an Agilent 1100 series LC-MS (Agilent Technologies Inc., Santa Clara, CA). Elemental analyses were performed on a Vario EL III (Elementar Analysensysteme GmbH, Langenselbold, Germany).

4.2. Synthesis of compound 1

To a solution of honokiol (5 mmol) in CH_2Cl_2 (10 ml) was added m-chloroperoxybenzoic acid (75%, 10 mmol) in five portions over 8 h and kept stirring for 2 h at room temperature. Then the reaction was quenched with saturated Na_2SO_3 solution, neutralized by $NaHCO_3$ and extracted with CH_2Cl_2. The combined extract was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in THF (10 ml) and NaOH solution (1 M, 10 ml) was added. After it was stirred for 1 h at room temperature, the mixture was acidized by diluted hydrochloric acid and extracted with ethyl acetate. The organic layer was dried, concentrated, and purified by silica gel chromatography (petroleum ether/ethyl acetate = 4/1, *v/v*) to afford **1** as a brown solid. Yield 38%; m.p. 32–34 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H, Ar-OH), 7.33 (s, 1H, Ar-H), 7.21 (d, $J = 8.2$ Hz, 1H, Ar-H), 6.99 (d, $J = 1.3$ Hz, 1H, Ar-H), 6.90 (dd, $J = 8.2$, 1.3 Hz, 1H, Ar-H), 6.82 (d, $J = 8.2$ Hz, 1H, Ar-H), 6.73 (d, $J = 8.2$ Hz, 1H, Ar-H), 6.00–5.88 (m, 1H, $CH_2\underline{C}H{=}CH_2$), 5.06 (d, $J = 17.1$ Hz, 1H, $CH_2CH{=}C\underline{H}_2$), 5.01 (d, $J = 9.9$ Hz, 1H, $CH_2\underline{C}H{=}C\underline{H}_2$), 4.85–4.77 (m, 1H, $CH_2\underline{C}H(OH)CH_2OH$), 3.63–3.55 (m, 2H, $CH_2CH(OH)C\underline{H}_2OH$), 3.27 (d, $J = 6.7$ Hz, 2H, $C\underline{H}_2CH{=}CH_2$), 3.21 (dd, $J = 15.7$, 8.3 Hz, 1H, $C\underline{H}_2CH(OH)CH_2OH$), 3.00 (dd, $J = 15.7$, 8.3 Hz, 1H, $C\underline{H}_2CH(OH)CH_2OH$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.9, 150.7, 137.7, 132.1, 130.2, 129.5, 129.0, 128.7, 127.9, 127.8, 125.9, 115.5, 115.5, 109.9, 83.4, 64.8, 39.3, 31.1. LC-MS *m/z*: 301.0 $[M+H]^+$. Elemental analysis: Found: C, 71.92%, H, 6.66%; calcd for $C_{18}H_{20}O_4$, C, 71.98%, H, 6.71%.

4.3. Synthesis of compound 2

To a solution of honokiol (5 mmol) in CH_2Cl_2 (10 ml) was added m-chloroperoxybenzoic acid (75%, 17.5 mmol) in five portions over 8 h and kept stirring for 2 h at room temperature. Then the reaction was quenched with saturated Na_2SO_3 solution, neutralized by $NaHCO_3$ and extracted with CH_2Cl_2. The combined extract was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in THF (10 ml) and NaOH solution (1M, 10 ml) was added. After it was stirred at room temperature for 1 h, the mixture was acidized by diluted hydrochloric acid and extracted with ethyl acetate. The organic layer was dried, concentrated, and purified by silica gel chromatography (petroleum ether/ethyl acetate = 4/1-2/1, *v/v*) to afford **2** as a yellow solid. Yield 71%; m.p. 56–58 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H, Ar-OH), 7.25 (s, 1H, Ar-H), 7.23 (d, J = 8.2 Hz, 1H, Ar-H), 7.09 (d, J = 1.8 Hz, 1H, Ar-H), 6.99 (dd, J = 8.2, 1.8 Hz, 1H, Ar-H), 6.84 (d, J = 8.2 Hz, 1H, Ar-H), 6.74 (d, J = 8.2 Hz, 1H, Ar-H), 5.03 (br, 1H, OH), 4.85–4.78 (m, 1H, CH_2CH(OH)CH_2OH), 3.65–3.53 (m, 2H, CH_2CH(OH)CH_2OH), 3.22 (dd, J = 15.8, 8.4 Hz, 1H, CH_2CH(OH)CH_2OH), 3.12–3.05 (m, 1H, CH_2CHOCH_2), 3.01 (dd, J = 15.8, 8.4 Hz, 1H, CH_2CH(OH)CH_2OH), 2.74 (dd, J = 10.4, 5.6 Hz, 1H, CH_2CHOCH_2), 2.70 (d, J = 5.0 Hz, 1H, CH_2CHOCH_2), 2.66 (dd, J = 14.3, 5.6 Hz, 1H, CH_2CHOCH_2), 2.54 (dd, J = 5.0, 2.6 Hz, 1H, CH_2CHOCH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.7, 153.0, 131.1, 130.9, 129.0, 128.5, 128.1, 127.0, 126.2, 116.2, 116.1, 108.6, 83.9, 63.5, 52.8, 46.6, 37.7, 31.4. LC-MS *m/z*: 317.1 $[M+H]^+$. Elemental analysis: Found: C, 68.29%, H, 6.33%; calcd for $C_{18}H_{20}O_5$, C, 68.34%, H, 6.37%.

4.4. Synthesis of compound 3

To a solution of honokiol (5 mmol) in CH_2Cl_2 (10 ml) was added m-chloroperoxybenzoic acid (75%, 17.5 mmol) in five portions over 8 h and kept stirring for 2 h at room temperature. Then the reaction was quenched with saturated Na_2SO_3 solution, neutralized by $NaHCO_3$, and extracted with CH_2Cl_2. The combined extract was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in THF (10 ml) and NaOH solution (1M, 10 ml) was added. After it was stirred and refluxed for 1 h, the mixture was acidized by diluted hydrochloric acid and extracted with ethyl acetate. The organic layer was dried, concentrated, and purified by silica gel chromatography (petroleum ether/ethyl acetate = 2/1-1/1, *v/v)* to afford **3** as a yellow solid. Yield 66%; m.p. 50–52 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H, Ar-OH), 7.35 (s, 1H, Ar-H), 7.23 (d, J = 8.2 Hz, 1H, Ar-H), 7.05 (s, 1H, Ar-H), 6.94 (dd, J = 8.2, 1.9 Hz, 1H, Ar-H), 6.81 (d, J = 8.2 Hz, 1H, Ar-H), 6.73 (d, J = 8.2 Hz, 1H, Ar-H), 5.14 (s, 2H, OH), 4.86–4.77 (m, 1H, CH_2CH(OH)CH_2OH), 3.88–3.78 (m, 1H, CH_2CH(OH)CH_2OH), 3.61–3.58 (m, 2H, CH_2CH(OH)CH_2OH), 3.55 (dd, J = 11.0, 5.0 Hz, 1H, CH_2CH(OH)CH_2OH), 3.46 (dd, J = 11.0, 5.0 Hz, 1H, CH_2CH(OH)CH_2OH), 3.22 (dd, J = 15.8, 8.4 Hz, 1H, CH_2CH(OH)CH_2OH), 3.00 (dd, J = 15.8, 8.4 Hz, 1H, CH_2CH(OH)CH_2OH), 2.72 (dd, J = 13.6, 6.3 Hz, 1H, CH_2CH(OH)CH_2OH), 2.61 (dd, J = 13.6, 6.3 Hz, 1H, CH_2CH(OH)CH_2OH); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.6, 152.7, 131.5, 131.1, 129.3, 128.9, 128.9, 127.9, 127.0, 126.2, 116.0, 108.6, 83.9, 71.7, 63.5, 49.8, 31.4. LC-MS *m/z*: 335.1 $[M+H]^+$.

Elemental analysis: Found: C, 64.71%, H, 6.61%; calcd for $C_{18}H_{22}O_6$, C, 64.66%, H, 6.63%.

4.5. Synthesis of compound 4

A mixture of honokiol (10 mmol), NaOAc (1 mmol) and $PdCl_2$ (0.15 mmol) in the co-solvent of DMA and H_2O (35 ml, v/v = 6:1) was stirred under O_2 (0.8 Mpa) at 60 °C for 16 h. After cooling, the mixture was diluted with water and extracted with ethyl acetate. The combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was then purified by silica gel chromatography (petroleum ether/ethyl acetate = 8/1, *v/v*) to afford **4** as light yellow oil. Yield 86%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, J = 1.5 Hz, 1H, benzofuran 4-H), 7.50 (d, J = 8.4 Hz, 1H, benzofuran 7-H), 7.26 (dd, J = 8.4, 1.5 Hz, 1H, benzofuran 6-H), 7.10–7.07 (m, 2H, C_6H_3 3,5-H), 6.94 (d, 1H, J = 8.8 Hz, C_6H_3 6-H), 6.41 (s, 1H, benzofuran 3-H), 6.04–5.93 (m, 1H, $CH_2\underline{CH}{=}CH_2$), 5.21 (s, 1H, OH), 5.12–5.04 (m, 2H, $CH_2CH{=}\underline{CH_2}$), 3.36 (d, J = 6.7 Hz, 2H, $\underline{CH_2}CH{=}CH_2$), 2.49 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.6, 154.4, 151.1, 138.1, 132.3, 131.8, 130.8, 130.2, 129.0, 128.8, 124.4, 120.9, 116.0, 115.8, 111.3, 102.9, 39.6, 14.2. LC-MS *m/z*: 265.1 $[M+H]^+$. Elemental analysis: Found: C, 81.85%, H, 6.08%; calcd for $C_{18}H_{16}O_2$, C, 81.79%, H, 6.10%.

4.6. Synthesis of compound 5

A mixture of **4** (10 mmol) and $PdCl_2$ (0.15 mmol) in the co-solvent of DMA and H_2O (40 ml, v/v = 4:1) was stirred under O_2 (0.8 Mpa) at 60 °C for 16 h. After cooling, the mixture was diluted with water and extracted with ethyl acetate. The combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was then purified by silica gel chromatography (petroleum ether/ethyl acetate = 4/1, *v/v*) to afford **5** as a white powder. Yield 64%; m.p. 118–120 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53–7.48 (m, 2H, benzofuran 4,7-H), 7.26–7.24 (m, 1H, benzofuran 6-H), 7.10–7.07 (m, 2H, Ar-H), 6.97 (d, 1H, J = 7.8 Hz, Ar-H), 6.41 (s, 1H, benzofuran 3-H), 5.35 (br, 1H, OH), 3.66 (s, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.18 (s, 3H, $COCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.0, 158.3, 157.1, 154.7, 132.8, 131.2, 130.5, 130.1, 129.5, 129.3, 123.9, 120.7, 116.3, 111.8, 102.7, 50.2, 29.2, 14.2. LC-MS *m/z*: 280.9 $[M+H]^+$. Elemental analysis: Found: C, 77.07%, H, 5.79%; calcd for $C_{18}H_{16}O_3$, C, 77.12%, H, 5.75%.

4.7. Synthesis of compound 6

To a solution of **5** (0.5 mmol) and acethydrazide (1.5 mmol) in EtOH (20 ml) acetic acid (0.2 ml) was added. After stirred at 50 °C for 5 h, the reaction was concentrated, poured into saturated salt water, shocked, and placed until precipitation. Then the precipitate was separated by filtration, washed with water, and dried to get **6** as a light yellow solid. Yield 59%; m.p. 92–95 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58–7.51 (m, 3H, Ar-H), 7.10–6.94 (m, 3H, Ar-H), 6.43–6.41 (m, 1H, Ar-H), 5.70 (br, 1H, OH), 5.38 (br, 1H, OH), 3.68–3.51 (m, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.49 (s, 1H,

CH_3), 2.35 (s, 2H, CH_3); LC-MS *m/z*: 337.0 $[M+H]^+$. Elemental analysis: Found: C, 71.34%, H, 5.96%, N, 8.35%; calcd for $C_{20}H_{20}N_2O_3$, C, 71.41%, H, 5.99%, N, 8.33%.

4.8. Synthesis of substituted benzoylhydrazide

Substituted benzoylhydrazide was prepared according to the literature protocol [29–33].

4.9. General procedure for the synthesis of substituted benzoylhydrazone (7–23)

To a solution of **5** (0.5 mmol) and substituted benzoylhydrazide (0.55 mmol) in EtOH (20 ml) acetic acid (0.2 ml) was added. After stirred and refluxed for 5 h, the reaction was concentrated, poured into saturated salt water, shocked, and placed until precipitation. Then the precipitate was separated by filtration, washed with water, and dried to afford substituted benzoylhydrazone (**7–23**).

4.9.1. Compound 7

White solid. Yield 90%; m.p. 99–101 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87–7.73 (m, 2H, Ar-H), 7.53–7.44 (m, 5H, Ar-H), 7.26–6.93 (m, 4H, Ar-H), 6.40 (s, 1H, Ar-H), 5.56 (br, 1H, OH), 3.78–3.62 (m, 2H, CH_2), 2.48 (s, 3H, CH_3), 2.18–1.88 (m, 3H, CH_3); ^{13}C NMR (101 MHz, Acetone-d_6) δ 164.4, 156.8, 155.0, 154.1, 149.3, 135.2, 134.2, 133.6, 132.5, 131.4, 130.3, 130.0, 129.7, 129.4, 128.6, 127.7, 125.9, 122.0, 117.6, 117.4, 110.8, 103.8, 45.2, 15.9, 14.1. LC-MS *m/z*: 399.2 $[M+H]^+$. Elemental analysis: Found: C, 75.30%, H, 5.54%, N, 6.99%; calcd for $C_{25}H_{22}N_2O_3$, C, 75.36%, H, 5.57%, N, 7.03%.

4.9.2. Compound 8

Light yellow solid. Yield 53%; m.p. 91–93 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (br, 1H), 7.70–7.60 (m, 1H, Ar-H), 7.52–7.26 (m, 4H, Ar-H), 7.17–6.82 (m, 5H, Ar-H), 6.60–6.54 (m, 1H, Ar-H), 3.67–3.47 (m, 2H, CH_2), 2.45 (s, 3H, CH_3), 1.87–1.69 (m, 3H, CH_3); ^{13}C NMR (101 MHz, Acetone-d_6) δ 163.3, 162.2, 161.6, 156.9, 155.0, 153.9, 134.4, 134.3, 132.8, 132.7, 132.6, 132.5, 130.3, 130.2, 129.8, 129.7, 125.9, 122.1, 122.0, 117.3, 110.9, 103.9, 45.2, 16.5, 14.2. LC-MS *m/z*: 414.1 $[M+H]^+$. Elemental analysis: Found: C, 72.70%, H, 5.64%, N, 10.14%; calcd for $C_{25}H_{23}N_3O_3$, C, 72.62%, H, 5.61%, N, 10.16%.

4.9.3. Compound 9

Light yellow solid. Yield 71%; m.p. 111–113 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99–9.97 (m, 1H, NH), 9.39 (br, 1H, OH), 7.67–7.56 (m, 3H, Ar-H), 7.50–7.46 (m, 1H, Ar-H), 7.41–7.36 (m, 1H, Ar-H), 7.16 (s, 1H, Ar-H), 7.03 (d, $J=7.5$ Hz, 1H, Ar-H), 6.90 (d, $J=7.5$ Hz, 1H, Ar-H), 6.62–6.52 (m, 3H, Ar-H), 5.71 (br, 2H, NH_2), 3.72–3.50 (m, 2H, CH_2), 2.45 (s, 3H, CH_3), 2.11–1.86 (m, 3H, CH_3); ^{13}C NMR (101 MHz, Acetone-d_6) δ 164.8, 162.8, 156.9, 155.0, 154.1, 133.1, 131.1, 130.8, 130.5, 130.3, 130.2, 129.8, 127.5, 127.3, 126.0, 125.9, 122.0, 117.6, 117.2, 114.1, 110.9, 103.8,

50.3, 16.7, 14.2. LC-MS *m/z*: 414.0 $[M+H]^+$. Elemental analysis: Found: C, 72.59%, H, 5.57%, N, 10.22%; calcd for $C_{25}H_{23}N_3O_3$, C, 72.62%, H, 5.61%, N, 10.16%.

4.9.4. Compound 10

Light yellow solid. Yield 84%; m.p. 127–129 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H, NH), 10.21–10.17 (m, 1H, NH), 9.39 (s, 1H, OH), 7.88–7.78 (m, 2H, Ar-H), 7.69–7.63 (m, 3H, Ar-H), 7.51–7.46 (m, 1H, Ar-H), 7.41–7.36 (m, 1H, Ar-H), 7.16 (s, 1H, Ar-H), 7.04 (d, $J = 8.0$ Hz, 1H, Ar-H), 6.91 (d, $J = 8.0$ Hz, 1H, Ar-H), 6.62–6.57 (m, 1H, Ar-H), 3.74–3.56 (m, 2H, CH_2), 2.45 (s, 3H, CH_3), 2.08 (s, 1H, CH_3), 2.07 (s, 3H, CH_3), 1.88 (s, 2H, CH_3). LC-MS *m/z*: 456.2 $[M+H]^+$. Elemental analysis: Found: C, 71.24%, H, 5.54%, N, 9.17%; calcd for $C_{27}H_{25}N_3O_4$, C, 71.19%, H, 5.53%, N, 9.22%.

4.9.5. Compound 11

Yellow solid. Yield 63%; m.p. 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 7.72 (d, $J = 7.4$ Hz, 1H, Ar-H), 7.67–7.64 (m, 1H, Ar-H), 7.63–7.58 (m, 1H, Ar-H), 7.51 (s, 1H, Ar-H), 7.49 (d, $J = 6.5$ Hz, 1H, Ar-H), 7.28 (dd, $J = 6.5$, 2.0 Hz, 1H, Ar-H), 7.21 (dd, $J = 8.3$, 1.7 Hz, 1H, Ar-H), 6.93 (d, $J = 8.3$ Hz, 1H, Ar-H), 6.42 (s, 1H, Ar-H), 5.55 (br, 1H), 5.30 (br, 1H), 3.68–3.27 (m, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.18–1.81 (m, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 156.9, 154.7, 154.5, 147.4, 145.5, 133.7, 130.9, 130.5, 130.3, 130.2, 129.7, 129.3, 128.8, 127.9, 126.0, 124.0, 123.5, 120.6, 116.2, 111.6, 102.7, 44.3, 14.8, 14.2. LC-MS *m/z*: 444.1 $[M+H]^+$. Elemental analysis: Found: C, 67.65%, H, 4.73%, N, 9.53%; calcd for $C_{25}H_{21}N_3O_5$, C, 67.71%, H, 4.77%, N, 9.48%.

4.9.6. Compound 12

Yellow solid. Yield 95%; m.p. 99–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.99–7.92 (m, 2H, Ar-H), 7.54–7.47 (m, 2H, Ar-H), 7.29–7.25 (m, 1H, Ar-H), 7.10 (s, 1H, Ar-H), 7.06 (m, 1H, Ar-H), 7.00–6.93 (m, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 3.79–3.65 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.18–1.92 (m, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 156.8, 154.4, 151.8, 149.8, 143.5, 131.4, 131.1, 130.9, 130.4, 130.2, 129.7, 129.4, 128.5, 128.2, 124.0, 123.9, 122.7, 120.7, 116.1, 111.4, 102.7, 44.4, 14.7, 14.2. LC-MS *m/z*: 444.2 $[M+H]^+$. Elemental analysis: Found: C, 67.63%, H, 4.77%, N, 9.55%; calcd for $C_{25}H_{21}N_3O_5$, C, 67.71%, H, 4.77%, N, 9.48%.

4.9.7. Compound 13

Light yellow solid. Yield 96%; m.p. 105–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17–9.04 (m, 3H, Ar-H), 7.53–7.45 (m, 2H, Ar-H), 7.24–7.18 (m, 1H, Ar-H), 7.10–7.02 (m, 2H, Ar-H), 6.97–6.91 (m, 1H, Ar-H), 6.40 (s, 1H, Ar-H), 3.83–3.52 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.19–1.97 (m, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.4, 156.8, 155.3, 154.4, 151.8, 147.8, 131.4, 131.1, 130.5, 130.2, 129.7, 129.4, 128.8, 124.0, 120.5, 116.0, 111.4, 102.6, 44.6, 15.0, 14.1. Elemental analysis: Found: C, 61.45%, H, 4.17%, N, 11.44%; calcd for $C_{25}H_{20}N_4O_7$, C, 61.47%, H, 4.13%, N, 11.47%.

4.9.8. Compound 14

White solid. Yield 82%; m.p. 113–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.51 (m, 2H, Ar-H), 7.50–7.42 (m, 2H, Ar-H), 7.28–7.26 (m, 1H, Ar-H), 7.10 (s, 1H, Ar-H), 7.08–7.03 (m, 2H, Ar-H), 6.97 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.93–6.88 (m, 1H, Ar-H), 6.44–6.40 (s, 1H, Ar-H), 5.63 (br, 1H), 5.27 (br, 1H), 3.76–3.63 (m, 2H, CH_2), 3.51 (br, 1H, OH), 2.50 (s, 3H, CH_3), 2.49–2.18 (m, 3H, CH_3); LC-MS *m/z*: 415.0 $[M + H]^+$. Elemental analysis: Found: C, 72.51%, H, 5.37%, N, 6.71%; calcd for $C_{25}H_{22}N_2O_4$, C, 72.45%, H, 5.35%, N, 6.76%.

4.9.9. Compound 15

White solid. Yield 68%; m.p. 117–119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.48 (m, 3H, Ar-H), 7.40 (s, 1H, Ar-H), 7.33 (d, $J = 7.6$ Hz, 1H, Ar-H), 7.26–7.24 (m, 1H, Ar-H), 7.17–7.07 (m, 2H, Ar-H), 7.03 (d, $J = 8.5$ Hz, 1H, Ar-H), 6.97 (d, $J = 8.5$ Hz, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 5.60 (br, 1H), 5.28 (br, 1H), 3.73–3.65 (m, 2H, CH_2), 3.52 (br, 1H), 2.49 (s, 3H, CH_3), 2.17–1.88 (m, 3H, CH_3); ^{13}C NMR (101 MHz, Acetone-d_6) δ 164.6, 164.2, 157.0, 155.1, 148.9, 136.5, 134.7, 133.7, 131.3, 130.6, 130.4, 130.3, 130.2, 128.6, 127.9, 125.9, 122.1, 119.4, 115.6, 111.0, 110.9, 103.9, 50.3, 15.9, 14.2. LC-MS *m/z*: 415.1 $[M + H]^+$. Elemental analysis: Found: C, 72.53%, H, 5.40%, N, 6.72%; calcd for $C_{25}H_{22}N_2O_4$, C, 72.45%, H, 5.35%, N, 6.76%.

4.9.10. Compound 16

White solid. Yield 77%; m.p. 134–136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87–7.68 (m, 2H, Ar-H), 7.55–7.47 (m, 3H, Ar-H), 7.32–7.28 (m, 1H, Ar-H), 7.15–7.07 (m, 2H, Ar-H), 7.01–6.89 (m, 2H, Ar-H), 6.42 (s, 1H, Ar-H), 5.28 (br, 1H), 3.72–3.66 (m, 2H, CH_2), 3.53 (br, 1H, OH), 2.50 (s, 3H, CH_3), 2.18 (s, 3H, CH_3). LC-MS *m/z*: 415.1 $[M + H]^+$. Elemental analysis: Found: C, 72.43%, H, 5.31%, N, 6.75%; calcd for $C_{25}H_{22}N_2O_4$, C, 72.45%, H, 5.35%, N, 6.76%.

4.9.11. Compound 17

Yellow solid. Yield 59%; m.p. 116–118 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, $J = 1.8$ Hz, 1H, Ar-H), 7.74 (dd, $J = 8.4$, 1.8 Hz, 1H, Ar-H), 7.56 (d, $J = 1.8$ Hz, 1H, Ar-H), 7.54–7.47 (m, 3H, Ar-H), 7.29 (dd, $J = 8.4$, 1.8 Hz, 1H, Ar-H), 7.07 (d, $J = 8.4$ Hz, 1H, Ar-H), 6.95–6.85 (m, 1H, Ar-H), 6.43 (s, 1H, Ar-H), 5.70 (br, 1H), 5.33 (br, 1H), 3.80–3.52 (m, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.18–1.78 (m, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.2, 160.7, 157.4, 156.6, 155.9, 155.7, 153.4, 133.8, 132.5, 131.3, 129.8, 128.8, 128.6, 128.2, 125.7, 124.8, 120.9, 116.6, 116.3, 115.1, 110.1, 103.0, 43.8, 16.5, 13.9. LC-MS *m/z*: 431.0 $[M + H]^+$. Elemental analysis: Found: C, 69.69%, H, 5.11%, N, 6.48%; calcd for $C_{25}H_{22}N_2O_5$, C, 69.76%, H, 5.15%, N, 6.51%.

4.9.12. Compound 18

White solid. Yield 77%; m.p. 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79–7.72 (m, 1H, Ar-H), 7.56–7.47 (m, 4H, Ar-H), 7.24–7.21 (m, 2H, Ar-H), 7.10–6.93 (m, 3H, Ar-H), 6.41 (s, 1H, Ar-H), 5.74 (br, 1H), 5.42 (br, 1H), 3.88–3.46 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.18 (s, 1H, CH_3), 1.89 (s, 2H, CH_3). LC-MS *m/z*: 417.0 $[M + H]^+$.

Elemental analysis: Found: C, 72.03%, H, 5.12%, N, 6.68%; calcd for $C_{25}H_{21}N_2O_3F$, C, 72.10%, H, 5.08%, N, 6.73%.

4.9.13. Compound 19

White solid. Yield 87%; m.p. 91–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.43 (m, 4H, Ar-H), 7.26 (dd, $J = 8.2$, 1.6 Hz, 1H, Ar-H), 7.20–7.12 (m, 2H, Ar-H), 7.10 (s, 1H, Ar-H), 7.09–7.00 (m, 1H, Ar-H), 6.98–6.94 (m, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 5.34 (br, 1H), 3.77–3.61 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.18–1.89 (m, 3H, CH_3). LC-MS *m/z*: 417.1 $[M+H]^+$. Elemental analysis: Found: C, 72.16%, H, 5.04%, N, 6.77%; calcd for $C_{25}H_{21}N_2O_3F$, C, 72.10%, H, 5.08%, N, 6.73%.

4.9.14. Compound 20

White solid. Yield 67%; m.p. 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.46 (m, 4H, Ar-H), 7.24 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.15–7.09 (m, 3H, Ar-H), 7.07–6.93 (m, 2H, Ar-H), 6.41 (s, 1H, Ar-H), 5.73 (br, 1H), 5.40 (br, 1H), 3.85–3.45 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.18–1.88 (m, 3H, CH_3). LC-MS *m/z*: 417.1 $[M+H]^+$. Elemental analysis: Found: C, 72.17%, H, 5.09%, N, 6.80%; calcd for $C_{25}H_{21}N_2O_3F$, C, 72.10%, H, 5.08%, N, 6.73%.

4.9.15. Compound 21

White solid. Yield 74%; m.p. 85–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31–8.16 (m, 1H, Ar-H), 7.54–7.46 (m, 2H, Ar-H), 7.25–7.19 (m, 1H, Ar-H), 7.16 (s, 1H, Ar-H), 7.10–6.94 (m, 3H, Ar-H), 6.93–6.84 (m, 1H, Ar-H), 6.40 (s, 1H, Ar-H), 5.46 (br, 1H), 3.80–3.58 (m, 2H, CH_2), 2.48 (s, 3H, CH_3), 2.17–1.88 (m, 3H, CH_3). LC-MS *m/z*: 435.0 $[M+H]^+$. Elemental analysis: Found: C, 69.19%, H, 4.59%, N, 6.48%; calcd for $C_{25}H_{20}N_2O_3F_2$, C, 69.12%, H, 4.64%, N, 6.45%.

4.9.16. Compound 22

Light yellow solid. Yield 86%; m.p. 85–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17–7.93 (m, 2H, Ar-H), 7.78–7.67 (m, 1H, Ar-H), 7.55–7.42 (m, 3H, Ar-H), 7.28–7.20 (m, 1H, Ar-H), 7.12–6.90 (m, 3H, Ar-H), 6.37 (s, 1H, Ar-H), 5.76 (br, 1H), 3.70–3.46 (m, 2H, CH_2), 2.46 (s, 3H, CH_3), 2.16–1.88 (m, 2H, CH_3). LC-MS *m/z*: 466.1 $[M+H]^+$. Elemental analysis: Found: C, 67.02%, H, 4.57%, N, 5.95%; calcd for $C_{26}H_{21}N_2O_3F_3$, C, 66.95%, H, 4.54%, N, 6.01%.

4.9.17. Compound 23

White solid. Yield 90%; m.p. 95–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–7.85 (m, 2H, Ar-H), 7.72 (d, $J = 7.5$ Hz, 2H, Ar-H), 7.53–7.48 (m, 2H, Ar-H), 7.27–7.23 (m, 1H, Ar-H), 7.18–7.13 (m, 1H, Ar-H), 7.11–7.06 (m, 1H, Ar-H), 6.99–6.93 (m, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 5.46 (br, 1H), 3.78–3.64 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.18–1.90 (m, 3H, CH_3). LC-MS *m/z*: 466.0 $[M+H]^+$. Elemental analysis: Found: C, 67.00%, H, 4.48%, N, 6.06%; calcd for $C_{26}H_{21}N_2O_3F_3$, C, 66.95%, H, 4.54%, N, 6.01%.

4.10. Anti-NA activity assay

All the target compounds and natural compound honokiol were tested for their anti-NA activity *in vitro*. The NA inhibition assay was performed according to the standard method [14]. 2'-(4-Methylumbelliferyl)-α-D-acetyl neuraminic acid (MUNANA) was used as a substrate of NA. The cleavage of MUNANA by NA could produce a fluorescent product, which would emit a fluorescence of 450 nm under the irradiation of excitation wavelength of 360 nm. The degree of fluorescence can reflect the activity of NA sensitively. In the enzyme reaction system, a certain concentration of the target compounds with the NA enzyme was suspended in MES buffer (pH = 6.5). The reaction was started by adding the substrate MUNANA. After the reaction was incubated for 40 min at 37 °C, it was terminated by adding sodium hydroxide solution. The plate was read within 20 min under the parameters of excitation wavelength at 360 nm and emission wavelength at 450 nm. The inhibition rates of the tested compounds were obtained according to the decrement of the fluorescence intensity. The IC_{50} (the concentration required for 50% inhibition) was calculated by plotting the percent of inhibition of NA activity versus the inhibitor concentration.

4.11. Docking study

Molecular docking was performed by AutoDock (version 4.2 [34] with default parameters, The Scripps Research Institute and Olson Laboratory, http://autodock.scripps.edu/). The crystal structure data of H1N1 neuraminidase oseltamivir complex (PDB Code: 3TI6) is downloaded from RSCB Protein Data Bank. The conformational energy of docked compound was minimized by MM2 force field. The protein and ligand were prepared in the standard manner using AutoDock Tools (version 1.5.6, The Scripps Research Institute and Olson Laboratory, http://autodock.scripps.edu/) [34]. A grid was centered on the catalytic active site [–28.60, 14.23, 21.11]. The dimension of binding box was set as 46 × 38 × 48 grid points with the points separated by 0.375 Å. The results were analyzed and visualized by PyMOL (http://www. pymol.org/).

Acknowledgments

The authors are thankful to the Institute of Materia Medica of Chinese Academy of Medical Sciences and Peking Union Medical College for providing the biological activity assays.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the National Natural Science Fundation of China (No. 21442014) and the National Natural Science Foundation of China (No. 81673480).

References

[1] J. R. Ortiz, J. A. Englund, and K. M. Neuzil, *Vaccine* **29**, 4439–4452 (2011).
[2] M. von Itzstein, *Nat Rev Drug Discov*. **6**, 967–974 (2007).
[3] F. J. Meng, T. Sun, W. Z. Dong, M. H. Li, and Z. Z. Tuo, *Arch. Pharm. (Weinheim)* **349**, 168–174 (2016).
[4] A. O. H. El-Nezhawy, A. F. Eweas, I. A. Maghrabi, A. S. Edalo, and S. F. Abdelwahab, *Arch. Pharm. (Weinheim)* **348**, 786–795 (2015).
[5] Y. Wu, F. Gao, J. Qi, Y. Bi, L. Fu, S. Mohan, Y. Chen, X. Li, B. M. Pinto, C. J. Vavricka, P. Tien, and G. F. Gao, *J. Virol.* **90**, 10693–10700 (2016).
[6] M. Okomo-Adhiambo, K. Sleeman, K. Ballenger, H. T. Nguyen, V. P. Mishin, T. G. Sheu, J. Smagala, Y. Li, A. I. Klimov, and L. V. Gubareva, *Viruses* **2**, 2269–2289 (2010).
[7] T. G. Sheu, V. M. Deyde, M. Okomo-Adhiambo, R. J. Garten, X. Xu, R. A. Bright, E. N. Butler, T. R. Wallis, A. I. Klimov, and L. V. Gubareva, *Antimicrob. Agents Chemother*. **52**, 3284–3292 (2008).
[8] J. Kirchmair, J. M. Rollinger, K. R. Liedl, N. Seidel, A. Krumbholz, and M. Schmidtke, *Future Med Chem*. **3**, 786–450 (2011).
[9] A. G. Mercader and A. B. Pomilio, *Eur. J. Med. Chem*. **45**, 1724–1730 (2011).
[10] X. Li, T. Ohtsuki, S. Shindo, M. Sato, T. Koyano, S. Preeprame, T. Kowithayakorn, and M. Ishibash, *Planta Med*. **73**, 1195–1196 (2007).
[11] K. Id, Y. Kawasaki, K. Kawakami, and H. Yamada, *Curr. Med. Chem*. **23**, 4773–4783 (2016).
[12] Y. B. Ryu, J. H. Kim, S. J. Park, J. S. Chang, M. C. Rho, K. H. Bae, K. H. Park, and W. S. Lee, *Bioorg. Med. Chem. Lett*. **20**, 437–974 (2010).
[13] T. T. Dao, P. H. Nguyen, H. S. Lee, E. Kim, J. Park, S. I. Lim, and W. K. Oh, *Bioorg. Med. Chem. Lett*. **21**, 294–298 (2011).
[14] A. L. Liu, F. Yang, M. Zhu, D. Zhou, M. Lin, S. M. Y. Lee, Y. T. Wang, and G. H. Du, *Planta Med*. **76**, 1874–1876 (2010).
[15] P. H. Nguyen, M. K. Na, T. T. Dao, D. T. Ndinteh, J. T. Mbafor, J. Park, H. Cheong, and W. K. Oh, *Bioorg. Med. Chem. Lett*. **20**, 6430–6434 (2010).
[16] U. Grienke, M. Schmidtke, S. V. Grafenstein, J. Kirchmair, K. R. Liedl, and J. M. Rollinger, *Nat. Prod. Rep*. **29**, 11–36 (2012).
[17] U. Grienke, H. Braun, N. Seidel, J. Kirchmair, M. Richter, A. Krumbholz, S. von Grafenstein, K. R. Liedl, M. Schmidtke, and J. M. Rollinger, *J. Nat. Prod*. **77**, 563–570 (2014).
[18] W. L. Kuo, C. Y. Chung, T. L. Hwang, and J. J. Chen, *Phytochemistry* **85**, 153–160 (2013).
[19] T. Mitsudome, T. Umetani, N. Nosaka, K. Mori, T. Mizugaki, K. Ebitani, and K. Kaneda, *Angew. Chem. Int. Ed. Engl*. **45**, 481–485 (2006).
[20] H. M. Abdel-Rahman, M. Abdel-Aziz, H. N. Tinsley, B. D. Gary, J. C. Canzoneri, and G. A. Piazza, *Arch. Pharm. (Weinheim)* **349**, 104–111 (2016).
[21] K. Yuan, M. Xiao, Y. Tan, J. Ye, Y. Xie, X. Sun, A. Hu, W. Lian, and A. Liu, *Mol. Divers*. **21**, 565–576 (2017).
[22] Y. Fang, M. Xiao, A. Hu, J. Ye, W. Lian, and A. Liu, *Chin. J. Chem*. **34**, 403–411 (2016).
[23] Z. Wu, J. Peng, A. Hu, J. Ye, and G. Li, *Med. Chem. Res*. **25**, 481–368 (2016).
[24] S. R. Shih, T. Y. Chu, G. Reddy, S. N. Tseng, H. L. Chen, W. F. Tang, M. S. Wu, J. Y. Yeh, Y. S. Chao, J. Hsu, H. P. Hsieh, and J. T. Horng, *J. Biomed. Sci*. **17**, 13(2010).
[25] J. J. Jablonski, D. Basu, D. A. Engel, and H. M. Geysen, *Bioorg. Med. Chem*. **20**, 487–497 (2012).
[26] S. Barman, L. You, R. Chen, V. Codrea, G. Kago, R. Edupuganti, J. Robertus, R. M. Krug, and E. V. Anslyn, *Eur. J. Med. Chem*. **71**, 81–90 (2014).
[27] M. Xiao, J. Ye, W. Lian, M. Zhang, B. Li, A. Liu, and A. Hu. *Med. Chem. Res*. **26**, 3216–3227 (2017).

[28] F. Liu, W. Cao, C. Deng, Z. Wu, G. Zeng, and Y. Zhou, *Chem. Cent. J.* **10**, 51–61 (2016).
[29] F. Palace-Berl, S. D. Jorge, K. F. M. Pasqualoto, A. K. Ferreira, D. A. Maria, R. R. Zorzi, L. D. S. Bortolozzo, J. Â. L. Lindoso, and L. C. Tavares, *Bioorg. Med. Chem.* **21**, 5395–5406 (2013).
[30] G. L. Backes, D. M. Neumann, and B. S. Jursic, *Bioorg. Med. Chem.* **22**, 4629–4636 (2014).
[31] L. Li, H. Ding, B. Wang, S. Yu, Y. Zou, X. Chai, and Q. Wu, *Bioorg. Med. Chem. Lett.* **24**, 192–194 (2014).
[32] S. M. Hashemi, H. Badali, M. A. Faramarzi, N. Samadi, M. H. Afsarian, H. Irannejad, and S. Emami, *Mol. Divers.* **19**, 15–27 (2015).
[33] I. Chaaban, E. S. M. E. Khawass, H. A. A. E. Razik, N. S. E. Salamouni, M. Redondo-Horcajo, I. Barasoain, J. F. Díaz, J. Yli-Kauhaluoma, and V. M. Moreira, *Arch. Pharm. (Weinheim)* **349**, 749–761 (2016).
[34] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, and A. J. Olson, *J. Comput. Chem.* **30**, 2785–2791 (2009).