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RESEARCH ARTICLE

Synthesis and biological evaluation of novel 3-substituted amino-4-hydroxylcoumarin derivatives as chitin synthase inhibitors and antifungal agents

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Abstract

A series of novel 3-substituted amino-4-hydroxycoumarin derivatives have been designed and synthesized as chitin synthase (CHS) inhibitors. All the synthesized compounds have been screened for their CHS inhibition activity and antimicrobial activity *in vitro*. The enzymatic assay indicated that most of the compounds have good inhibitory activity against CHS, in which compound **6o** with IC_{50} of 0.10 mmol/L had stronger activity than that of polyoxins B, which acts as control drug with IC_{50} of 0.18 mmol/L. As far as the antifungal activity is concerned, most of the compounds possessed moderate to excellent activity against some representative pathogenic fungi. Especially, compound **6b** was found to be the most potent agent against *Cryptococcus neoformans* with minimal inhibitory concentration (MIC) of 4 μg/mL. Moreover, the results of antibacterial screening showed that these compounds have negligible actions to some tested bacteria. Therefore, these compounds would be promising to develop selective antifungal agents.

Keywords

Antifungal agent, chitin synthase, chitin synthase inhibitor, 3-substituted amino-4-hydroxycoumarin

History

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Introduction

In the past decades, as the major causes of high morbidity and mortality rates in patients who received antineoplastic chemotherapy, organ transplants or suffered AIDS, the invasive fungal infections have become a more and more alarming problem to be settled[1]. The treatment of fungal infections, particularly those caused by drug-resistant fungal pathogens, is often complicated by high toxicity, low tolerability or narrow spectrum of activity[2–4]. Furthermore, the representative antifungal drugs which were used in clinic at present have certain limitation. For instance, azoles exhibit broad antifungal spectrum and are vulnerable to resistance; adverse side effects of amphotericin B are infusional toxicity, nephrotoxicity; flucytosine is restricted to pathogenic yeasts and fungal strains resistant to echinocandins have emerged[5–7]. Consequently, antifungal agents of new molecular scaffolds with high efficiency, broad spectrum and low toxicity are highly desirable.

Chitin is widely distributed in invertebrates, the cuticles of arthropod exoskeletons and the fungal cell walls[8], but it is absent in plants and humans[9,10]. It is a linear polysaccharide chain that is composed of thousands of *N*-acetyl-D-glucosamine (GlcNAc) residues joined by β-1,4-glycosidic bonds[11,12]. The biosynthesis of chitin is catalyzed by chitin synthase (CHS) which uses the uridine diphosphoryl-*N*-acetyl-D-glucosamine (UDP-GlcNAc) as the substrate donor to provide the GlcNAc (Figure 1)[13,14]. Thus, the CHS signifies an ideal target for the development of pesticides and antifungal agents[15]. Although many efforts have been made to discover the CHS inhibitors in the past decades, not much progress has been made. Such inhibitors possess the potential to inhibit the biosynthesis of chitin efficiently. The nucleoside polyoxins and nikkomycins, the representative competitive CHS inhibitors[16] isolated from culture filtrates of *Streptomyces* strains, possess some of the structural features of the natural substrate UDP-GlcNAc and can interfere with the synthesis of fungal cell wall and ecdysis of insect *in vivo* by inhibiting the CHS. The inhibition constants (K_i) of nucleoside polyoxins are in range of 0.1–1 μM[17,18]. The low inhibitory activity and weak efficacy of these compounds maybe due to the degradation of their dipeptide side chains in the organism. Many analogues of nikkomycins and polyoxins were designed and developed, but none of them has entered in clinical trials[19–21]. Therefore, new CHS inhibitors will be needed.

Coumarin is chemically known as 2*H*-chromen-2-one heterocycle containing an oxygen atom[22]. The structural type of coumarin enables its derivatives to interact readily with all kinds of enzymes and receptors in organisms through weak bond interactions. So they exhibit wide potentiality as medicinal drugs[23] with various biological and pharmacological activities such as antibacterial, antifungal[24], antioxidant[25],

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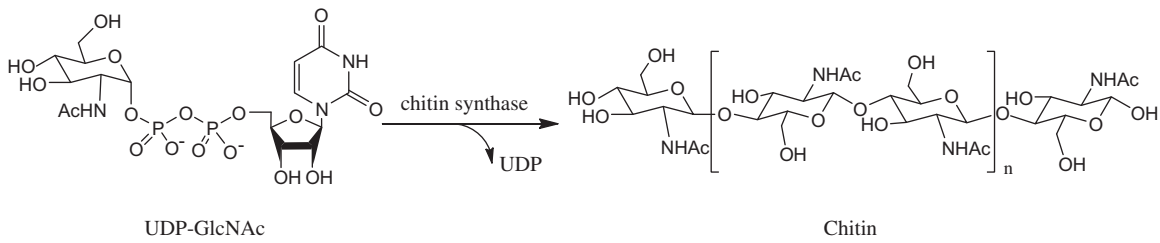
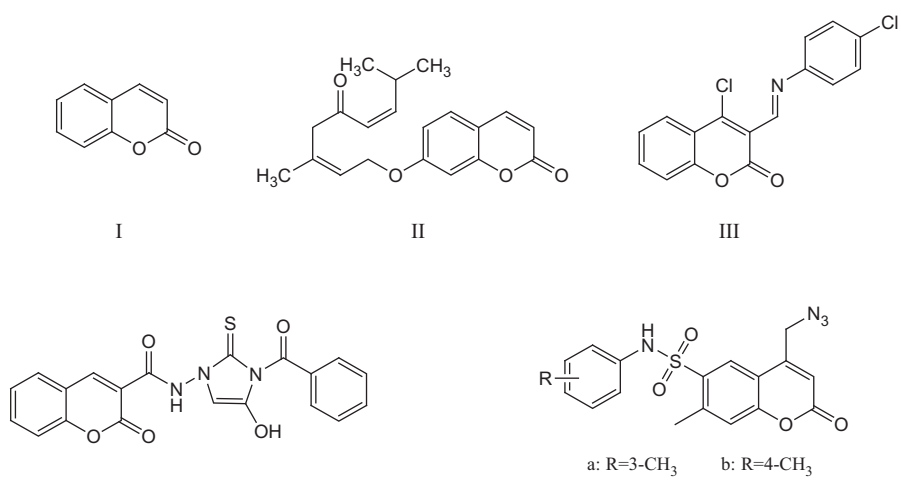


Figure 1. The biosynthesis of chitin.

Figure 2. Some reported antimicrobial agents containing coumarin moiety.



anti-inflammatory[26], analgesic[27], anticancer[28], anthelmintic[29], anti-HIV[30] and antiviral[31] activities. In recent studies, the coumarin heterocycles (Figure 2), as antimicrobial agents, have attracted extensive interest. For instance, the 7-substituted coumarin (**II**) showed a moderate efficacy (minimal inhibitory concentration, MIC = 125 μg/mL) against *Cryptococcus neoformans*[32]; the 4-chloro-3-phenylimino coumarin (**III**) exhibited moderate antifungal activity (MIC = 15 μg /mL) against *Aspergillus niger* and *Candida albicans* in comparison with fluconazole[33]; the Imidazolethione coumarin hydrazide derivatives (**IV**) had good antibacterial activity against *Escherichia coli* with inhibitory zone diameter of 32 mm in 25 μg/mL[34] and the 4-azidomethyl coumarin sulfonamides derivatives (**V**) showed excellent antifungal efficacies against *C. albicans, A. niger* and *Fusarium oxysporum* with MIC values of 1–4 μg/mL, which were 2–8 times more potent than fluconazole (MIC = 8 μg/mL)[35]. Thus, the coumarin is a versatile fragment used to design the novel compounds with pharmacological activity.

In continuation of previous study for developing new CHS inhibitors[37], in this work we focused our attention on 3-substituted amino-4-hydroxycoumarin derivatives bearing an *N*-phenylpiperazine group on L-tartaric amide side chain. We expected that substituting the 3-position of the coumarin with a linear moiety which resembles roughly the side chain of polyoxins B and Nikkomycin Z (Figure 3) would lead to novel derivatives of coumarin which possess broad-spectrum antifungal activity and CHS inhibition activity. The flexible and different substituents were introduced to the coumarin ring or the aryl of phenylpiperazine in order to investigate their bioactivity and to draw the structure–activity relationship of these derivatives. Thus, we report herein synthesis and biological evaluation of the 3-substituted amino-4-hydroxycoumarin derivatives as novel CHS inhibitors.

Experimental protocols

Chemistry

All the reagents, solvents and instruments used in this article are produced in China unless indicated. All reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel plates. The melting points were determined by X-6 melting point apparatus without correction. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AV 300 MHz spectrometer (Switzerland) using $CDCl_3$ or DMSO-d_6 as solvents and tetramethylsilane (TMS) as an internal standard. The chemical shifts are expressed in δ, ppm. The mass spectra were recorded using Agilent single quadrupole liquid chromatogram–mass spectrograph (LC–MS) (Santa Clara, CA). Highresolution mass spectra were determined by Finnigan/AMT95 (San Jose, CA).

General procedure for the synthesis of compounds

*Synthesis of diethyl-L-tartarate (**1**)*

To a solution of L-tartaric acid (10.00 g, 66.67 mmol) and EtOH (100 mL), $SOCl_2$ (10.7 mL, 146.7 mmol) was added dropwise in an ice bath. The mixture was stirred at room temperature for 24 h. Then the solvent was evaporated *in vacuum* and CH_2Cl_2 (30 mL) was added to the mixture. The resulting mixture was washed with saturated aqueous $NaHCO_3$ and the aqueous layer was extracted with CH_2Cl_2 (30 mL). The combined organic extracts were washed with brine and dried by anhydrous Na_2SO_4. After filtering, the organic layer was concentrated *in vacuum*. The residue was purified by flash column chromatography on silica gel eluting with ethyl acetate–petroleum ether (P.E.) (1:5) to give the diethyl-L-tartarate (**1**) (12.6 g, yield, 91%) as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.54 (s, 2H, CH), 4.33 (q, J = 7.1 Hz, 4H, CH_2), 3.07 (s, 2H, OH), 1.34 (t, J = 7.1 Hz, 6H, CH_3).

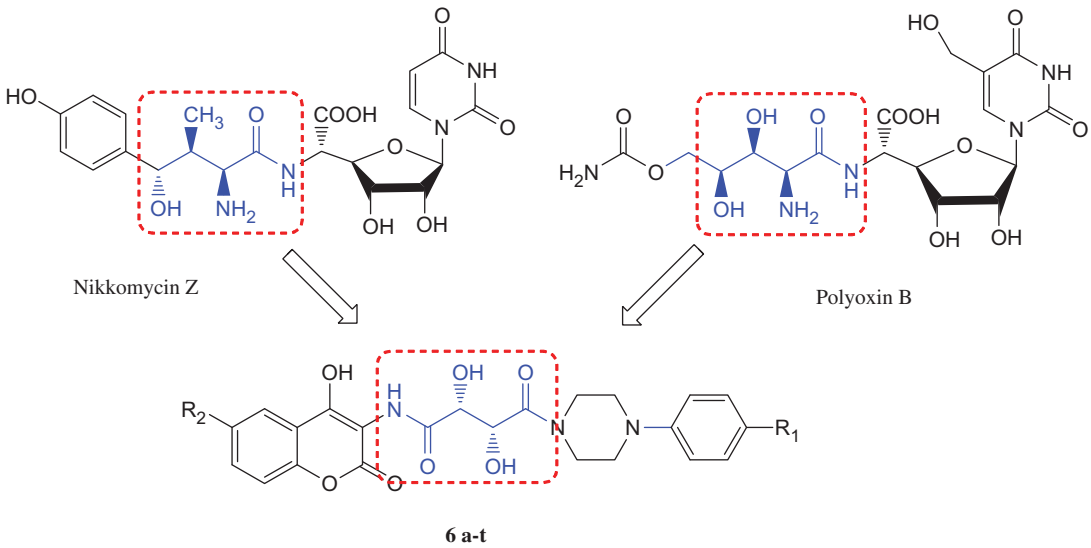


Figure 3. The structures of polyoxin B, Nikkomycin Z and designed compounds.

*Synthesis of L-(4*R*,5*R*)-2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylic acid monoethyl ester (**2**)*

To a solution of diethyl-L-tartrate (**1**) (26.4 g, 128 mmol) and *p*-TsOH·H_2O (0.3 g) in dry benzene (300 mL) 2,2-dimethoxypropane (20 g, 192 mmol) was added and the mixture was stirred for 4 h at 80 °C. After cooling to room temperature, the resulting mixture was washed with saturated aqueous $NaHCO_3$ and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine and water. Then solution was dried by anhydrous Na_2SO_4. After being filtered, the solution was concentrated *in vacuum* to give diethyl(2*R*,3*R*)-2,3-*O*-isopropylidenetartrate (29.8 g, yield, 95%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.81 (d, $J = 4.2$ Hz, 1H, CH), 4.79 (d, $J = 4.8$ Hz, 1H, CH), 4.29 (q, $J = 7.1$ Hz, 4H, CH_2), 1.50 (s, 6H, C–CH_3), 1.32 (t, $J = 7.1$ Hz, 6H, CH_3). To a solution of diethyl(2*R*,3*R*)-2,3-*O*-isopropylidenetartrate (10 g, 40.5 mmol), distilled water (100 mL) and 1,4-dioxine (100 mL) the NaOH solution (1 mol/L, 43 mL) was added dropwise in half hour. The mixture was stirred at room temperature for 2–3 h and extracted with CH_2Cl_2 (200 mL). The aqueous layer was acidized with concentrated hydrogen chloride to pH 2 and extracted with CH_2Cl_2 (200 mL). The combining organic solution was dried by anhydrous Na_2SO_4. After being filtered, the solution was concentrated *in vacuum*. The residue was purified by flash column chromatography on silica gel eluting with ethyl acetate–P.E. (1:5, 1:1) to give the desired product **2** (7.3 g, yield, 83%) as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.07 (s, 1H, COOH), 4.88 (d, $J = 5.2$ Hz, 1H, CH), 4.81 (d, $J = 5.4$ Hz, 1H, CH), 4.31 (q, $J = 7.1$ Hz, 2H, CH_3CH_2O), 1.53 (s, 3H, C–CH_3), 1.51 (s, 3H, C–CH_3), 1.33 (t, $J = 7.1$ Hz, 3H, CH_3CH_2O).

*General procedure for preparation of the 1-(4-substituted phenyl)piperazine (**4a–e**)*

The mixture of 4-substituted aniline (10 g, 78.4 mmol), bis-(2-chloroethylamine)hydrochloride (14.7 g, 82.4 mmol) and *para*-toluenesulphonic acid (PTSA) (0.5, 3%) in xylene (44 mL) was heated to reflux at 140–145 °C for 12–24 h. When the reaction was completed, the mixture was cooled to room temperature to crystallize. The crystal was filtrated and recrystallized in the distilled water to give the desired product with yield 73–85%.

*General procedure of synthesizing intermediate **5***

L-(4*R*,5*R*)-2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylic acid monoethyl ester (**2**) (5 g, 22.9 mmol) was dissolved in 30 mL CH_2Cl_2, then *N*-hydroxybenzotriazole (3.1 g, 22.9 mmol), 4-dimethylaminopyridine (0.25 g, 2.2 mmol) and *N*,*N*-dicyclohexylcarbodiimide (5.2 g, 25.2 mmol) were added in the solution. The mixture was stirred in room temperature for 0.5 h and 1-(4-subsituted-phenyl)piperazine (**4**) (18.4 mmol) was added in 15 mL CH_2Cl_2 and then stirred for 20 h in room temperature. After the reaction was completed, the mixture was filtered. The solution was washed with saturated aqueous $NaHCO_3$ and 1 mol/L hydrochloric acid, and dried by anhydrous Mg_2SO_4. After being filtered, the solution was concentrated *in vacuum*. The residue was purified by flash column chromatography on silica gel eluting with ethyl acetate–P.E. (1:5) to give product **5**.

*L-(4*R*,5*R*)-5-(4-(4-fluorophenyl)piperazine-1-carbonyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid (**5a**).* Grey liquid; yield 54%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.18 (s, 1H, COOH), 7.04 (d, $J = 8.1$ Hz, benzene-3,5-2H), 6.82 (d, $J = 8.1$ Hz, benzene-2.6-2H), 5.27 (d, $J = 6.0$ Hz, 1H, CH), 4.94 (d, $J = 6.0$ Hz, 1H, CH), 3.81 (s, 4H, piperazine), 3.27 (s, 4H, piperazine), 1.52 (s, 3H, CH_3), 1.44 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.48, 168.22, 155.83, 147.70, 126.75, 117.12, 112.65, 76.43, 75.65, 49.85, 49.37, 45.52, 42.78, 26.24.

*L-(4*R*,5*R*)-5-(4-(4-chlorophenyl)piperazine-1-carbonyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid (**5b**).* White solid; yield 40%; mp 120–121 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.27 (s, 1H, COOH), 7.24 (d, $J = 8.9$ Hz, benzene-3,5-2H), 6.86 (d, $J = 8.9$ Hz, benzene-2.6-2H), 5.25 (d, $J = 6.0$ Hz, 1H, CH), 4.90 (d, $J = 6.0$ Hz, 1H, CH), 3.96 (t, $J = 12.1$ Hz, 2H, piperazine), 3.73 (t, $J = 18.0$ Hz, 2H, piperazine), 3.30–3.17 (m, 2H, piperazine), 3.10 (dd, $J = 19.6$, 8.3 Hz, 2H, piperazine), 1.51 (s, 3H, CH_3), 1.43 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.28, 167.12, 149.20, 129.13, 125.75, 118.00, 112.97, 76.33, 75.65, 49.86, 49.27, 45.51, 42.58, 26.25.

*L-(4*R*,5*R*)-5-(4-(4-bromophenyl)piperazine-1-carbonyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid (**5c**).* Yellow solid; yield 67%; mp 129–130 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.15 (s, 1H, COOH), 7.31 (d, $J = 7.8$ Hz, benzene-3,5-2H), 6.75

(d, $J = 7.8$ Hz, benzene-2.6-2H), 5.20 (d, $J = 6.0$ Hz, 1H, CH), 4.99 (d, $J = 6.0$ Hz, 1H, CH), 3.89 (t, $J = 13.6$ Hz, 2H, piperazine), 3.68 (t, $J = 18.5$ Hz, 2H, piperazine), 3.27 (s, 2H, piperazine), 3.10 (t, $J = 19.0$ Hz, 2H, piperazine), 1.50 (s, 3H, CH_3), 1.44 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.27, 167.32, 150.12, 127.33, 118.75, 118.10, 112.96, 76.43, 75.65, 49.87, 49.26, 45.49, 42.57, 26.23.

*L-(4R,5R)-5-(4-(4-methylphenyl)piperazine-1-carbonyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid (**5d**).* Light yellow solid; yield 45%; mp 125–127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.27 (s, 1H, COOH), 7.13 (d, $J = 8.2$ Hz, 2H, benzene), 6.95 (d, $J = 8.2$ Hz, 2H, benzene), 5.23 (d, $J = 6.1$ Hz, 1H, CH), 4.90 (d, $J = 6.1$ Hz, 1H, CH), 4.10–3.90 (m, 2H, piperazine), 3.90–3.77 (m, 2H, piperazine), 3.26 (m, 2H, piperazine), 3.21–3.05 (m, 2H, piperazine), 2.30 (s, 3H, benzene–CH_3), 1.52 (s, 3H, C–CH_3), 1.45 (s, 3H, C–CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.78, 167.55, 149.22, 130.14, 125.75, 115.70, 112.67, 76.37, 75.65, 49.75, 49.32, 45.49, 42.54, 26.21.

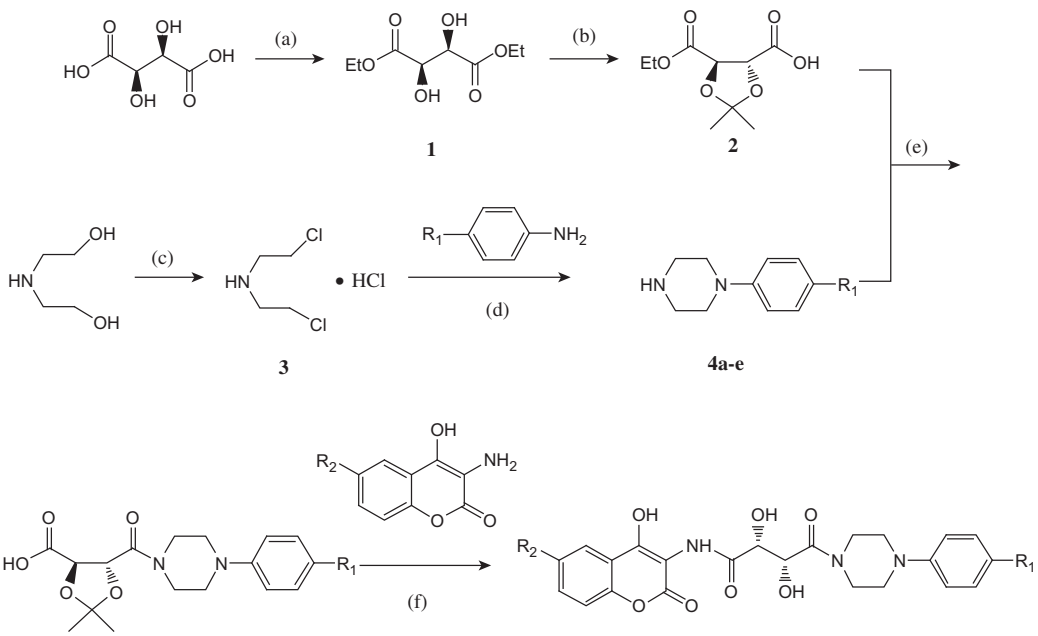
*L-(4R,5R)-5-(4-(4-methoxyphenyl)piperazine-1-carbonyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid (**5e**).* Light yellow solid; yield 52%; mp 136–137 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.27 (s, 1H, COOH), 6.78 (d, $J = 7.9$ Hz, benzene-3,5-2H), 6.69 (d, $J = 7.9$ Hz, benzene-2.6-2H), 5.25 (d, $J = 6.0$ Hz, 1H, CH), 4.90 (d, $J = 6.0$ Hz, 1H, CH), 3.94 (m, 2H, piperazine), 3.85–3.63 (m, 2H, piperazine), 3.32–3.16 (m, 2H, piperazine), 3.10–2.96 (m, 2H, piperazine), 3.80 (s, 3H, OCH_3), 1.51 (s, 3H, CH_3), 1.43 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.38, 167.22, 150.82, 149.20, 118.12, 115.75, 112.97, 76.34, 75.55, 49.87, 49.46, 45.75, 42.62, 26.32.

*General procedure of synthesis of target compounds **6***

The intermediate compounds **5** (1.35 mmol) were dissolved in 10 mL CH_2Cl_2 and HOBt (1.35 mmol), DMAP (1.4 mmol), DCC (1.45 mmol) were added. The mixture was stirred in room temperature for 0.5 h and then a 3-amino-4-hydroxycoumarin derivative (1.24 mmol) in 10 mL CH_2Cl_2 was added. The mixture was stirred for 20 h in 35 °C and filtered. The organic layer was washed with saturated $NaHCO_3$ aqueous and 1 mol/L hydrochloric acid, and dried over anhydrous Mg_2SO_4. After being filtered, the solution was concentrated *in vacuum* to give a yellow solid. The solid was dissolved in 50% (TFA and water v/v) TFA (10 mL) and stirred for 24 h and then the solution was concentrated. The resulting oil was purified by silica gel chromatography eluting with ethyl acetate–P.E. (1:5) to give the target compound **6** (Scheme 1).

*(2R,3R)-4-(4-(4-fluorophenyl)piperazin-1-yl)-2,3-dihydroxy-N-(4-hydroxycoumarin-3-yl)-4-oxobutanamide (**6a**).* Yellow solid; yield 20%; [α]20D = +46 ($c = 1$, CH_3OH). mp 218–219 °C; IR (KBr disk): ν 3446 (OH), 3398, 3340 (NH), 2940, 2828 (CH), 1690, 1638 (C = O), 1589, 1536, 1495, 1457 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.19 (s, 1H, coumarin-OH), 8.88 (s, 1H, NH), 7.90 (d, $J = 7.4$ Hz, 1H, coumarin-5-H), 7.83 (dd, $J = 8.4$, 5.1 Hz, 2H, coumarin-7,8-H), 7.56 (t, $J = 7.4$ Hz, 1H, coumarin-6-H), 7.33 (d, $J = 7.6$ Hz, 2H, benzene-3,5-H), 7.18 (d, $J = 8.7$ Hz, 2H, benzene-2,6-H), 4.55 (d, $J = 27.7$ Hz, 2H, CH), 4.25–3.93(m, 4H, piperazine-H), 3.56 (m, 4H, piperazine-H), 1.99 (s, 1H, OH), 1.92 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.65, 169.46, 160.23, 158.87, 154.75, 151.12, 148.17, 132.15, 125.45, 124.11, 118.16, 117.60, 116.95, 116.47, 99.89, 72.78, 71.63, 48.86, 48.07, 44.89, 42.23. LC–MS: m/z 472 $[M + H]^+$, HRMS: calcd for $C_{23}H_{22}FN_3O_7$ $[M + H]^+$, 472.1442; found, 472.1500.

*(2R,3R)-4-(4-(4-chlorophenyl)piperazin-1-yl)-2,3-dihydroxy-N-(4-hydroxycoumarin-3-yl)-4-oxobutanamide (**6b**).* Yellow solid; yield 15%; [α]20D = +52 ($c = 1$, CH_3OH). mp 184–186 °C; IR (KBr disk): ν 3445 (OH), 3379, 3339 (NH), 2928,



R_1=F, Cl, Br, CH_3, OCH_3 R_2=H, OH, CH_3, CI

Scheme 1. Synthesis of compounds **6a–t**. Reagents and conditions: (a) $SOCl_2$, EtOH, rt, 12 h. (b) $CH_3C(OCH_3)_2CH_3$, p-TsOH, benzene, 80 °C; dioxine, H_2O, NaOH. (c) $SOCl_2$, $CHCl_3$, 40–50 °C, 4 h. (d) xylol, p-TsOH, 145 °C. (e) DCC, HOBt, DMAP, CH_2Cl_2. (f) DCC, HOBt, DMAP, CH_2Cl_2;50%CF_3COOH, 35 °C.

2830 (CH), 1690, 1640 (C = O), 1599, 1538, 1493, 1455 (C = C). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 13.21 (s, 1H, coumarin-OH), 9.02 (s, 1H, NH), 7.92 (d, J = 7.5 Hz, 1H, coumarin-5-H), 7.79 (d, 2H, coumarin-7,8-H), 7.54 (t, J = 7.5 Hz, 1H, coumarin-6-H), 7.35 (d, J = 7.9 Hz, 2H, benzene-3,5-H), 7.09 (d, J = 8.2 Hz, 2H, benzene-2,6-H), 4.55 (d, J = 27.7 Hz, 2H, CH), 3.89 (m, 4H, piperazine-H), 3.45 (m, 4H, piperazine-H), 2.47 (s, 1H, OH), 2.35 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 172.95, 169.62, 160.19, 155.13, 150.85, 148.32, 132.54, 126.87, 125.32, 124.01, 118.23, 117.15, 116.87, 116.42, 101.13, 72.42, 71.25, 48.78, 48.11, 44.81, 42.14. LC–MS: *m/z* 488 [M + H]$^+$, HRMS: calcd for $C_{23}H_{22}ClN_3O_7$ [M + H]$^+$, 488.1146; found, 488.1223.

*(2*R*,3*R*)-4-(4-(4-bromophenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4-hydroxycoumarin-3-yl)-4-oxobutanamide* ***(6c)***. White solid; yield 34%; [α]20D = +22 (c = 1, $CH_3COOCH_2CH_3$). mp 190–191 °C; IR (KBr disk): ν 3445 (OH), 3407, 3343 (NH), 2928, 2830 (CH), 1691, 1640 (C = O), 1606, 1540, 1492, 1455 (C = C). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 13.22 (s, 1H, coumarin-OH), 9.29 (s, 1H, NH), 7.89 (d, J = 7.7 Hz, 1H, coumarin-5-H), 7.72 (t, J = 7.7 Hz, 1H, coumarin-7-H), 7.53 (d, 2H, coumarin-6,8-H), 7.35 (d, J = 7.9 Hz, 2H, benzene-3,5-H)), 6.72 (d, J = 8.1 Hz, 2H, benzene-2,6-H)), 4.62 (d, 2H, CH), 3.95–3.61 (m, 4H, piperazine-H), 3.21 (s, 4H, piperazine-H), 2.86 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 173.68, 169.59, 160.07, 153.82, 150.81, 150.27, 132.60, 131.95, 125.15, 124.08, 118.06, 116.60, 116.58, 110.87, 103.85, 72.52, 71.23, 48.66, 48.17, 44.85, 42.14. LC–MS: *m/z* 532 [M + H]$^+$, HRMS: calcd for $C_{23}H_{22}BrN_3O_7$ [M + H]$^+$, 532.0641; found, 532.0665.

*(2*R*,3*R*)-4-(4-(4-methylphenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4-hydroxycoumarin-3-yl)-4-oxobutanamide* ***(6d)***. Yellow solid; yield 19%; $[\alpha]_D^{20}$ = +41 (c = 1.1, CH_3OH). mp 195–197 °C; IR (KBr disk): ν 3446 (OH), 3405, 3344 (NH), 2959, 2931, 2829 (CH), 1690, 1639 (C = O), 1600, 1541, 1495, 1456 (C = C). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 13.20 (s, 1H, coumarin-OH), 9.40 (s, 1H, NH), 7.91 (d, J = 7.8 Hz, 1H, coumarin-5-H), 7.68 (t, J = 7.8 Hz, 1H, coumarin-7-H), 7.44 (dd, J = 12.2, 7.9 Hz, 2H, coumarin-6,8-H), 7.06 (d, J = 8.2 Hz, 2H, benzene-3,5-H)), 6.89 (d, J = 8.3 Hz, 2H, benzene-2,6-H)), 4.82 (s, 1H, CH), 4.52 (s, 1H, CH), 4.03 (d, J = 7.1 Hz, 2H, OH), 3.85–3.51 (m, 4H, piperazine-H), 3.14 (s, 4H, piperazine-H), 2.21 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 173.42, 169.81, 160.14, 155.82, 150.56, 148.35, 132.41, 128.15, 126.52, 125.42, 124.31, 118.07, 116.60, 112.87, 101.16, 72.78, 71.12, 48.63, 48.27, 44.92, 42.12. LC–MS: *m/z* 468 [M + H]$^+$, HRMS: calcd for $C_{24}H_{25}N_3O_7$ [M + H]$^+$, 468.1693; found, 468.1725.

*(2*R*,3*R*)-4-(4-(4-methoxyphenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4-hydroxycoumarin-3-yl)-4-oxobutanamide* ***(6e)***. Yellow solid; yield 15%; $[\alpha]_D^{20}$ = +62 (c = 1, CH_3OH). mp 222–224 °C; IR (KBr disk): ν 3445 (OH), 3404, 3340 (NH), 3000, 2930, 2829 (CH), 1690, 1640 (C = O), 1601, 1545, 1496, 1458 (C = C). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 13.21 (s, 1H, coumarin-OH), 9.36 (s, 1H, NH), 7.98 (d, J = 7.5 Hz, 1H, coumarin-5-H), 7.54 (dd, J = 15.9, 8.1 Hz, 1H, coumarin-7-H), 7.35 (t, J = 7.8 Hz, 2H, coumarin-6,8-H), 6.93 (d, J = 9.0 Hz, 2H, benzene-3,5-H), 6.86 (d, J = 9.0 Hz, 2H, benzene-2,6-H), 4.81 (d, J = 4.3 Hz, 1H, CH), 4.12 (d, J = 4.4 Hz, 1H, CH), 3.97 (t, J = 14.9 Hz, 2H, piperazine-H), 3.78 (s, 3H, OCH_3), 3.73 (s, 2H, piperazine-H), 3.16 (s, 2H, piperazine-H), 3.10–2.89 (m, 2H, piperazine-H), 2.63 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 173.45, 169.60, 160.02, 153.78, 150.52, 149.87, 148.27, 132.59, 125.41, 124.01, 117.96, 116.95, 116.42, 114.58, 99.89, 72.51, 71.32, 48.55, 48.16, 44.89, 42.09. LC–MS: *m/z* 484 [M + H]$^+$, HRMS: calcd for $C_{24}H_{25}N_3O_8$ [M + H]$^+$, 484.1642; found, 484.1692.

*(2*R*,3*R*)-4-(4-(4-fluorophenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4-hydroxy-6-methylcoumarin-3-yl)-4-oxobutanamide* ***(6f)***. Yellow solid; yield 15%; $[\alpha]_D^{20}$ = +55 (c = 1, CH_3OH). mp 246–247 °C; IR (KBr disk): ν 3493 (OH), 3381, 3326 (NH), 2949, 2835 (CH), 1690, 1640 (C = O), 1588, 1541, 1495, 1450 (C = C). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 13.21 (s, 1H, coumarin-OH), 9.36 (s, 1H, NH), 7.69 (s, 1H, coumarin-5-H), 7.45 (d, J = 8.4 Hz, 1H, coumarin-7-H), 7.36 (d, J = 8.5 Hz, 1H, coumarin-8-H), 7.02 (d, J = 8.8 Hz, 2H, benzene-3,5-H), 6.76 (d, J = 8.8 Hz, 2H, benzene-2,6-H), 4.72 (d, 2H, CH), 3.75 (d, 4H, piperazine-H), 3.56 (s, 2H, OH), 3.23 (s, 4H, piperazine-H), 2.42 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 172.98, 169.59, 160.03, 158.82, 152.75, 150.12, 146.76, 135.45, 132.15, 128.11, 118.06, 117.60, 116.95, 115.97, 102.29, 72.58, 71.23, 48.75, 48.05, 44.91, 42.21, 22.21. LC–MS: *m/z* 486 [M + H]$^+$, HRMS: calcd for $C_{24}H_{24}FN_3O_7$ [M + H]$^+$, 486.1598; found, 486.1625.

*(2*R*,3*R*)-4-(4-(4-chlorophenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4-hydroxy-6-methylcoumarin-3-yl)-4-oxobutanamide* ***(6g)***. Yellow solid; yield 11%; $[\alpha]_D^{20}$ = +54 (c = 1.2, CH_3OH). mp 195–197 °C; IR (KBr disk): ν 3494 (OH), 3382, 3330 (NH), 2947, 2831 (CH), 1691, 1639 (C = O), 1581, 1539, 1470, 1441 (C = C). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 13.35 (s, 1H, coumarin-OH), 9.42 (s, 1H, NH), 7.62 (s, 1H, coumarin-5-H), 7.46 (d, J = 8.5 Hz, 1H, coumarin-7-H), 7.35 (d, J = 8.5 Hz, 1H, coumarin-8-H), 7.30 (d, J = 8.1 Hz, 2H, benzene-3,5-H), 6.65 (d, J = 8.1 Hz, 2H, benzene-2,6-H), 4.85 (s, 2H, CH), 3.82 (s, 4H, piperazine-H), 3.28 (s, 4H, piperazine-H), 2.91 (s, 2H, OH), 2.35 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 173.52, 169.55, 160.09, 157.13, 151.35, 148.22, 135.87, 132.58, 129.87, 128.46, 127.13, 118.07, 117.25, 116.62, 101.25, 72.85, 71.21, 48.77, 48.12, 44.84, 42.14, 21.20. LC–MS: *m/z* 502 [M + H]$^+$, HRMS: calcd for $C_{24}H_{24}ClN_3O_7$ [M + H]$^+$, 502.1303; found, 502.1326.

*(2*R*,3*R*)-4-(4-(4-bromophenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4-hydroxy-6-methylcoumarin-3-yl)-4-oxobutanamide* ***(6h)***. Light yellow solid; yield 33%; $[\alpha]_D^{20}$ = +47 (c = 1, CH_3OH). mp 199–200 °C; IR (KBr disk): ν 3495 (OH), 3378, 3329 (NH), 2946, 2830 (CH), 1690, 1638 (C = O), 1587, 1541, 1475, 1445 (C = C). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 13.22 (s, 1H, coumarin-OH), 9.39 (s, 1H, NH), 7.70 (s, 1H, coumarin-5-H), 7.48 (d, J = 8.4 Hz, 1H, coumarin-7-H), 7.36 (t, J = 7.5 Hz, 3H, coumarin-8-H, benzene-3,5-H), 6.94 (d, J = 8.8 Hz, 2H, benzene-2,6-H), 4.82 (s, 1H, CH), 4.52 (s, 1H, CH), 3.72 (d, J = 17.0 Hz, 4H, piperazine-H), 3.60 (s, 2H, OH), 3.20 (s, 4H, piperazine-H), 2.41 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 173.59, 169.61, 160.05, 153.85, 151.26, 150.19, 135.15, 132.60, 131.95, 127.12, 118.05, 116.90, 116.58, 115.21, 104.02, 72.56, 71.25, 48.69, 48.15, 44.79, 42.08, 21.23. LC–MS: *m/z* 546 [M + H]$^+$, HRMS: calcd for $C_{24}H_{24}BrN_3O_7$ [M + H]$^+$, 546.0798; found, 546.0822.

*(2*R*,3*R*)-4-(4-(4-methylphenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4-hydroxy-6-methylcoumarin-3-yl)-4-oxobutanamide* ***(6i)***. Yellow solid; yield 21%; $[\alpha]_D^{20}$ = +43 (c = 1, CH_3OH). mp 217–218 °C; IR (KBr disk): ν 3500 (OH), 3382, 3330 (NH), 2962, 2945, 2830 (CH), 1691, 1636 (C = O), 1581, 1540, 1474, 1441 (C = C). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 13.19(s, 1H, coumarin-OH), 9.36 (s, 1H, NH), 7.53 (s, 1H, coumarin-5-H), 7.21 (s, 2H, benzene-3,5-H), 7.14 (s, 2H, benzene-2,6-H), 7.04 (d, J = 8.2 Hz, 2H, coumarin-7,8-H), 5.14 (s, 1H, CH), 4.68 (s, 1H, CH), 4.21–3.79 (m, 4H, piperazine-H), 3.47 (d, J = 22.0 Hz, 4H, piperazine-H), 3.25 (s, 2H, OH), 2.33 (s, 3H, CH_3), 2.26 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 173.62, 169.60, 160.04, 155.81, 150.06, 148.65, 135.42, 132.41, 128.15, 127.31, 126.52, 124.97,

118.07, 114.60, 102.25, 72.72, 71.13, 48.66, 48.20, 44.82, 42.12, 21.81, 21.25. LC–MS: *m/z* 482 $[M+H]^+$, HRMS: calcd for $C_{25}H_{27}N_3O_7$ $[M+H]^+$, 482.1849; found, 182.1867.

*(2*R*,3*R*)-4-(4-(4-methoxyphenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4-hydroxy-6-methylcoumarin-3-yl)-4-oxobutanamide* ***(6j)***. Yellow solid; yield 25%; $[\alpha]_D^{20} = +65$ ($c = 1.1$, CH_3OH). mp 231–232 °C; IR (KBr disk): ν 3493 (OH), 3380, 3327 (NH), 3001, 2954, 2837 (CH), 1689, 1632 (C = O), 1579, 1536, 1467, 1440 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.25 (s, 1H, coumarin-OH), 9.37 (s, 1H, NH), 7.68 (s, 1H, coumarin-5-H), 7.39 (d, 2H, coumarin-7,8-H), 6.85 (d, $J = 8.6$ Hz, 2H, benzene-3,5-H), 6.58 (d, $J = 8.6$ Hz, 2H, benzene-2,6-H), 4.85 (d, $J = 4.3$ Hz, 1H, CH), 4.21 (d, $J = 4.4$ Hz, 1H, CH), 3.95 (t, 2H, piperazine-H), 3.76 (s, 3H, OCH_3), 3.73 (s, 2H, piperazine-H), 3.15 (s, 2H, piperazine-H), 3.10 (s, 2H, piperazine-H), 2.84 (s, 2H, OH), 2.35(s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.65, 169.62, 160.07, 155.78, 150.52, 149.87, 148.07, 135.41, 132.59, 126.91, 118.06, 117.35, 116.42, 114.58, 101.89, 72.55, 71.22, 55.81, 48.59, 48.18, 44.83, 42.19, 21.42. LC–MS: *m/z* 498 $[M+H]^+$, HRMS: calcd for $C_{25}H_{27}N_3O_8$ $[M+H]^+$, 498.1798; found, 498.1830.

*(2*R*,3*R*)-4-(4-(4-fluorophenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4,6-dihydroxycoumarin-3-yl)-4-oxobutanamide* ***(6k)***. Yellow solid; yield 30%; $[\alpha]_D^{20} = +27$ ($c = 1$, $CH_3COOCH_2CH_3$). mp 198–200 °C; IR (KBr disk): ν 3445 (OH), 3398, 3327 (NH), 2932, 2826 (CH), 1695, 1640 (C = O), 1585, 1541, 1498, 1450 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.30 (s, 1H, coumarin-OH), 9.45 (s, 1H, NH), 7.85 (s, 1H, coumarin-5-H), 7.71 (d, $J = 8.3$ Hz, 1H, coumarin-7-H), 7.51 (d, $J = 8.3$ Hz, 1H, coumarin-8-H), 7.16 (d, $J = 7.6$ Hz, 2H, benzene-2,6-H), 7.02 (d, $J = 7.7$ Hz, 2H, benzene-3,5-H), 6.45 (s, 1H, coumarin-6-OH), 4.78 (s, 1H, CH), 4.49 (s, 1H, CH), 3.75 (s, 4H, piperazine-H), 3.24 (s, 4H, piperazine-H), 2.95 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.68, 169.60, 160.07, 158.85, 155.75, 154.45, 150.72, 148.71, 126.15, 118.06, 117.59, 116.95, 115.67, 112.11, 101.28, 72.52, 71.24, 48.76, 48.08, 44.91, 42.12. LC–MS: *m/z* 488 $[M+H]^+$, HRMS: calcd for $C_{23}H_{22}FN_3O_8$ $[M+H]^+$, 488.1391; found, 488.1426.

*(2*R*,3*R*)-4-(4-(4-chlorophenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4,6-dihydroxycoumarin-3-yl)-4-oxobutanamide* ***(6l)***. Light yellow solid; yield 35%; $[\alpha]_D^{20} = +52$ ($c = 1$, CH_3OH). mp 170–172 °C; IR (KBr disk): ν 3446 (OH), 3340, 3328 (NH), 2932, 2826 (CH), 1691, 1639 (C = O), 1594, 1540, 1494, 1451 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.32 (s, 1H, coumarin-OH), 9.41 (s, 1H, NH), 7.82 (s, 1H, coumarin-5-H), 7.69 (d, $J = 8.2$ Hz, 1H, coumarin-7-H), 7.49 (d, $J = 8.3$ Hz, 1H, coumarin-8-H), 7.25 (d, $J = 7.6$ Hz, 2H, benzene-2,6-H), 6.98 (d, $J = 7.7$ Hz, 2H, benzene-3,5-H), 6.70 (s, 1H, coumarin-6-OH), 4.83 (s, 1H, CH), 4.54 (s, 1H, CH), 3.69 (d, $J = 43.7$ Hz, 4H, piperazine-H), 3.20 (s, 4H, piperazine-H), 2.87 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.58, 169.66, 160.09, 155.75, 153.82, 148.75, 147.55, 144.45, 128.59, 125.95, 118.11, 116.76, 115.55, 112.19, 101.01, 72.58, 71.14, 48.85, 48.15, 44.89, 42.14. LC–MS: *m/z* 504 $[M+H]^+$, HRMS: calcd for $C_{23}H_{22}ClN_3O_8$ $[M+H]^+$, 504.1095; found, 504.1123.

*(2*R*,3*R*)-4-(4-(4-bromophenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4,6-dihydroxycoumarin-3-yl)-4-oxobutanamide* ***(6m)***. Light yellow solid; yield 40%; $[\alpha]_D^{20} = +67$ ($c = 1.2$, CH_3OH). mp 185–187 °C; IR (KBr disk): ν 3445 (OH), 3341, 3327 (NH), 2930, 2829 (CH), 1690, 1640 (C = O), 1597, 1546, 1498, 1451 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.36 (s, 1H, coumarin-OH), 9.39 (s, 1H, NH), 7.75 (d, $J = 8.5$ Hz, 1H, coumarin-8-H), 7.45 (d, $J = 7.6$ Hz, 2H, benzene-2,6-H), 7.19 (d, $J = 8.4$ Hz, 1H, coumarin-7-H), 7.05 (s, 1H, coumarin-5-H), 6.85 (d, $J = 7.7$ Hz, 2H, benzene-3,5-H), 6.36 (s, 1H, coumarin-6-OH), 4.76 (d, 2H, CH), 3.73 (d, $J = 43.7$ Hz, 4H, piperazine-H), 3.19 (s, 4H, piperazine-H), 3.05 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.68, 169.61, 160.07, 155.76, 153.89, 148.69, 146.46, 131.15, 125.89, 118.23, 116.76, 116.15, 115.49, 112.21, 101.32, 72.61, 71.11, 48.88, 48.20, 44.85, 42.12. LC–MS: *m/z* 548 $[M+H]^+$, HRMS: calcd for $C_{23}H_{22}BrN_3O_8$ $[M+H]^+$, 548.0590; found, 548.0612.

*(2*R*,3*R*)-4-(4-(4-methylphenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4,6-dihydroxycoumarin-3-yl)-4-oxobutanamide* ***(6n)***. White solid; yield 33%; $[\alpha]_D^{20} = +56$ ($c = 1$, CH_3OH). mp 189–190 °C; IR (KBr disk): ν 3445 (OH), 3398 (NH), 2926, 2825 (CH), 1695, 1640 (C = O), 1583, 1539, 1491, 1456 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.32 (s, 1H, coumarin-OH), 9.41 (s, 1H, NH), 7.85 (d, $J = 8.1$ Hz, 1H, coumarin-8-H), 7.39 (d, $J = 7.9$ Hz, 2H, benzene-2,6-H), 7.12 (d, $J = 8.0$ Hz, 1H, coumarin-7-H), 6.98 (s, 1H, coumarin-5-H), 6.81 (d, $J = 7.6$ Hz, 2H, benzene-3,5-H), 5.86 (s, 1H, coumarin-6-OH), 4.76 (s, 1H, CH), 4.52 (s, 1H, CH), 3.76 (s, 4H, piperazine-H), 3.25 (s, 4H, piperazine-H), 2.95 (s, 2H, OH), 2.36 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.98, 169.59, 160.11, 155.88, 153.75, 148.58, 147.45, 129.36, 128.15, 125.63, 118.22, 115.51, 114.76, 112.53, 103.15, 72.69, 71.06, 48.79, 48.19, 44.83, 42.09, 21.45. LC–MS: *m/z* 484 $[M+H]^+$, HRMS: calcd for $C_{24}H_{25}N_3O_8$ $[M+H]^+$, 484.1642; found, 484.1651.

*(2*R*,3*R*)-4-(4-(4-methoxyphenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4,6-dihydroxycoumarin-3-yl)-4-oxobutanamide* ***(6o)***. White solid; yield 26%; $[\alpha]_D^{20} = +50$ ($c = 1$, CH_3OH). mp 201–202 °C; IR (KBr disk): ν 3445 (OH), 3399, 3329 (NH), 3001, 2928, 2827 (CH), 1698, 1640 (C = O), 1585, 1540, 1496, 1451 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.35 (s, 1H, coumarin-OH), 9.43 (s, 1H, NH), 7.79 (d, $J = 8.6$ Hz, 1H, coumarin-8-H), 7.14 (d, $J = 8.7$ Hz, 1H, coumarin-7-H), 6.95(t, 3H, $J = 7.5$ Hz benzene-2,6-H, coumarin-5-H), 6.81 (d, $J = 7.4$ Hz, 2H, benzene-3,5-H), 5.91 (s, 1H, coumarin-6-OH), 4.85 (s, 1H, CH), 4.58 (s, 1H, CH), 3.86 (s, 3H, OCH_3), 3.81 (s, 4H, piperazine-H), 3.19 (s, 4H, piperazine-H), 2.86 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.36, 169.60, 160.06, 155.47, 153.26, 148.56, 146.25, 145.11, 125.58, 118.21, 116.25, 115.36, 114.78, 112.69, 103.26, 72.64, 71.03, 55.78, 48.74, 48.21, 44.90, 42.10. LC–MS: *m/z* 500 $[M+H]^+$, HRMS: calcd for $C_{24}H_{25}N_3O_9$ $[M+H]^+$, 500.1591; found, 500.1666.

*(2*R*,3*R*)-4-(4-(4-fluorophenyl)piperazin-1-yl)-2,3-dihydroxy*-N-*(4-hydroxy-6-chlorocoumarin-3-yl)-4-oxobutanamide* ***(6p)***. Light yellow solid; yield 41%; $[\alpha]_D^{20} = +47$ ($c = 1.1$, CH_3OH). mp 266–267 °C; IR (KBr disk): ν 3452 (OH), 3369, 3339 (NH), 2941, 2830 (CH), 1664, 1632 (C = O), 1601, 1530, 1479, 1453 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.25 (s, 1H, coumarin-OH), 9.39 (s, 1H, NH), 7.34–7.29 (m, 3H, coumarin-7-H, benzene-2,6-H), 7.21 (s, 1H, coumarin-5-H), 7.09 (s, 1H, coumarin-8-H), 6.98 (d, $J = 8.2$ Hz, 2H, benzene-3,5-H), 4.80 (s, 1H, CH), 4.51 (s, 1H, CH), 3.67 (d, $J = 44.5$ Hz, 4H, piperazine-H), 3.19 (s, 4H, piperazine-H), 2.89 (s, 1H, OH), 2.73 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.69, 169.59, 160.03, 158.25, 155.75, 150.19, 147.32, 135.24, 129.43, 127.14, 122.47, 118.06, 117.53, 115.95, 101.89, 72.53, 71.21, 48.69, 48.11, 44.85, 42.25. LC–MS: *m/z* 506 $[M+H]^+$, HRMS: calcd for $C_{23}H_{21}ClFN_3O_7$ $[M+H]^+$, 506.1052; found, 506.1075.

(2R,3R)-4-(4-(4-chlorophenyl)piperazin-1-yl)-2,3-dihydroxy-N-(4-hydroxy-6-chlorocoumarin-3-yl)-4-oxobutanamide ***(6q)***.
White solid; yield 29%; $[\alpha]_D^{20} = +25$ ($c = 1$, $CH_3COOCH_2CH_3$). mp 189–192 °C; IR (KBr disk): ν 3451 (OH), 3370, 3340 (NH), 2940, 2828 (CH), 1671, 1630 (C = O), 1600, 1535, 1481, 1450 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.20 (s, 1H, coumarin-OH), 9.40 (s, 1H, NH), 7.26 (dd, $J = 21.1$, 12.3 Hz, 4H, coumarin-5,7-H, benzene-2,6-H), 7.07 (d, $J = 7.5$ Hz, 1H, coumarin-8-H), 6.98 (d, $J = 8.2$ Hz, 2H, benzene-3,5-H), 4.80 (s, 1H, CH), 4.51 (s, 1H, CH), 3.67 (d, $J = 44.5$ Hz, 4H, piperazine-H), 3.19 (s, 4H, piperazine-H), 2.89 (s, 1H, OH), 2.73 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.85, 169.55, 159.70, 152.70, 149.92, 149.42, 132.18, 129.24, 129.08, 123.21, 123.07, 118.79, 118.20, 117.62, 104.57, 72.52, 71.18, 48.79, 48.35, 44.87, 42.14. LC–MS: *m/z* 522 $[M + H]^+$, HRMS: calcd for $C_{23}H_{21}Cl_2N_3O_7$ $[M + H]^+$, 522.0757; found, 522.0765.

(2R,3R)-4-(4-(4-bromophenyl)piperazin-1-yl)-2,3-dihydroxy-N-(4-hydroxy-6-chlorocoumarin-3-yl)-4-oxobutanamide ***(6r)***.
Yellow solid; yield 15%; $[\alpha]_D^{20} = +64$ ($c = 1$, CH_3OH). mp 187–188 °C; IR (KBr disk): ν 3452 (OH), 3371, 3340 (NH), 2949, 2827 (CH), 1669, 1630 (C = O), 1600, 1539, 1481, 1451 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.19 (s, 1H, coumarin-OH), 9.42 (s, 1H, NH), 7.46 (s, 1H, coumarin-5-H), 7.35 (d, $J = 7.9$ Hz, 2H, benzene-2,6-H), 7.21–7.09 (m, 2H, coumarin-7,8-H), 6.89 (d, $J = 7.9$ Hz, 2H, benzene-3,5-H), 4.91 (s, 1H, CH), 4.49 (s, 1H, CH), 3.82 (d, $J = 44.5$ Hz, 4H, piperazine-H), 3.26 (s, 4H, piperazine-H), 3.02 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.79, 169.67, 159.91, 153.76, 149.96, 148.89, 132.26, 129.58, 129.24, 124.78, 120.20, 118.81, 118.12, 114.21, 103.98, 72.55, 71.21, 48.80, 48.41, 44.90, 42.15. LC-–MS: *m/z* 566 $[M + H]^+$, HRMS: calcd for $C_{23}H_{21}ClBrN_3O_7$ $[M + H]^+$, 566.0251; found, 566.0274.

(2R,3R)-4-(4-(4-methylphenyl)piperazin-1-yl)-2,3-dihydroxy-N-(4-hydroxy-6-chlorocoumarin-3-yl)-4-oxobutanamide ***(6s)***.
Yellow solid; yield 21%; $[\alpha]_D^{20} = +53$ ($c = 1.1$, CH_3OH). mp 179–182 °C; IR (KBr disk): ν 3451 (OH), 3370, 3340 (NH), 2968, 2940, 2830 (CH), 1669, 1631 (C = O), 1601, 1536, 1480, 1453 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.35 (s, 1H, coumarin-OH), 9.46 (s, 1H, NH), 7.51–7.36 (m, 4H, coumarin-5,7-H, benzene-2,6-H), 7.11 (d, 1H, coumarin-8-H), 6.97 (d, $J = 7.9$ Hz, 2H, benzene-3,5-H), 4.79 (s, 1H, CH), 4.36 (s, 1H, CH), 3.78 (d, $J = 44.5$ Hz, 4H, piperazine-H), 3.23 (s, 4H, piperazine-H), 2.91 (s, 2H, OH), 2.36 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.86, 169.55, 159.89, 152.96, 150.03, 149.39, 132.25, 129.36, 128.55, 126.21, 123.18, 120.03, 118.21, 115.12, 103.79, 72.58, 71.26, 48.79, 48.46, 44.92, 42.16, 21.36. LC–MS: *m/z* 502 $[M + H]^+$, HRMS: calcd for $C_{24}H_{24}ClN_3O_7$ $[M + H]^+$, 502.1303; found, 502.1321.

(2R,3R)-4-(4-(4-methoxyphenyl)piperazin-1-yl)-2,3-dihydroxy-N-(4-hydroxy-6-chlorocoumarin-3-yl)-4-oxobutanamide ***(6t)***.
Light yellow solid; yield 18%; $[\alpha]_D^{20} = +66$ ($c = 1$, CH_3OH). mp 215–216 °C; IR (KBr disk): ν 3452 (OH), 3374, 3339 (NH), 3001, 2946, 2831 (CH), 1665, 1631 (C = O), 1600, 1539, 1480, 1450 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.35 (s, 1H, coumarin-OH), 9.46 (s, 1H, NH), 7.48 (s, 1H, coumarin-5-H), 7.26 (d, $J = 8.5$ Hz, 1H, coumarin-7-H), 7.11 (d, $J = 8.8$ Hz,1H, coumarin-8-H), 6.98 (d, $J = 7.5$ Hz, 2H, benzene-2,6-H), 6.75 (d, $J = 7.6$ Hz, 2H, benzene-3,5-H), 4.85 (s, 1H, CH), 4.41 (s, 1H, CH), 3.86 (d, $J = 44.5$ Hz, 4H, piperazine-H), 3.76 (s, 3H, OCH_3), 3.32 (s, 4H, piperazine-H), 2.99 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.78, 169.59, 159.99, 153.85, 151.21,150.09, 149.42, 132.19, 129.24, 123.18, 119.95, 118.46, 117.56, 114.49, 103.52, 72.61, 71.19, 55.36, 48.82, 48.45, 44.96, 42.11. LC–MS: *m/z* 518 $[M + H]^+$, HRMS: calcd for $C_{24}H_{24}ClN_3O_8$ $[M + H]^+$, 518.1252; found, 518.1269.

Biological activity assay

Inhibition of CHS assay

Yeast cells (*Saccharomyces cerevisiae* CGMCC2.145) were grown in 400 mL yeast extract peptone dextrose (YPD) medium to an OD 600 nm of 2–3 corresponding to about 2–3 g (wet weight) of cells and collected through centrifugation at 1500*g* for 15 min at 4 °C, then washed with water. The precipitates were suspended in 20 mL 50 mmol/L Tris-HCl solution at pH 7.0 which contains 40 µL fungal protease inhibitor cocktail and 50 µL solution of 200 mmol/L phenylmethanesulfonyl fluoride in DMSO, and were lysed by sonication treatment at 4 °C for 80 min. Insoluble materials were removed. One volume of supernatant was mixed with two volumes solution of 10% (w/w) sucrose dissolved in 100 mmol/L Tris-HCl buffer at pH 7.5, and centrifuged at 55 000*g* for 2 h at 4 °C. After centrifugation, the supernatant was discarded and the pellet was re-suspended in 50 mmol/L Tris-HCl at pH 7.5 and 33% glycerol to serve as CHS sample, and stored at −80 °C. The stock solutions of the candidate compounds were made by dissolving compounds with DMSO for no more than 10.0 g/L and diluting with sterile water for final concertration of DMSO below 3%. The solution of the candidate compounds was made by diluting the stock solution with 50 mM Tris-HCl buffer solution at pH 7.5[36,37].

Two hundred microliters 30 µg/mL wheatgerm agglutinin (WGA) stock solutions in 50 mmol/L Tris-HCl at pH 7.5 were added to each well of the microplate and were incubated at room temperature for 16 h, then the solutions in the wells were removed and the plates were washed at least three times with distilled water. Three hundred microliters of 3 mg/mL bovine serum albumin in 50 mmol/L Tris-HCl buffer solution at pH 7.5 were added to each wells and incubated at 37 °C for 2 h. After removing the solution, the wells were washed by Tris-HCl solution for three times. Fifty microliters solution of 80 mmol/L GlcNAc plus 4 mmol/L UDP-GlcNAc, 50 µL solution of a candidate compound and 50 µL of CHS sample were added to a well and added 50 mM Tris-HCl buffer solution to a total volume of 200 µL. Microplates were incubated at 25 °C for 60 min. Then, the unbound components were removed and wells were washed with distilled water for three times.

To each well, 200 µL solution of 1 µg/ml wheatgerm agglutinin-Horse Reddish Peroxidase (WGA-HRP) in 50 mmol/L Tris-HCl at pH 7.5 was added. After being gently shaken for 6–15 min, the microplates were further kept at 37 °C for 15 min, and then washed five times with distilled water. Finally, 150 µL peroxidase substrate buffer solution (0.8 mmol/L TMB, 2 mmol/L H_2O_2, 50 mmol/L Na_2HPO_4–citric acid, pH3.7) was added and the mixtures reacted in lucifuge place for 30 min at 37 °C. The reaction was stopped with 50 µL 2 mol/L H_2SO_4 and measured with Biotek ELX 800 Microplate reader (Winooski, VT) at 450 nm. Standard chitin of 0.50 g/L was used to construct a response of absorbance at 450 nm with logarithmic quantities of chitin in wells. There was a linear response of absorbance at 450 nm to logarithmic quantities of chitin from 3.1 to 50 mg/L in a total volume of 200 µL solution in microplate wells. Parameters of such a linear response showed good consistency during independent repetitive assays. Chitin in each well was calculated accordingly to estimate half-inhibition concentration (IC_{50}) of a test compound.

Antibacterial and antifungal assays

MIC (mg/mL) is defined as the lowest concentration of target compounds that completely inhibited the growth of

microorganism[38]. All the synthesized compounds **6a–t** were tested for antimicrobial activity *in vitro* by the standard two-folds serial dilution method in 96-well microplates according to the National Committee for Clinical Laboratory Standards (NCCLS). DMSO was used as a solvent control to ensure that the solvent had no effect on microorganism growth. All the bacteria and fungi growth was monitored visually and spectrophotometrically, and the experiments were performed in triplicate.

Antifungal activity assay

Antifungal activity was screened against four main pathogenic fungal species (*C. albicans* CMCC 76615, *Aspergillus fumigatus* GIMCC 3.19, *C. neoformans* ATCC 32719 and *Aspergillus flavus* ATCC 16870) in clinic. Fluconazole and polyoxin B were used as standard antifungal drugs. DMSO was used as a solvent control. A spore suspension in sterile distilled water was prepared from 1-day old culture of the fungi growing on the media containing 1% peptone, 2% glucose and solid media as well as 15% agar. The final spore concentration was $1–5 \times 10^3$ spore mL^{-1}. All target compounds were dissolved in DMSO to prepare the stock solutions. The tests were made resulting in 12 wanted concentrations (0.25–512 mg/mL). These dilutions were incubated at 37 °C for 24 h. The MIC values of antifungal activity in μg/mL are summarized in Table 1.

Antibacterial activity assays

Antibacterial activity was screened against three Gram positive (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, Methicillin-resistant *Staphylococcus aureus* N 315) and three Gram negative (*E. coli* JM 109, *Proteusbacillus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 9027) bacteria by using streptomycin and ofloxacin as a standard antibacterial drugs. The bacterial suspension was adjusted with culture medium to a concentration of 1×10^5 Colony Forming Unit (CFU). The culture medium consisted of 1% peptone, 0.3% beef extract, 0.5% sodium chloride in distilled water, the solid media as well as 15% agar. All compounds were dissolved in DMSO to prepare the stock solutions. The tests were carried out at a required concentration of 512, 256,128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 μg/mL. These dilutions were incubated at 37 °C for 24 h. The MIC values of antibacterial activity in μg/mL are summarized in Table 2 (see the supplementary information).

Results and discussion

CHS inhibitory activity

All the synthesized compounds **6a–t** showed the potency of inhibiting the CHS, but some compounds, such as compounds **6e**, **6j**, **6l**, **6m**, **6p**, **6q**, **6s** and **6t** were not further screened because their inhibition ratio were less than 15% at a concentration of 300 μg/mL. The inhibition ratios of other compounds are depicted in Figure 4, and these derivatives with higher inhibition ratios were further screened for their IC_{50} values which were calculated and shown in Figure 5. Among them, compounds **6b**, **6c**, **6d**, **6h**, **6i**, **6n** and **6r** had IC_{50} values of 0.19, 0.17, 0.19, 0.21, 0.18, 0.20 and 0.16 mmol/L, respectively, which were almost equal to that of polyoxin B whose IC_{50} value was 0.18 mmol/L. Especially, compound **6o** with IC_{50} of 0.10 mmol/L had the most potential CHS inhibitory activity in these compounds. Compounds **6a**, **6f**, **6g** and **6k** exerted slightly lower inhibitory activity.

Antimicrobial activity

All target compounds **6a–t** showed weak or no antibacterial efficacy against all the tested bacterial strains (the results are listed in Table 2 in the supplementary information). The strongest activity of these compounds against all tested bacteria is **6h** with MIC of 32 μg/mL against *E. coli*, but it is 8-fold weaker than streptomycin and 64-fold weaker than ofloxacin both with MIC values of less than 4 μg/mL. Furthermore, none of the MIC values of other compounds against the six tested strains was less than 64 μg/mL. It turned out that these compounds **6a–t** have no effect on the tested strains.

The results of antifungal activity in Table 1 showed that target compounds **6a–t** exhibited moderate even excellent efficacy against all the tested fungal strains. Compounds **6b**, **6c**, **6h**, **6k**, **6o**, **6r** and **6t** against *C. albicans* had the MIC values of 32 μg/mL, which were equal to that of polyoxin B. Compounds **6b**, **6c**, **6e**, **6i**, **6k**, **6o** and **6r** have good antifungal activity against *A. flavus* with the MIC values of 64 μg/mL, which were comparable with

Table 1. The MIC values (μg/mL) of compounds **6a–t** against fungi *in vitro*.

Compound	R_1	R_2	Yield (%)	*Candida albicans*	*Aspergillus flavus*	*Aspergillums fumigates*	*Cryptococcus neoformans*
6a	F	H	20	64	128	128	256
6b	Cl	H	15	32	64	64	4
6c	Br	H	34	32	64	64	32
6d	CH_3	H	19	64	32	128	64
6e	OCH_3	H	15	256	64	128	256
6f	F	CH_3	15	512	512	128	512
6g	Cl	CH_3	11	64	256	128	256
6h	Br	CH_3	33	32	128	128	64
6i	CH_3	CH_3	21	64	64	128	128
6j	OCH_3	CH_3	25	128	128	128	256
6k	F	OH	30	32	64	128	64
6l	Cl	OH	35	256	256	256	256
6m	Br	OH	40	64	128	64	128
10n	CH_3	OH	33	64	32	64	16
6o	OCH_3	OH	26	32	64	16	32
6p	F	Cl	41	256	512	256	512
6q	Cl	Cl	29	128	128	128	256
6r	Br	Cl	15	32	64	64	64
10s	CH_3	Cl	21	128	256	256	256
10t	OCH_3	Cl	18	128	128	256	128
Fluconazole	–	–	–	16	64	32	8
Polyoxin B	–	–	–	32	64	64	16

Figure 4. The inhibition ratio of compounds at 300 μg/mL.

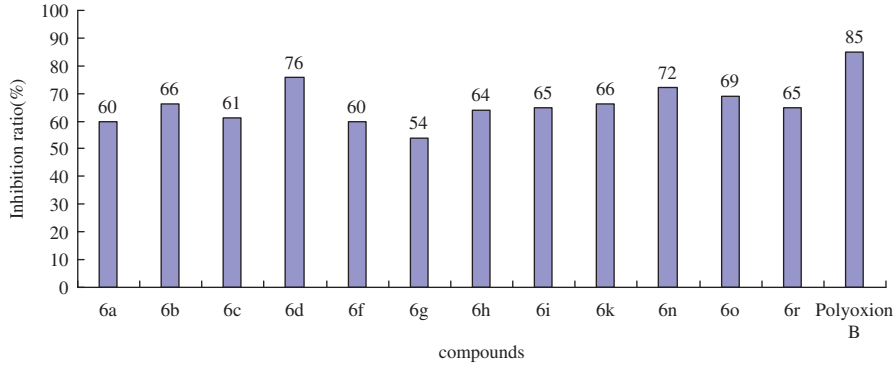
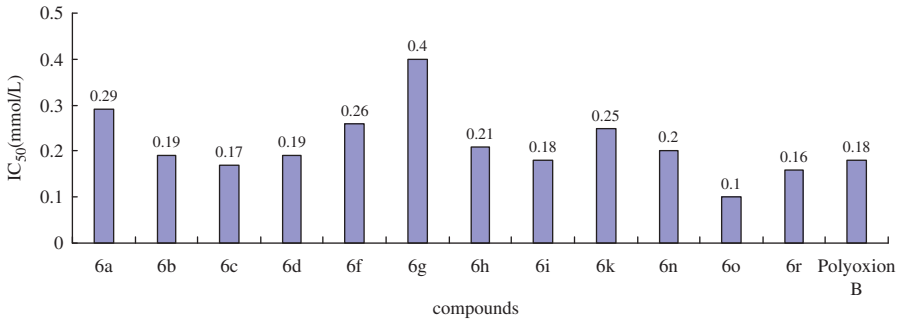


Figure 5. The IC_{50} values of the some compounds against CHS.



fluconazole and polyoxin B whose MIC values both were 64 μg/mL. Meanwhile, compounds **6d** and **6n** with MIC of 32 μg/mL exhibit better activity than fluconazole and polyoxin B. To *Aspergillums fumigatus*, compounds **6b**, **6c**, **6m**, **6n** and **6r** were comparable with polyoxin B whose MIC value was 64 μg/mL. Especially, compound **6o** with MIC values of 16 μg/mL exhibits better activity than fluconazole. Compound **6b** has very high activity against *C. neoformans* with MIC value of 4 μg/mL which is twice more potent than fluconazole and four times more potent than polyoxin B.

From these assays data, we can see that these synthesized compounds have selective bioactivity against fungi, but they have no effects on bacteria. These results indicated that the design of these compounds as antifungal agents was rational. In general, the compounds which have good inhibitory activity against CHS showed good antifungal activity. To some extent, the antifungal activity of these compounds has positive correlation with their inhibitory activity against CHS.

In terms of the structural features of these compounds, it is seen that a suitable lipid–water partition coefficient could be beneficial to the bioactivity. For instance, compound **6o**, in which the hydroxy group might increase the hydrophilicity of the molecule while the methoxy group could improve the lipophilicity of the compound, and the double effects resulted in a better lipid–water partition coefficient of this compound, exerted excellent CHS inhibitory activity. Meanwhile, the properties of substituted groups (R_2) on coumarin ring greatly affected the inhibitory activity against CHS. The compounds with electron-donating group such as H, OH groups exhibit to be more active than these compounds with electron-withdrawing groups. For example, these compounds **6p**, **6q**, **6s** and **6t** bearing the Cl group in coumarin ring have little inhibitory activity against CHS with inhibition ratio less than 15% in 300 μg/mL. In contrast, the compounds which bear the electron-withdrawing groups, such as Cl, Br groups, in phenylpiperazine ring showed broad-spectrum antifungal activity. However, compounds **6d**, **6i** and **6n** exhibited actively anti-fungal activity to all tested pathogenic fungi.

Conclusion

A series of novel 3-amino-4-hydroxycoumarin derivatives containing an *N*-phenylpiperazine moiety, a L-tartrate acid amide and 4-hydroxycoumarin scaffold in one molecule have been designed and synthesized in order to find new lead compounds that possess the excellent bioactivity against CHS and fungal activity. The enzymatic assay results showed that all these target compounds have CHS inhibitory activity. Among them, compounds **6a**, **6b**, **6c**, **6d**, **6i**, **6n**, **6o** and **6r** exhibited comparatively good activity against CHS; especially, **6o** with IC_{50} value of 0.1 mmol/L is the strongest CHS inhibitor in these compounds. The antifungal assay showed that most of these compounds exhibit moderate even excellent activity against the tested strains which are the common pathogen in clinic. The microbiological results revealed that these compounds exhibited more significant antifungal activity than activity against bacteria. This indicated that it is possible to develop new selective CHS inhibitors from our series of compounds which may have potential for the treatment of fungal infections.

Declaration of interest

The authors report no conflicts of interest.

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References

1. Pfaller MA, Diekem DJ. Epidemiology of invasive Candidiasis: a persistent public health problem. Clin Microbiol Rev Clin Microbiol Rev 2007;20:133–63.
2. Shapiro RS, Robbins Ni, Cowen LE. Regulatory circuitry governing fungal development, drug resistance, and disease. Microbiol Mol Biol Rev 2011;75:213–67.
3. Mukherjee PK, Sheehan DJ, Hitchcock CA, et al. Combination treatment of invasive fungal infections. Clin Microbiol Rev 2005;18:163–94.

4. Kathiravan MK, Salake AB, Chothe AS, et al. The biology and chemistry of antifungal agents: a review. Bioorg Med Chem 2012; 20:5678–98.
5. Perlin DS. Resistance to echinocandin-class antifungal drugs. Drug Resist Updat 2007;10:121–30.
6. Walker LA, Gow NA, Munro CA. Fungal echinocandin resistance. Fungal Genet Biol 2010;47:117–26.
7. Verweij PE, Snelders E, Kema GH, et al. Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? Lancet Infect Dis 2009;9:789–95.
8. Tellam RL, Vuocolo T, Johnson SE, et al. Insect chitin synthase cDNA sequence, gene organization and expression. Eur J Biochem 2000;267:6025–42.
9. Magellan H, Boccara M, Drujon T, et al. Discovery of two new inhibitors of *Botrytis cinerea* chitin synthase by a chemical library screening. Bioorg Med Chem 2013;21:4997–5003.
10. Di Domenico B. Novel antifungal drugs. Curr Opin Microbiol 1999; 2:509–15.
11. Merzendorfer H, Zimoch L. Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. J Exp Biol 2003;206:4393–412.
12. Grol AH, Lucca AJD, Walsh TJ. Emerging targets for the development of novel antifungal therapeutics. Trends Microbiol 1998;6:117.
13. Ke S, Liu Fy, Wang N, et al. 1,3,4-Oxadiazoline derivatives as novel potential inhibitors targeting chitin biosynthesis: design, synthesis and biological evaluation. Bioorg Med Chem Lett 2009;19:332–5.
14. Lenardon MD, MunrCA, Gow NAR. Chitin synthesis and fungal pathogenesis. Curr Opin Microbiol 2010;13:416–23.
15. Chaudhary PM, Tupe SG, Deshpande MV. Chitin synthase inhibitors as antifungal agents. Mini-Rev Med Chem 2013;13:222–36.
16. Chen WQ, Dai DF, Wang CC, et al. Genetic dissection of the polyoxin building block-carbamoylpolyoxamic acid biosynthesis revealing the ''pathway redundancy'' in metabolic networks. Microb Cell Fact 2013;12:121.
17. Xu WM, Yang S, Bhadury P, et al. Synthesis and bioactivity of novel sulfone derivatives containing 2,4-dichlorophenyl substituted 1,3,4-oxadiazole/thiadiazole moiety as chitinase inhibitors. Pestic Biochem Phys 2011;101:6–15.
18. Georgopapadakou NH, Tkacz JS. The fungal cell wall as a drug target. Trends Microbial 1995;3:98.
19. Plant A, Thompson P, Williams DM. Stereoselective synthesis of novel uracil polyoxin C conjugates as substrate analogues of chitin synthase. J Org Chem 2008;73:3714–24.
20. Yeager AR, Finney NS. Second-generation dimeric inhibitors of chitin synthase. Bioorg Med Chem 2004;12:6451–60.
21. Chaudhary PM, Chavan SR, Shirazi F, et al. Exploration of click reaction for the synthesis of modified nucleosides as chitin synthase inhibitors. Bioorg Med Chem 2009;17:2433–40.
22. Renuka N, Kumar KA. Synthesis and biological evaluation of novel formyl-pyrazoles bearing coumarin moiety as potent antimicrobial and antioxidant agents. Bioorg Med Chem Lett 2013;23:6406–9.
23. Peng XM, Damu GLV, Zhou CH. Current developments of coumarin compounds in medicinal chemistry. Curr Pharm Des 2013;19: 3884–930.
24. Mitra I, Saha A, Roy K. Quantification of contributions of different molecular fragments for antioxidant activity of coumarin derivatives based on QSAR analyses. Can J Chem 2013; 91:428–41.
25. Bansal Y, Sethi P, Bansal G. Coumarin: a potential nucleus for anti-inflammatory molecules. Med Chem Res 2013;22:3049–60.
26. Keri RS, Hosamani KM, Shingalapur RV, et al. Analgesic, anti-pyretic and DNA cleavage studies of novel pyrimidine derivatives of coumarin moiety. Eur J Med Chem 2010;45:2597–605.
27. Via LD, Gia O, Magno SM, et al. New tetracyclic analogues of photochemotherapeutic drugs 5-MOP and 8-MOP: synthesis, DNA interaction, and antiproliferative activity. J Med Chem 1999;42: 4405–13.
28. Lee BH, Clothier MF, Dutton FE, et al. Anthel mintic β-hydroxyketoamides (bkas). Bioorg Med Chem Lett 1988;8:3317–20.
29. Spino C, Dodier M, Sotheeswaran S. Anti-HIV coumarins from calophyllum seed oil. Bioorg Med Chem Lett 1998;8:3475–8.
30. Hwu JR, Singha R, Hong SC, et al. Synthesis of new benzimidazole–coumarin conjugates as anti-hepatitis C virus agents. Antiviral Res 2008;77:157–62.
31. Tian LW, Pei Y, Zhang YJ, et al. 7-O-Methylkaempferol and -quercetin glycosides from the whole plant of *Nervilia fordii*. J Nat Prod 2009;72:1057–60.
32. Kurdelas RR, Lima B, Tapia A, et al. Antifungal activity of extracts and prenylated coumarins isolated from Baccharis darwinii Hook & Arn. (Asteraceae). Molecules 2010;15:4898–907.
33. Mladenović M, Vuković N, Nićiforović N, et al. Synthesis and molecular descriptor characterization of novel 4-hydroxy-chromene-2-one derivatives as antimicrobial agents. Molecules 2009;14: 1495–512.
34. Shams HZ, Mohareb RM, Helal MH, Mahmoud AE. Synthesis, structure elucidation, and biological evaluation of some fused and/or pendant thiophene, pyrazole, imidazole, thiazole, triazole, triazine, and coumarin systems based on cyanoacetic 2-[(benzoylamino)thioxomethyl] hydrazide. Phosphorus Sulfur 2007;182:237–63.
35. Basanagouda M, Shivashankar K, Kulkarni MV, et al. Synthesis and antimicrobial studies on novel sulfonamides containing 4-azidomethyl coumarin. Eur J Med Chem 2010;45:1151–7.
36. Lucero HA, Kuranda MJ, Bulik DA. A nonradioactive, high throughput assay for chitin synthase activity. Anal Biochem 2002; 305:97–105.
37. Ji QG, Yang D, Wang X, et al. Design, synthesis and evaluation of novel quinazoline-2,4-dione derivatives as chitin synthase inhibitors and antifungal agents. Bioorg Med Chem 2014;22: 3405–13.
38. NCCLS (National Committee for Clinical Laboratory Standards). Performance standards for antimicrobial susceptibility testing: Twelfth informational supplement 2002; M100-S12(M7).

Supplementary material available online

Supplementary Table 2