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Short communication

Synthesis and antimicrobial activities of 7-*O*-modified genistein derivatives

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Abstract

Three series of genistein derivatives with heterocycles were prepared, in which genistein and heterocyclic moieties were separated by 2-carbon, 3-carbon and 4-carbon spacers. Among the 33 compounds we prepared 11 of them (**2c** and **5a–j**) are reported for the first time, while the preparation of **2a**,**b**, **3a–j** and **4a–j** was reported in our recent paper. All the derivatives were screened for antibacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescence*) and antifungal (*Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*) activities by MTT method. Among the compounds tested, **4a**, **4e**, **4f**, **4h**, **5e** and **5f** exhibited good antibacterial activities while **4a** also showed notable antifungal activity. Especially, **5f** exhibited stronger antibacterial activity against *B. subtilis* and *S. aureus* comparable to positive controls.

Keywords: Genistein; Structure modifications; Heterocyclic moieties; Antibacterial activity; Antifungal activity; Structure–activity relationship

1. Introduction

Isoflavonoids are a broad class of polyphenolic secondary metabolites that are abundant in plants and in various common foods such as apples, onions, tea, and red wine. Apart from their important biological roles in nitrogen fixation and chemical defense, isoflavonoids possess a broad range of pharmacological properties, including antioxidant, anticancer and anti-inflammatory properties [1], and hence received considerable therapeutic importance.

Genistein (**1**, shown in Scheme 1), a major metabolite of soy, is reported to have many biological activities including antiestrogenic [2], anti-inflammatory [3], antiproliferative [4], antioxidant [5], antiviral [6], antiallergic [7], antibacterial effects [8], and so on. The versatile biological activities of genistein prompted us to prepare a new series of its derivatives and evaluate their biological significance. Moreover, literature survey revealed that various types of compounds possessing *N*-containing heterocyclic moieties have antimicrobial features [9–11]. Herein, we describe the syntheses of genistein derivatives in which genistein and heterocyclic moieties were linked by spacers, and our investigation of the effects of the size of the spacers and substitution patterns of the heterocyclic moieties.

Our interest in this area is to design and synthesize diverse biologically active genistein derivatives. In continuation of our earlier studies [12–14], in this paper we would like to illustrate the synthesis and antimicrobial activities of three series of analogues of genistein. To our knowledge, this is the first report on the screening of 7-*O*-modified genistein derivatives for their antimicrobial activities.

2. Chemistry

The synthesis of compounds **3a–j** and **5a–j** was accomplished according to the general pathway illustrated in Scheme 1. Compounds **2a–c** were the key intermediates for the synthesis of the compounds investigated. They were usually prepared from alkylation of 7-OH group by using

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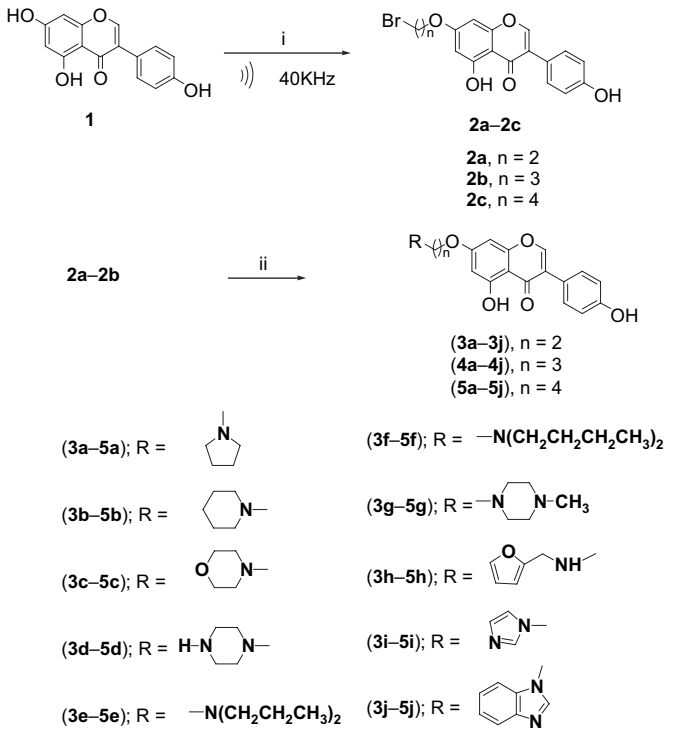

Scheme 1. Synthesis of 7-*O*-heterocycle derivatives of genistein. Reagents and conditions: (i) $BrCH_2CH_2Br$, $BrCH_2CH_2CH_2Br$ or $BrCH_2CH_2CH_2CH_2Br$, K_2CO_3, DMF; (ii) R, K_2CO_3, dioxane, DMF, heating.

1,2-, 1,3- or 1,4-dihaloalkanes in the presence of bases such as NaOH or K_2CO_3 in anhydrous acetone [15,16]. However, to investigate the application of ultrasound in the selective structure modification of natural polyphenols, ultrasound was applied hereby for the semisynthesis of genistein derivatives, and the method [17] we used could, under the same or similar reaction conditions, remarkably reduce the reaction time with discernible improvement in the regioselectivity. Thus, derivatives **2a–c** were prepared through 'step i' under ultrasound irradiation by treating **1** with excessive amounts of 1,2-dibromoethane, 1,3-dibromopropane or 1,4-dibromobutane. To increase the antimicrobial properties of genistein, genistein derivatives in which the genistein ring system is linked to the alkylamines by different spacers at C-7 position were investigated, with a view to modify their lipophilicity. The methods for alkylation of **2a–c** were well-documented [18]. The literature survey indicated that the method reported by Liu et al. [19] is appropriate for *N*-alkylation of imidazole or benzimidazole, since the formation of quaternary imidazolium or benzimidazolium salts, as unwanted side product, was usually limited. In this method, **2a–c** were directly treated with imidazole or benzimidazole in dioxane to afford the expected products **3i,j** and **4i,j**, respectively. Compounds **3a–h** and **4a–h** were synthesized by a convenient synthetic route. Reaction of **2a–c** with different cyclic and noncyclic alkylamines yielded **3a–h** and **4a–h**, respectively.

All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

3. Biological evaluation and discussion

All genistein derivatives were screened for their antimicrobial activities by MTT method against *Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli*, *Pseudomonas fluorescence* (Gram-negative bacteria), and *Trichophyton rubrum*, *Candida albicans* (fungi), which may be the causal agents of some serious infections in humans. The minimal inhibitory concentration (MIC) values for the bacteria and fungi are listed in Table 1. Also included are the activities of reference compounds kanamycin, penicillin (for bacteria) and ketoconazole (for fungi).

A few compounds in series 1, which contain 2-carbon spacer, exhibited moderate activities against *S. aureus*, *B. subtilis* and *P. fluorescence*. In this series, compound **3d** showed pronounced activity against *S. aureus* and *P. fluorescence*. In addition, compound **3a** also showed moderate activity against *P. fluorescence*.

Several compounds in series 2, which contain 3-carbon spacer, displayed good activities against the test microorganisms. In this series, compounds **4e**, **4f** and **4h** showed good activities against *S. aureus*, *B. subtilis* and *P. fluorescence*. Among them **4h** showed strong activity against *S. aureus* with its MIC value (3.4 μg/mL) near to those of positive controls. Notably, compound **4a** exhibiting a broad antimicrobial spectrum against both bacteria and fungi, was the only compound with considerable antifungal activity in this work.

Similarly, several compounds in series 3, which contain 4-carbon spacer, showed good activities against *S. aureus*, *B. subtilis* and *P. fluorescence*. Among them, compounds **5e** and **5f** containing dipropylamine moiety and dibutylamine moiety, respectively, displayed strong activities against these three bacteria. Compound **5f** exhibited remarkable activities against *S. aureus* and *B. subtilis* with the MICs (1.7 μg/mL and 3.7 μg/mL, respectively) comparable to that of penicillin. As shown in Table 1, none of the compounds showed activity against *E. coli* with the concentration lower than 50 μg/mL.

From the results it could be deduced that among the compounds those containing alkyl amino side chains showed better activities against the test bacteria than those containing aromatic ring amino side chains. Especially, the derivatives with diproylamine moiety and dibutylamine moiety were more active than most of the other analogues. A possible explanation for this result is that the lipophilicity of the genistein derivatives affected by their side chains played an important role in their antimicrobial activities.

In conclusion, three series of analogues of genistein were synthesized in approach of new antimicrobial compounds. All compounds were examined for their antimicrobial activities against four bacteria and two fungi, and some structure–activity relationships were explored. Compounds in series 3, which contain 4-carbon spacer between genistein and the aliphatic amines, displayed a good deal of activities. These results open the way for investigation of new potential pharmacophores in the study of antimicrobial agents. The convenient syntheses of these compounds make them potential candidates for further development.

Table 1
Antimicrobial activity of the synthesized compounds

Compound	Minimum inhibitory concentrations (μg/mL)						
	Gram-positive bacteria		Gram-negative bacteria		Fungi		
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas fluorescence*	*Escherichia coli*	*Trichophyton rubrum*	*Candida albicans*	*Aspergillus niger*
1	>50	>50	>50	>50	>50	>50	>50
2a	>50	>50	>50	>50	>50	>50	>50
2b	>50	>50	>50	>50	>50	>50	>50
2c	>50	>50	>50	>50	>50	>50	>50
3a	>50	49.6	28.5	>50	>50	>50	39.8
3b	>50	>50	>50	>50	>50	>50	>50
3c	>50	>50	>50	>50	>50	>50	>50
3d	47.5	17.5	23.5	>50	>50	>50	>50
3e	>50	41.2	47.5	>50	>50	>50	>50
3f	30.0	32.8	37.0	>50	>50	>50	41.6
3g	>50	35.5	>50	>50	>50	>50	>50
3h	>50	>50	>50	>50	>50	>50	>50
3i	>50	>50	>50	>50	>50	>50	>50
3j	>50	>50	>50	>50	>50	>50	>50
4a	48.5	18.5	20.5	>50	21.5	20.5	23.5
4b	>50	>50	>50	>50	>50	>50	>50
4c	>50	>50	>50	>50	>50	>50	>50
4d	>50	40.0	>50	>50	>50	>50	>50
4e	32.0	15.3	45.0	>50	>50	>50	>50
4f	>50	17.1	18.5	>50	>50	>50	>50
4g	43.0	>50	>50	>50	>50	>50	>50
4h	12.5	3.4	10.5	>50	>50	>50	45.5
4i	>50	>50	>50	>50	>50	>50	>50
4j	>50	>50	>50	>50	>50	>50	>50
5a	38.5	23.7	23.1	>50	48.5	>50	>50
5b	>50	>50	>50	>50	>50	>50	>50
5c	35.5	37.5	>50	>50	>50	>50	>50
5d	15.5	20.1	37.5	>50	>50	>50	>50
5e	19.5	10.5	19.5	>50	>50	>50	>50
5f	3.7	1.7	13.2	>50	>50	>50	>50
5g	30.3	27.2	37.4	>50	>50	>50	>50
5h	>50	35.3	12.0	>50	>50	>50	>50
5i	>50	>50	>50	>50	>50	>50	>50
5j	>50	21.3	>50	>50	>50	>50	>50
Ketoconazole					3.9	3.9	7.8
Kanamycin	1.0	1.0	3.9	3.9			
Penicillin	0.78	2.0					

4. Experimental protocols

4.1. General

Reactions and the resulted products were monitored by thin-layer chromatography (TLC) on Merck pre-coated silica gel F254 plates with separated compounds visualized at 254 nm under a UV lamp. Melting points (uncorrected) were determined on an XT4 MP apparatus (Taike Corp, Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker DPX500 or DPX300 spectrometer with solvent signals allotted as internal standard. Elemental analyses were performed on a CHN–O-Rapid instrument and were within ±0.4% of the theoretical values. Sonication was performed in a Kunshan KQ 500E ultrasonic cleaner (Jiangsu, China) with irradiation delivered at 40 kHz and 500 W. The reaction flask was positioned in the maximum-energy area in the cleaner with water circulated around to control the temperature of the water bath.

4.2. Chemistry

The reagents (chemicals), all being of A.R. grade, were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Genistein (**1**, >96%) provided by Shanxi Huike Botanical Development Co. Ltd was further refined prior to use by its recrystallization from ethanol as pale yellow needles, mp: 298–299 °C. ^{1}H NMR (DMSO-d_6): 6.21 (d, $J = 1.9$ Hz, 1H), 6.37 (d, $J = 1.9$ Hz, 1H), 6.80 (d, $J = 8.5$ Hz, 2H), 7.35 (d, $J = 8.5$ Hz, 2H), 8.32 (s, 1H), 9.64 (s, 1H), 10.94 (s, 1H), 12.96 (s, 1H). ESI-MS $C_{15}H_{10}O_5$ $[M-H]^-$ 269. Anal. Calcd for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73. Found: C, 66.68; H, 3.87.

The purity of each active compound mentioned in the study was checked to be >99% through elemental analyses plus HRMS and ^{1}H NMR spectra.

*4.2.1. 7-(2-Bromoethoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**2a**)*

Genistein (0.27 g, 1 mmol), dibromoethane (4.7 g, 25 mmol) and potassium carbonate (0.07 g, 0.5 mmol) in 60 mL of dry DMF were sonicated. After the completion of reaction, the resultant mixture was cooled to room temperature and filtered. The filtrate was distilled to form yellow solid. Recrystallization of the solid from 15 mL acetone gave compound **2a** (0.33 g, 88%) as yellow needles, mp: 175–178 °C. ^{1}H NMR (DMSO-d_6): 4.43 (t, J = 5.5 Hz, 2H), 6.41 (d, J = 2.0 Hz, 1H), 6.67 (d, J = 2.0 Hz, 1H), 6.82 (dd, J = 8.5 Hz, 1.0 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 8.39 (d, J = 1.0 Hz, 1H), 9.64 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{17}H_{13}BrO_5$ $[M-H]^-$ 375. Anal. Calcd for $C_{17}H_{13}BrO_5$: C, 54.13; H, 3.47. Found: C, 54.06; H, 3.51.

*4.2.2. 7-(3-Bromopropoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**2b**)*

Genistein (0.27 g, 1 mmol), 1,3-dibromopropane (5.1 g, 25 mmol) and potassium carbonate (0.07 g, 0.5 mmol) in 60 mL of dry DMF were sonicated. After the completion of reaction, the obtained mixture was cooled to room temperature and filtered. The filtrate is distilled to give yellow solid. Recrystallization of the solid from 15 mL acetone gave compound **2b** (0.34 g, 87%) as yellow needles, mp: 124–126 °C. ^{1}H NMR (DMSO-d_6): 2.25 (m, 2H), 3.65 (t, J = 5.5 Hz, 2H), 4.18 (t, J = 5.5 Hz, 2H), 6.42 (s, 1H), 6.67 (s, 1H), 6.82 (d, J = 8.5 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 8.40 (s, 1H), 9.63 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{18}H_{15}BrO_5$ $[M-H]^-$ 389. Anal. Calcd for $C_{18}H_{15}BrO_5$: C, 55.26; H, 3.86. Found: C, 55.14; H, 3.80.

*4.2.3. 7-(4-Bromobutoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**2c**)*

Genistein (0.27 g, 1 mmol), 1,4-dibromobutane (5.4 g, 25 mmol) and potassium carbonate (0.07 g, 0.5 mmol) in 60 mL of dry DMF were sonicated. After the completion of reaction, the resultant mixture was cooled to room temperature and filtered. The filtrate was distilled to form yellow solid. Recrystallization of the solid from 15 mL acetone gave compound **2c** (0.33 g, 83%) as yellow needles, mp: 130–131 °C. ^{1}H NMR (DMSO-d_6): 1.83 (m, 2H), 1.94 (m, 2H), 3.60 (t, J = 6.2 Hz, 2H), 4.12 (t, J = 6.4 Hz, 2H), 6.40 (d, J = 2.0 Hz, 1H), 6.65 (d, J = 2.0 Hz, 1H), 6.80 (d, J = 8.5 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 8.40 (s, 1H), 9.65 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{19}H_{17}BrO_5$ $[M+H]^+$ 405. Anal. Calcd for $C_{19}H_{17}BrO_5$: C, 56.31; H, 4.23. Found: C, 56.28; H, 4.27.

*4.2.4. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(2-(pyrrolidin-1-yl)ethoxy)-4H-chromen-4-one (**3a**)*

To a solution of **2a** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added pyrrolidine (0.36 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **3a** (0.28 g, 75%), mp: 216–218 °C. ^{1}H NMR (DMSO-d_6): 1.91 (br s, 4H), 3.10 (br s, 2H), 3.59 (s, 4H), 4.48 (t, J = 5.6 Hz, 2H), 6.49 (d, J = 2.0 Hz, 1H), 6.75 (d, J = 2.0 Hz, 1H), 6.83 (d, J = 8.5 Hz, 1.0 Hz, 2H), 7.39 (d, J = 8.5 Hz, 2H), 8.44 (d, J = 1.0 Hz, 1H), 9.66 (s, 1H), 12.97 (s, 1H). ESI-MS $C_{21}H_{21}NO_5$ $[M+H]^+$ 368. Anal. Calcd for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81. Found: C, 68.73; H, 5.62; N, 3.90.

*4.2.5. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(2-(piperidin-1-yl)ethoxy)-4H-chromen-4-one (**3b**)*

To a solution of **2a** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added piperidine (0.43 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **3b** (0.32 g, 85%), mp: 218–220 °C. The purified product was dissolved in HCl/water (1:20) and single crystals (Fig. 1) were obtained after 6 d. ^{1}H NMR (DMSO-d_6): 1.37 (m, 2H), 1.47–1.50 (m, 4H), 2.08 (br s, 4H), 2.67 (t, J = 5.6 Hz, 2H), 4.19 (t, J = 5.6 Hz, 2H), 6.41 (d, J = 2.0 Hz, 1H), 6.67 (d, J = 2.0 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1.0 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 8.39 (d, J = 1.0 Hz, 1H), 9.64 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{22}H_{23}NO_5$ $[M+H]^+$ 382. Anal. Calcd for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 69.31; H, 5.92; N, 3.71.

*4.2.6. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(2-morpholinoethoxy)-4H-chromen-4-one (**3c**)*

To a solution of **2a** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added morpholine (0.44 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to give **3c** (0.31 g, 82%), mp: 174–176 °C. ^{1}H NMR (DMSO-d_6): 2.50 (4H, overlapped with H_2O protons' peak), 2.71 (t, J = 5.6 Hz, 2H), 3.58 (t, J = 5.6 Hz, 4H), 4.22 (t, J = 5.6 Hz, 2H), 6.41 (d, J = 2.0 Hz, 1H), 6.67 (d, J = 2.0 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1.0 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 8.39 (d, J = 1.0 Hz, 1H),

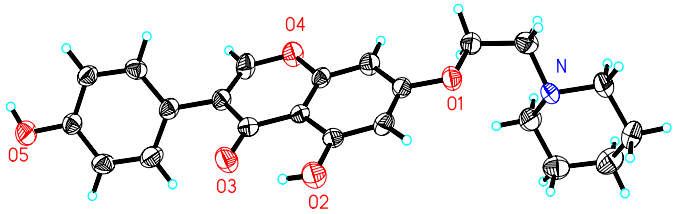

Fig. 1. Molecular structure of compound **3b** with 30% probability of the ellipsoids.

9.64 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{21}H_{21}NO_6$ $[M+H]^+$ 384. Anal. Calcd for $C_{21}H_{21}NO_6$: C, 65.79; H, 5.52; N, 3.65. Found: C, 65.83; H, 5.42; N, 3.69.

4.2.7. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(2-(piperazin-1-yl)ethoxy)-4H-chromen-4-one (**3d**)

To a solution of **2a** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added piperazine (0.43 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **3d** (0.30 g, 78%), mp: 225–226 °C. ^1H NMR (DMSO-d_6): 2.10 (s, 1H), 2.40 (br s, 4H), 2.67–2.72 (m, 6H), 4.19 (t, $J = 9.0$ Hz, 2H), 6.41 (s, 1H), 6.68 (s, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.38 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 9.61 (s, 1H), 12.94 (s, 1H). ESI-MS $C_{21}H_{22}N_2O_5$ $[M+H]^+$ 383. Anal. Calcd for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 65.87; H, 5.86; N, 7.28.

4.2.8. 7-(2-(Dipropylamino)ethoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**3e**)

To a solution of **2a** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added dipropylamine (0.51 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **3e** (0.33 g, 82%), mp: 223–224 °C. ^1H NMR (DMSO-d_6): 0.84 (t, $J = 7.2$ Hz, 6H), 1.39–1.42 (m, 4H), 2.42 (t, $J = 7.1$ Hz, 4H), 2.78 (t, $J = 5.6$ Hz, 2H), 4.13 (t, $J = 5.6$ Hz, 2H), 6.37 (d, $J = 2.0$ Hz, 1H), 6.64 (d, $J = 2.0$ Hz, 1H), 6.83 (d, $J = 8.5$ Hz, 1.0 Hz, 2H), 7.39 (d, $J = 8.5$ Hz, 2H), 8.40 (d, $J = 1.0$ Hz, 1H), 9.60 (s, 1H), 12.92 (s, 1H). ESI-MS $C_{21}H_{21}NO_5$ $[M+H]^+$ 398. Anal. Calcd for $C_{21}H_{21}NO_5$: C, 69.50; H, 6.85; N, 3.52. Found: C, 69.62; H, 6.78; N, 3.59.

4.2.9. 7-(2-(Dibutylamino)ethoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**3f**)

To a solution of **2a** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added dibutylamine (0.65 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **3f** (0.34 g, 81%), mp: 120–121 °C. ^1H NMR (DMSO-d_6): 0.87 (t, $J = 7.1$ Hz, 6H), 1.28 (m, 4H), 1.38 (m, 4H), 2.50 (4H, overlapped with H_2O protons' peak), 2.81 (br s, 2H), 4.15 (br s, 2H), 6.37 (d, $J = 2.0$ Hz, 1H), 6.64 (d, $J = 2.0$ Hz, 1H), 6.83 (d, $J = 8.5$ Hz, 1.0 Hz, 2H), 7.39 (d, $J = 8.5$ Hz, 2H), 8.40 (d, $J = 1.0$ Hz, 1H), 9.60 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{25}H_{31}NO_5$ $[M+H]^+$ 426. Anal. Calcd for $C_{25}H_{31}NO_5$: C, 70.57; H, 7.34; N, 3.29. Found: C, 70.49; H, 7.26; N, 3.32.

4.2.10. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(2-(4-methylpiperazin-1-yl)ethoxy)-4H-chromen-4-one (**3g**)

To a solution of **2a** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added 1-methyl-piperazine (0.50 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **3g** (0.36 g, 91%), mp: 114–115 °C. ^1H NMR (DMSO-d_6): 2.14 (s, 3H), 2.32 (br s, 8H), 2.69 (t, $J = 5.5$ Hz, 2H), 6.40 (s, 1H), 6.67 (s, 1H), 6.82 (d, $J = 8.4$ Hz, 2H), 7.39 (d, $J = 8.4$ Hz, 2H), 8.40 (s, 1H), 9.61 (s, 1H), 12.94 (s, 1H). ESI-MS $C_{22}H_{24}N_2O_5$ $[M+H]^+$ 367. Anal. Calcd for $C_{22}H_{24}N_2O_5$: C, 66.65; H, 6.10; N, 7.07. Found: C, 65.89; H, 6.02; N, 7.76.

4.2.11. 7-(2-(Furan-2-yl-methylamino)ethoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**3h**)

To a solution of **2a** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added 2-furan-2-yl-methylamine (0.49 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **3h** (0.31 g, 79%), mp: 211–212 °C. ^1H NMR (DMSO-d_6): 3.23 (br s, 2H), 4.18 (s, 2H), 4.32 (s, 2H), 6.45 (s, 1H), 6.51 (m, 1H), 6.56 (m, 1H), 6.70 (s, 1H), 6.83 (d, $J = 8.0$ Hz, 2H), 7.39 (d, $J = 8.0$ Hz, 2H), 7.73 (m, 1H), 8.44 (s, 1H), 9.65 (s, 1H), 12.98 (s, 1H). ESI-MS $C_{22}H_{19}NO_6$ $[M+H]^+$ 394. Anal. Calcd for $C_{22}H_{19}NO_6$: C, 67.17; H, 4.87; N, 3.56. Found: C, 67.10; H, 4.76; N, 3.61.

4.2.12. 7-(2-(1H-Imidazol-1-yl)ethoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**3i**)

To a solution of **2a** (0.37 g, 1 mmol) in 20 mL of dioxane was added imidazole (0.41 g, 6 mmol), followed by heating at reflux for 12 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **3i** (0.41 g, 68%), mp: 210–212 °C. ^1H NMR (DMSO-d_6): 4.39 (s, 4H), 6.42 (d, $J = 1.98$ Hz, 1H), 6.67 (d, $J = 1.98$ Hz, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 6.90 (s, 1H), 7.25 (s, 1H), 7.38 (d, $J = 8.5$ Hz, 2H), 7.69 (s, 1H), 8.40 (s, 1H), 9.59 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{20}H_{16}N_2O_5$ $[M+H]^+$ 365. Anal. Calcd for $C_{20}H_{16}N_2O_5$: C, 65.93; H, 4.43; N, 7.69. Found: C, 65.97; H, 4.41; N, 7.68.

4.2.13. 7-(2-(1H-benzimidazolium-1-yl)ethoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**3j**)

To a solution of **2a** (0.37 g, 1 mmol) in 20 mL of dioxane was added benzimidazole (0.71 g, 6 mmol), followed by heating at reflux for 24 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The

mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **3j** (0.27 g, 86%), mp: 230–232 °C. ^1H NMR (DMSO-d_6): 4.47 (br s, 2H), 4.69 (br s, 2H), 6.34 (s, 1H), 6.59 (s, 1H), 6.82 (d, $J = 7.8$ Hz, 2H), 7.21 (t, $J = 7.1$ Hz, 1H), 7.29 (t, $J = 7.1$ Hz, 1H), 7.36 (d, $J = 7.8$ Hz, 2H), 7.64 (d, $J = 7.9$ Hz, 1H), 7.70 (d, $J = 7.9$ Hz, 1H), 8.28 (s, 1H), 8.37 (s, 1H), 9.62 (s, 1H), 12.96 (s, 1H). ESI-MS $C_{24}H_{18}N_2O_5$ $[M+H]^+$ 415. Anal. Calcd for $C_{24}H_{18}N_2O_5$: C, 69.56; H, 4.38; N, 6.76. Found: C, 69.57; H, 4.30; N, 6.77.

4.2.14. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(3-(pyrrolidin-1-yl)propoxy)-4H-chromen-4-one (**4a**)

To a solution of **2b** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added pyrrolidine (0.36 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4a** (0.30 g, 80%), mp: 180–181 °C. ^1H NMR (DMSO-d_6): 1.68 (br s, 4H), 1.89 (m, 2H), 2.43 (br s, 4H), 2.52 (t, $J = 7.2$ Hz, 2H), 4.13 (t, $J = 6.25$ Hz, 2H), 6.38 (s, 1H), 6.64 (s, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.38 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 9.59 (s, 1H), 12.92 (s, 1H). ESI-MS $C_{22}H_{23}NO_5$ $[M+H]^+$ 382. Anal. Calcd for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 69.32; H, 6.01; N, 3.73.

4.2.15. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(3-(piperidin-1-yl)propoxy)-4H-chromen-4-one (**4b**)

To a solution of **2b** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added piperidine (0.43 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4b** (0.34 g, 86%), mp: 192–194 °C. ^1H NMR (DMSO-d_6): 1.37 (br s, 2H), 1.48–1.49 (m, 4H), 1.87 (m, 2H), 2.32–2.34 (m, 4H), 2.37 (t, $J = 7.1$ Hz, 2H), 4.12 (t, $J = 6.0$ Hz, 2H), 6.39 (s, 1H), 6.65 (s, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.40 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 9.59 (s, 1H), 12.92 (s, 1H). ESI-MS $C_{23}H_{25}NO_5$ $[M+H]^+$ 396. Anal. Calcd for $C_{23}H_{25}NO_5$: C, 69.86; H, 6.37; N, 3.54. Found: C, 68.91; H, 6.32; N, 3.61.

4.2.16. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(3-morpholinopropoxy)-4H-chromen-4-one (**4c**)

To a solution of **2b** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added morpholine (0.44 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4c** (0.32 g, 86%), mp: 218–219 °C. ^1H NMR (DMSO-d_6): 1.88 (m, 2H), 2.36–2.37 (m, 4H), 2.41 (t, $J = 7.0$ Hz, 2H), 3.56–3.57 (m, 4H), 4.14 (t, $J = 6.0$ Hz, 2H), 6.39 (s, 1H), 6.65 (s, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.40 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 9.59 (s, 1H), 12.94 (s, 1H). ESI-MS $C_{22}H_{23}NO_6$ $[M+H]^+$ 398. Anal. Calcd for $C_{22}H_{23}NO_6$: C, 66.49; H, 5.83; N, 3.52. Found: C, 66.51; H, 5.76; N, 3.47.

4.2.17. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(3-(piperazin-1-yl)propoxy)-4H-chromen-4-one (**4d**)

To a solution of **2b** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added piperazine (0.43 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4d** (0.30 g, 76%), mp: 210–211 °C. ^1H NMR (DMSO-d_6): 1.87 (m, 2H), 2.10 (s, 1H), 2.30 (br s, 4H), 2.37 (t, $J = 7.0$ Hz, 2H), 2.68 (br s, 4H), 4.12 (t, $J = 6.2$ Hz, 2H), 6.40 (s, 1H), 6.64 (s, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.40 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 9.59 (s, 1H), 12.94 (s, 1H). ESI-MS $C_{22}H_{24}N_2O_5$ $[M+H]^+$ 397. Anal. Calcd for $C_{22}H_{24}N_2O_5$: C, 66.65; H, 6.10; N, 7.07. Found: C, 66.71; H, 6.02; N, 7.13.

4.2.18. 7-(3-(Dipropylamino)propoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**4e**)

To a solution of **2b** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added dipropylamine (0.51 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4e** (0.35 g, 86%), mp: 218–220 °C. ^1H NMR (DMSO-d_6): 0.92 (t, $J = 7.3$ Hz, 6H), 1.66 (m, 4H), 2.13 (m, 2H), 3.06 (br s, 4H), 3.23 (br s, 2H), 4.20 (t, $J = 5.7$ Hz, 2H), 6.43 (s, 1H), 6.67 (s, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.40 (d, $J = 8.5$ Hz, 2H), 8.43 (s, 1H), 9.60 (s, 1H), 12.97 (s, 1H). ESI-MS $C_{24}H_{29}NO_5$ $[M+H]^+$ 412. Anal. Calcd for $C_{24}H_{29}NO_5$: C, 70.05; H, 7.10; N, 3.40. Found: C, 69.93; H, 7.08; N, 3.47.

4.2.19. 7-(3-(Dibutylamino)propoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**4f**)

To a solution of **2b** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added dibutylamine (0.65 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4f** (0.37 g, 85%), mp: 176–178 °C. ^1H NMR (DMSO-d_6): 0.90 (t, $J = 7.1$ Hz, 6H), 1.32 (m, 4H), 1.55 (m, 4H), 2.08 (m, 2H), 2.87 (t, $J = 7.8$ Hz, 2H), 3.11 (br s, 2H), 4.20 (t, $J = 5.6$ Hz, 2H), 6.42 (s, 1H), 6.66 (s, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.40 (d, $J = 8.5$ Hz, 2H), 8.43 (s, 1H), 9.60 (s, 1H), 12.96 (s, 1H). ESI-MS $C_{26}H_{33}NO_5$ $[M+H]^+$ 440.

Anal. Calcd for $C_{26}H_{33}NO_5$: C, 71.05; H, 7.57; N, 3.19. Found: C, 71.12; H, 7.40; N, 3.18.

*4.2.20. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-4H-chromen-4-one (**4g**)*

To a solution of **2b** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added 1-methyl-piperazine (0.50 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4g** (0.37 g, 90%), mp: 110–111 °C. ^{1}H NMR (DMSO-d_6): 1.87 (br s, 2H), 2.15 (s, 3H), 2.40 (br s, 8H), 4.12 (br s, 2H), 6.39 (s, 1H), 6.64 (s, 1H), 6.82 (d, $J = 8.4$ Hz, 2H), 7.38 (d, $J = 8.4$ Hz, 2H), 8.40 (s, 1H), 9.61 (s, 1H), 12.94 (s, 1H). ESI-MS $C_{23}H_{26}N_2O_5$ $[M+H]^+$ 411. Anal. Calcd for $C_{23}H_{26}N_2O_5$: C, 67.30; H, 6.38; N, 6.82. Found: C, 67.21; H, 6.43; N, 6.75.

*4.2.21. 7-(3-(Furan-2-yl-methylamino)propoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**4h**)*

To a solution of **2b** (0.37 g, 1 mmol) in 5 mL of anhydrous DMF was added 2-furan-2-yl-methylamine (0.49 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4h** (0.29 g, 70%), mp: 209–210 °C. ^{1}H NMR (DMSO-d_6): 2.10 (br s, 2H), 3.05 (br s, 2H), 4.18 (s, 2H), 4.25 (s, 2H), 6.40 (s, 1H), 6.51 (m, 1H), 6.64 (m, 2H), 6.83 (d, $J = 8.6$ Hz, 2H), 7.39 (d, $J = 8.6$ Hz, 2H), 7.77 (m, 1H), 8.40 (s, 1H), 9.65 (s, 1H), 12.98 (s, 1H). ESI-MS $C_{23}H_{21}NO_6$ $[M+H]^+$ 408. Anal. Calcd for $C_{23}H_{21}NO_6$: C, 67.80; H, 5.20; N, 3.44. Found: C, 67.72; H, 5.23; N, 3.49.

*4.2.22. 7-(3-(1H-Imidazol-1-yl)propoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**4i**)*

To a solution of **2b** (0.37 g, 1 mmol) in 20 mL of dioxane was added imidazole (0.41 g, 6 mmol), followed by heating at reflux for 12 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4i** (0.26 g, 70%), mp: 230–231 °C. ^{1}H NMR (DMSO-d_6): 2.19 (m, 2H), 4.03 (t, $J = 5.8$ Hz, 2H), 4.14 (t, $J = 6.8$ Hz, 2H), 6.40 (s, 1H), 6.64 (s, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 6.91 (s, 1H), 7.21 (s, 1H), 7.40 (d, $J = 8.5$ Hz, 2H), 7.66 (s, 1H), 8.40 (s, 1H), 9.61 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{21}H_{18}N_2O_5$ $[M+H]^+$ 379. Anal. Calcd for $C_{21}H_{18}N_2O_5$: C, 66.66; H, 4.79; N, 7.40. Found: C, 66.59; H, 4.82; N, 7.46.

*4.2.23. 7-(3-(1H-benzimidazolium-1-yl)propoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**4j**)*

To a solution of **2b** (0.37 g, 1 mmol) in 20 mL of dioxane was added benzimidazole (0.71 g, 6 mmol), followed by heating at reflux for 24 h until the starting material **2b** disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **4j** (0.28 g, 66%), mp: 268–270 °C. ^{1}H NMR (DMSO-d_6): 2.29 (m, 2H), 4.06 (t, $J = 5.4$ Hz, 2H), 4.44 (t, $J = 6.5$ Hz, 2H), 6.38 (s, 1H), 6.60 (s, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.19 (t, $J = 6.8$ Hz, 1H), 7.24 (t, $J = 7.1$ Hz, 1H), 7.40 (d, $J = 8.5$ Hz, 2H), 7.62 (d, $J = 7.9$ Hz, 1H), 7.65 (d, $J = 7.9$ Hz, 1H), 8.23 (s, 1H), 8.40 (s, 1H), 9.60 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{25}H_{20}N_2O_5$ $[M+H]^+$ 429. Anal. Calcd for $C_{25}H_{20}N_2O_5$: C, 70.08; H, 4.71; N, 6.54. Found: C, 70.15; H, 4.64; N, 6.50.

*4.2.24. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(4-(pyrrolidin-1-yl)butoxy)-4H-chromen-4-one (**5a**)*

To a solution of **2c** (0.40 g, 1 mmol) in 5 mL of anhydrous DMF was added pyrrolidine (0.36 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **5a** (0.28 g, 70%), mp: 190 °C. ^{1}H NMR (DMSO-d_6): 1.74–1.89 (m, 8H), 2.87–2.96 (m, 6H), 4.10 (t, $J = 6.3$ Hz, 2H), 6.40 (d, $J = 2.0$ Hz, 1H), 6.65 (d, $J = 2.0$ Hz, 1H), 6.81 (d, $J = 8.5$ Hz, 2H), 7.38 (d, $J = 8.5$ Hz, 2H), 8.39 (s, 1H), 9.65 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{23}H_{25}NO_5$ $[M+H]^+$ 396. Anal. Calcd for $C_{23}H_{25}NO_5$: C, 69.86; H, 6.37; N, 3.54. Found: C, 69.80; H, 6.31; N, 3.61.

*4.2.25. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(4-(piperidin-1-yl)butoxy)-4H-chromen-4-one (**5b**)*

To a solution of **2c** (0.40 g, 1 mmol) in 5 mL of anhydrous DMF was added piperidine (0.43 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated .The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **5b** (0.35 g, 86%), mp: 176 °C. ^{1}H NMR (DMSO-d_6): 1.35–1.55 (m, 8H), 1.69 (m, 2H), 2.22–2.27 (m, 6H), 4.08 (t, $J = 6.4$ Hz, 2H), 6.38 (d, $J = 2.0$ Hz, 1H), 6.62 (d, $J = 2.0$ Hz, 1H), 6.80 (d, $J = 8.5$ Hz, 2H), 7.37 (d, $J = 8.5$ Hz, 2H), 8.39 (s, 1H), 9.63 (s, 1H), 12.94 (s, 1H). ESI-MS $C_{24}H_{27}NO_5$ $[M+H]^+$ 410. Anal. Calcd for $C_{24}H_{27}NO_5$: C, 70.40; H, 6.65; N, 3.42. Found: C, 70.35; H, 6.68; N, 3.47.

*4.2.26. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(4-morpholinobutoxy)-4H-chromen-4-one (**5c**)*

To a solution of **2c** (0.40 g, 1 mmol) in 5 mL of anhydrous DMF was added morpholine (0.44 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to give **5c**

(0.34 g, 83%), mp: 178–179 °C. ^{1}H NMR (DMSO-d_6): 1.58 (m, 2H), 1.75 (m, 2H), 2.30–2.34 (m, 6H), 3.57 (m, 6H), 4.11 (t, $J = 6.3$ Hz, 2H), 6.39 (d, $J = 2.0$ Hz, 1H), 6.64 (d, $J = 2.0$ Hz, 1H), 6.80 (d, $J = 8.5$ Hz, 2H), 7.38 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 9.61 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{23}H_{25}NO_6$ $[M + H]^+$ 412. Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.21; H, 6.03; N, 3.47.

*4.2.27. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(4-(piperazin-1-yl)butoxy)-4H-chromen-4-one (**5d**)*

To a solution of **2c** (0.40 g, 1 mmol) in 5 mL of anhydrous DMF was added piperazine (0.43 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **5d** (0.31 g, 75%), mp: 170–171 °C. ^{1}H NMR (DMSO-d_6): 1.54 (m, 2H), 1.72 (m, 2H), 2.24–2.27 (m, 6H), 2.66 (m, 4H), 4.09 (t, $J = 6.3$ Hz, 2H), 6.39 (d, $J = 2.0$ Hz, 1H), 6.64 (d, $J = 2.0$ Hz, 1H), 6.80 (d, $J = 8.5$ Hz, 2H), 7.38 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 9.61 (s, 1H), 12.95 (s, 1H). ESI-MS $C_{23}H_{26}N_2O_5$ $[M + H]^+$ 411. Anal. Calcd for $C_{23}H_{26}N_2O_5$: C, 67.30; H, 6.38; N, 6.82. Found: C, 67.20; H, 6.40; N, 6.85.

*4.2.28. 7-(4-(Dipropylamino)butoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**5e**)*

To a solution of **2c** (0.40 g, 1 mmol) in 5 mL of anhydrous DMF was added dipropylamine (0.51 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **5e** (0.34 g, 80%), mp: 132 °C. ^{1}H NMR (DMSO-d_6): 0.82 (t, $J = 7.2$ Hz, 6H), 1.35 (m, 4H), 1.50 (m, 2H), 1.70 (m, 2H), 2.28 (t, $J = 7.0$ Hz, 4H), 2.37 (t, $J = 6.9$ Hz, 2H), 4.06 (t, $J = 6.5$ Hz, 2H), 6.37 (d, $J = 2.0$ Hz, 1H), 6.62 (d, $J = 2.0$ Hz, 1H), 6.80 (d, $J = 8.5$ Hz, 2H), 7.36 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 9.61 (s, 1H), 12.92 (s, 1H). ESI-MS $C_{25}H_{31}NO_5$ $[M + H]^+$ 426. Anal. Calcd for $C_{25}H_{31}NO_5$: C, 70.57; H, 7.34; N, 3.29. Found: C, 70.60; H, 7.30; N, 3.37.

*4.2.29. 7-(4-(Dibutylamino)butoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**5f**)*

To a solution of **2c** (0.40 g, 1 mmol) in 5 mL of anhydrous DMF was added dibutylamine (0.65 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **5f** (0.37 g, 82%), mp: 108 °C. ^{1}H NMR (DMSO-d_6): 0.88 (t, $J = 7.2$ Hz, 6H), 1.30 (m, 4H), 1.58 (m, 4H), 1.77 (m, 4H), 3.00 (m, 4H), 3.09 (m, 2H), 4.11 (t, $J = 6.5$ Hz, 2H), 6.39 (d, $J = 2.0$ Hz, 1H), 6.64 (d, $J = 2.0$ Hz, 1H), 6.80 (d, $J = 8.5$ Hz, 2H), 7.36 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 9.61 (s, 1H), 12.92 (s, 1H). ESI-MS $C_{27}H_{35}NO_5$ $[M + H]^+$ 454. Anal. Calcd for $C_{27}H_{35}NO_5$: C, 71.50; H, 7.18; N, 3.09. Found: C, 71.42; H, 7.24; N, 3.18.

*4.2.30. 5-Hydroxy-3-(4-hydroxyphenyl)-7-(4-(4-methylpiperazin-1-yl)butoxy)-4H-chromen-4-one (**5g**)*

To a solution of **2c** (0.40 g, 1 mmol) in 5 mL of anhydrous DMF was added 1-methyl-piperazine (0.50 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **5g** (0.36 g, 85%), mp: 140 °C. ^{1}H NMR (DMSO-d_6): 1.54 (m, 2H), 1.70 (m, 2H), 2.12 (s, 3H), 2.26–2.31 (m, 8H), 2.50 (overlapped with solvent, 2H), 4.08 (t, $J = 6.5$ Hz, 2H), 6.39 (d, $J = 2.0$ Hz, 1H), 6.64 (d, $J = 2.0$ Hz, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.38 (d, $J = 8.5$ Hz, 2H), 8.39 (s, 1H), 9.61 (s, 1H), 12.92 (s, 1H). ESI-MS $C_{24}H_{28}N_2O_5$ $[M + H]^+$ 425. Anal. Calcd for $C_{24}H_{28}N_2O_5$: C, 67.91; H, 6.65; N, 6.60. Found: C, 67.85; H, 6.69; N, 6.68.

*4.2.31. 7-(4-(Furan-2-yl-methylamino)butoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**5h**)*

To a solution of **2c** (0.40 g, 1 mmol) in 5 mL of anhydrous DMF was added 2-furan-2-yl-methylamine (0.49 g, 5 mmol), followed by heating at 80 °C for 1 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **5h** (0.31 g, 74%), mp: 121 °C. ^{1}H NMR (DMSO-d_6): 1.54 (m, 2H), 1.72 (m, 2H), 2.50 (overlapped with solvent, 2H), 3.64 (s, 2H), 4.07 (t, $J = 6.5$ Hz, 2H), 6.22 (d, $J = 2.0$ Hz, 1H), 6.38 (m, 2H), 6.62 (d, $J = 2.0$ Hz, 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 7.38 (d, $J = 8.5$ Hz, 2H), 7.52 (m, 1H), 8.37 (s, 1H), 9.60 (s, 1H), 12.93 (s, 1H). ESI-MS $C_{24}H_{23}NO_6$ $[M + H]^+$ 422. Anal. Calcd for $C_{24}H_{23}NO_6$: C, 68.40; H, 5.50; N, 3.32. Found: C, 68.47; H, 5.53; N, 3.28.

*4.2.32. 7-(4-(1H-Imidazol-1-yl)butoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**5i**)*

To a solution of **2c** (0.40 g, 1 mmol) in 20 mL of dioxane was added imidazole (0.41 g, 6 mmol), followed by heating at reflux for 12 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **5i** (0.24 g, 62%), mp: 234 °C. ^{1}H NMR (DMSO-d_6): 1.63 (m, 2H), 1.83 (m, 2H), 4.01 (t, $J = 6.2$ Hz, 2H), 4.07 (t, $J = 6.5$ Hz, 2H), 6.37 (d, $J = 2.0$ Hz, 1H), 6.61 (d, $J = 2.0$ Hz, 1H), 6.80 (d, $J = 8.5$ Hz, 2H), 6.88 (br s, 1H), 7.18 (br s, 1H), 7.37 (d, $J = 8.5$ Hz, 2H), 7.64 (br s, 1H), 8.37 (s, 1H), 9.60 (s, 1H), 12.93 (s, 1H). ESI-MS $C_{22}H_{20}N_2O_5$ $[M + H]^+$ 393. Anal.

Calcd for $C_{22}H_{20}N_2O_5$: C, 67.34; H, 5.14; N, 7.14. Found: C, 67.40; H, 5.21; N, 7.06.

4.2.33. 7-(4-(1H-benzimidazolium-1-yl)butoxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (**5j**)

To a solution of **2c** (0.40 g, 1 mmol) in 20 mL of dioxane was added benzoimidazole (0.71 g, 6 mmol), followed by heating at reflux for 24 h until the starting material disappeared. To the reaction mixture was added ice water, dropwise. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with CH_2Cl_2/CH_3OH 9:1 to afford **5j** (0.36 g, 81%), mp: 261 °C. ^{1}H NMR (DMSO-d_6): 2.28 (m, 2H), 2.50 (overlapped with solvent, 2H), 4.02 (t, $J = 6.2$ Hz, 2H), 4.07 (t, $J = 6.5$ Hz, 2H), 6.36 (d, $J = 2.0$ Hz, 1H), 6.58 (d, $J = 2.0$ Hz, 1H), 6.81 (d, $J = 8.5$ Hz, 2H), 7.19–7.25 (m, 2H), 7.38 (d, $J = 8.5$ Hz, 2H), 7.60–7.65 (m, 2H), 8.23 (s, 1H), 8.38 (s, 1H), 9.60 (s, 1H), 12.93 (s, 1H). ESI-MS $C_{26}H_{22}N_2O_5$ $[M+H]^+$ 443. Anal. Calcd for $C_{26}H_{22}N_2O_5$: C, 70.58; H, 5.01; N, 6.33. Found: C, 70.65; H, 4.92; N, 6.35.

4.3. Antimicrobial activity

The antibacterial activity of the synthesized compounds was tested against *B. subtilis*, *E. coli*, *P. fluorescence* and *S. aureus* using MH medium. The antifungal activity of the compounds was tested against *Aspergillus niger*, *C. albicans* and *T. rubrum* using RPMI-1640 medium. The MICs of the test compounds were determined by a colorimetric method using the dye MTT [20]. A stock solution of the synthesized compound (50 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid medium (MH medium for antibacterial activity and RPMI-1640 medium for antifungal activity). A specified quantity of the (10 μL) medium (with or without the compound) was poured into microtitration plates (the one without the compound was used to test whether the dye MTT affects growth of the microorganism). A suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL, and 90 μL suspension was applied to microtitration plates with serially diluted compounds in DMSO to be tested (each concentration was repeated 3 times) and incubated at 37 °C for 24 h and 48 h for antibacterial and antifungal activities, respectively. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm. The observed MICs are presented in Table 1.

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