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Short communication

Synthesis and biological evaluation of novel luteolin derivatives as antibacterial agents

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Abstract

A series of luteolin derivatives **2**–**20** were prepared, **3**–**20** of which were first reported. The chemical structures of these compounds were confirmed by means of ^{1}H NMR, ESI-MS and elemental analyses. The compounds were assayed for antibacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescens* and *Escherichia coli*) activities by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl trtrazolium bromide) method. Among the compounds tested, most of them displayed significant activity against the tested strains, and 2-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-hydroxy-7-(2-(3-morpholinopropylamino)ethoxy)-4*H*-chromen-4-one (**17**) showed the most favorable antibacterial activity *in vitro* with MICs of 1.562, 3.125, 3.125, and 6.25 μg/mL against *B. subtilis, S. aureus, P. fluorescens and E. coli*, respectively. Structure–activity relationships (SAR) were also discussed based on the obtained experimental data.
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1. Introduction

Luteolin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one), a polyphenolic compound available in foods of plant origin, belongs to the flavone subclass of flavonoids, usually occurring as glycosylated forms in celery, green pepper, perilla leaf and camomile tea [1–3]. It has been reported to have many different biological activities such as antimutagenic, antiplatelet aggregation [4], antitumorigenic, antioxidant and anti-inflammatory properties [1]. Luteolin can also display anticancer effect [5], and inhibit a series of human cancer cell lines (renal A-549, ovary SK-OV-3, melanoma SK-MEL-2, XF-498, HCT15, gastric HGC-27) [6]. In addition, luteolin has been shown to have antibacterial activity against a number of bacteria [7–9], however, few reports have been dedicated to the improvement of the antibacterial activities and the structure–activity relationships of luteolin derivatives. Based on our recent efforts on the improvement of isoflavonoids [10–12], our research interest now is focused on the modification of luteolin.

It is reported that the 2,3-dihydro-1,4-benzodioxin nucleus bearing an acidic acetic moiety conferred anti-inflammatory activity as non-steroidal acid anti-inflammatory drugs (NSAIDS) [13]. Furthermore, it is also found that ethyl 1,4-benzodioxan-2-carboxylate is used as an intermediate compound for the production of drug doxazosin mesylate, which is a drug for the treatment of hypertension and benign prostatic hyperplasia [14]. It belongs to the quinazoline group of drugs and is presently produced in the racemic form [15]. In addition, strobilurin E, which has a similar 1,4-benzodioxan structure, has been reported to have strong antifungal activity [16].

In view of realizing these results and the special structure of luteolin which has two adjacent phenolic hydroxyl in the B ring, we have synthesized a series of luteolin derivatives which have the potential pharmacophore 1,4-benzodioxin and 2-carbon spacer at C-7 position. At the same time, these derivatives of luteolin were assayed for antibacterial (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas fluorescens*

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and Escherichia coli) activities by MTT method. Fortunately, it was found that some compounds displayed high antibacterial activities against the tested strains *in vitro*.

2. Chemistry

In this paper a series of luteolin derivatives containing a 2-carbon spacer at C-7 position are prepared, which also have potential pharmacophore 1,4-benzodioxin. The synthesis of compounds **2**–**20** followed the general pathway illustrated in Scheme 1. Compounds **2** and **3** were the key intermediates for synthesis of the compounds investigated. Treating compound **1** with 1,2-dibromoethane at 70 °C for 30 min in anhydrous DMF catalyzed by potassium carbonate yielded compound **2**, which has the potential pharmacophore 1,4-benzodioxin. Secondly, in order to increase the antibacterial properties of luteolin, whose ring A system linked to the alkylamine by a 2-carbon spacer at C-7 position was investigated, with a view to modify their lipophilicity. Thus, derivative **3** was prepared through 'step ii' by treating compound **2** with excessive amounts of 1,2-dibromoethane at 120 °C for 2 h in anhydrous DMF catalyzed by potassium carbonate. Thirdly, reaction of **3** with different alkylamines and aromatic amines in anhydrous DMF at 80 °C for 1 h yielded compounds **4**–**20**. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

3. Results and discussion

All the synthesized compounds were screened for antibacterial activity against two Gram-positive bacterial strains (*B. subtilis* and *S. aureus*) and two Gram-negative bacterial strains (*E. coli* and *P. fluorescens*) by MTT method. The MICs (minimum inhibitory concentrations) of the compounds against four bacteria are presented in Table 1.

Compounds **13**, **16** and **17** displayed prominent activity equal to that of penicillin G against *B. subtilis* (1.562 μg/mL) while compounds **10**–**12**, **14**, **15** and **18**–**20** exhibited moderate activity (3.125–6.25 μg/mL). Compounds **13**, **17** showed significant activity against *S. aureus* (3.125 μg/mL) while compounds **12**, **15** and **16** displayed moderate activity (6.25 μg/mL). Compounds **16** and **17** showed highest activity equal to that of kanamycin B against *P. fluorescens* (3.125 μg/mL) while compounds **5**, **7**, **9**, **12**, **13**, **15**, **19** and **20** exhibited moderate activity (6.25 μg/mL). Compounds **13** and **17** showed good activity against *E. coli* (6.25 μg/mL) while compounds **12**–**16** and **18**–**20** displayed moderate activity (12.5 μg/mL). In addition, compound **2** which has the structure of 1,4-benzodioxin showed better antibacterial activity against the four tested strains compared to luteolin.

Among compounds prepared, compounds **12** and **13** showed higher antibacterial activity than lots of other compounds. This result disclosed that compounds with aromatic rings at C-7 position of luteolin were more active than compounds with aliphatic chains. Compounds **15**–**17** had a slight difference in the structure, all of them contained a morpholine ring, but their antibacterial activity was different. Compound **17** displayed significant activity while **15** exhibited moderate activity compared to the former. A possible explanation for this result is the former had better lipophilicity than the latter.

Among all the synthetic compounds, two species can be divided, one containing heterocyclic moieties, including compounds **10**, **11** and **14**–**20**, and compounds **4**–**9** containing aliphatic chain substituents. From Table 1 we can see that compounds **10**, **11** and **14**–**20** showed obvious antibacterial activity against the tested strains, while **4**–**9** show moderate antibacterial activity. So the species containing heterocyclic substituents have a much higher inhibitory potency than the other species.

All the compounds prepared contain a 2-carbon spacer at C-7 position. This modification is likely to make the compounds more lipophilic, which may increase their permeability of the cell membrane. The biological activity of a particular substance depends on a complex sum of individual properties including compound structure, affinity for the target site, and survival in the medium of application, survival within the biological system, transport properties, and state of the target organism [17]. In this study, we focused our attention on the structure–activity relationships. Structural analysis of these compounds may provide some explanation for the structure–activity relationships.

4. Conclusions

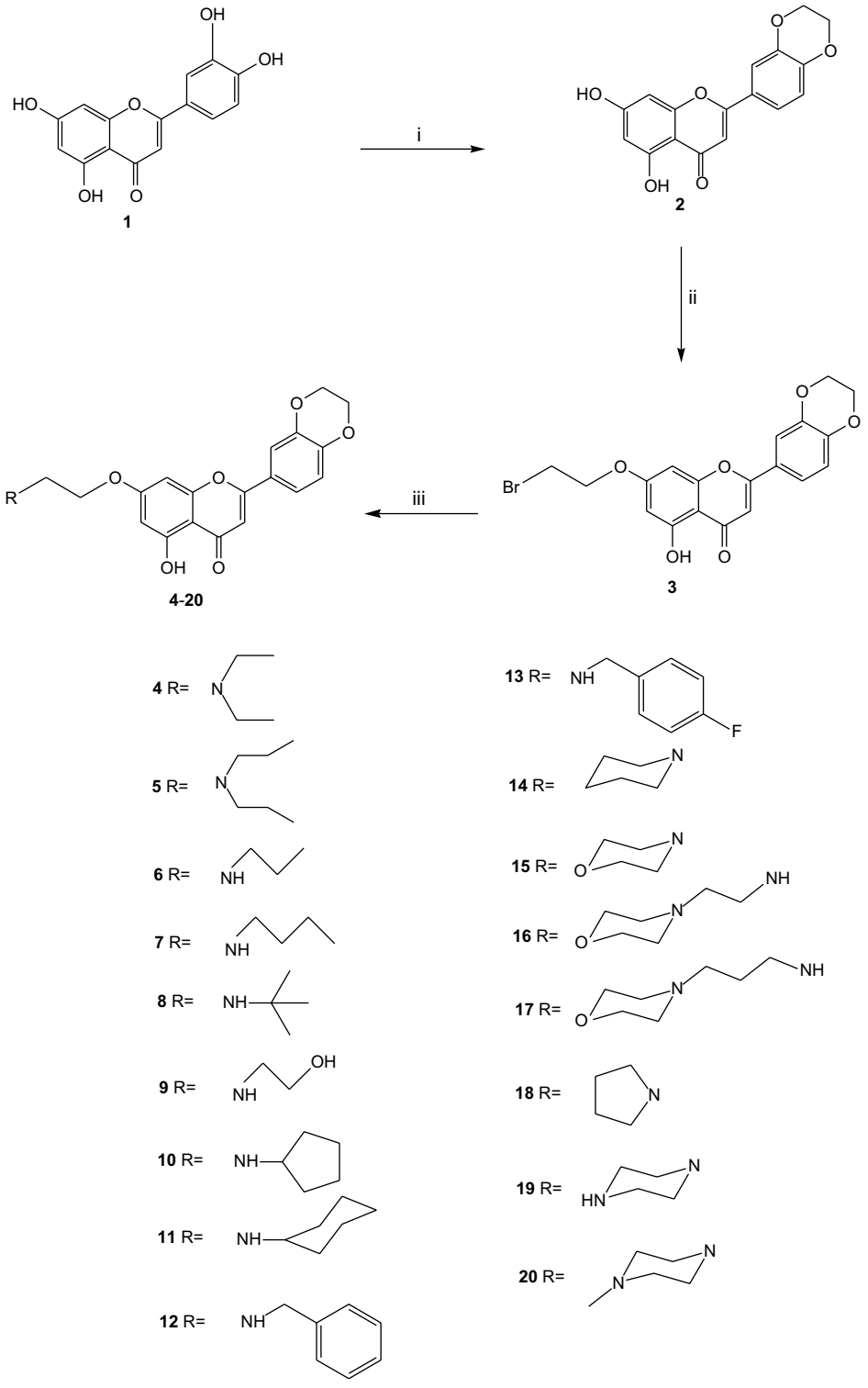
In this paper a series of luteolin derivatives containing a 2-carbon spacer at C-7 position are prepared and evaluated for antibacterial activity against *B. subtilis*, *S. aureus*, *P. fluorescens* and *E. coli*. Bioassays indicated that some of the compounds showed high antibacterial activity *in vitro*, especially morpholine derivative **17** was found to be more active than others at an MIC value of 1.562, 3.125, 3.125, and 6.25 μg/mL against *B. subtilis, S. aureus, P. fluorescens* and *E. coli*, respectively. The study on structure–activity relationships of these luteolin derivatives indicated that the hydrophilicity and aromaticity seemed to be important for the antibacterial activity.

Further SAR studies and mechanistic studies on this new class of antibacterial compounds are currently under active investigation and will be reported in due course.

5. Experimental protocols

5.1. Chemistry

Reaction 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 a 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



Scheme 1. Synthesis route of luteolin derivatives. Reagents and conditions: (i) $BrCH_2CH_2Br$, K_2CO_3, DMF, 70 °C, 30 min; (ii) $BrCH_2CH_2Br$, K_2CO_3, DMF, 120 °C, 2 h; (iii) alkylamines and aromatic amines, DMF, 80 °C, 1 h.

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. The reaction flask was positioned in the maximum-energy area in the cleaner with cycled water running to control the temperature of the water bath. The reagents (chemicals), all being of AR grade, were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Luteolin (**1**, >96%, mp 328–330 °C) provided by Shanxi Huike Botanical Development Co. Ltd. was used without purification. The antibacterial activity of reference compounds kanamycin B (Nanjing Zhuyan Biotechnology Co. Ltd, Amresco 060D0504, Nanjing

Table 1
Minimum inhibitory concentrations (MIC − μg/mL) of luteolin derivatives

Compound	Microorganisms			
	Gram-positive		Gram-negative	
	B. subtilis	*S. aureus*	*P. fluorescens*	*E. coli*
1	25	50	25	50
2	12.5	25	12.5	50
3	12.5	25	12.5	25
4	12.5	25	12.5	25
5	12.5	12.5	6.25	25
6	12.5	25	12.5	25
7	12.5	25	6.25	25
8	12.5	25	12.5	25
9	12.5	25	6.25	25
10	6.25	12.5	12.5	25
11	6.25	12.5	12.5	25
12	3.125	6.25	6.25	12.5
13	1.562	3.125	6.25	12.5
14	6.25	12.5	12.5	12.5
15	3.125	6.25	6.25	12.5
16	1.562	6.25	3.125	12.5
17	1.562	3.125	3.125	6.25
18	6.25	12.5	12.5	12.5
19	6.25	12.5	6.25	12.5
20	6.25	12.5	6.25	12.5
Kanamycin B	0.39	1.562	3.125	3.125
Penicillin G	1.562	1.562	6.25	6.25

210002, China) and penicillin G (North China Pharmaceutical Co. Ltd, D0211107, Hebei 050015, China) were included.

5.2. Experimental procedure for the synthesis of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5,7-dihydroxy-4H-chromen-4-one (2)

To a solution of **1** (0.286 g, 1 mmol) in 20 mL of anhydrous DMF was added 1,2-dibromoethane (0.38 g, 2 mmol) and potassium carbonate (0.07 g, 0.5 mmol), followed by heating at 70 °C for 30 min. 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 ethyl acetate:petroleum ether = 1:8 to afford **2**, yellow powder, yield 78%, mp: 307−309 °C. ^{1}H NMR (DMSO-d_6): 4.32 (d, $J = 5.0$ Hz, 4H), 6.19 (d, $J = 1.8$ Hz, 1H), 6.50 (d, $J = 2.1$ Hz, 1H), 6.85 (s, 1H), 7.01 (d, $J = 8.1$ Hz, 1H), 7.56 (d, $J = 7.8$ Hz, 2H), 10.82 (s, 1H), 12.88 (s, 1H). ESI-MS: 313.1 ($[M + H]^+$). Anal. Calcd for $C_{17}H_{12}O_6$: C, 65.39%; H, 3.87%. Found: C, 65.57; H, 3.76%.

5.3. Experimental procedure for the synthesis of 7-(2-bromoethoxy)-2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-4H-chromen-4-one (3)

To a solution of **2** (0.312 g, 1 mmol) in 20 mL of anhydrous DMF was added 1,2-dibromoethane (4.7 g, 25 mmol) and potassium carbonate (0.14 g, 1 mmol), followed by heating at 120 °C for 2 h until the starting material **2** disappeared. To the reaction mixture was added ice water, dropwise. The mixture was distilled to form yellow solid. Recrystallization of the solid from 20 mL acetone gave compound **3**, yellow powder, 0.282 g, yield 85%, mp: 278−279 °C. ^{1}H NMR (DMSO-d_6): 3.83 (t, $J = 5.0$ Hz, 2H), 4.32 (dd, $J = 5.0$ Hz, 8.5 Hz, 4H), 4.45 (t, $J = 8.5$ Hz, 2H), 6.39 (d, $J = 2.0$ Hz, 1H), 6.86 (d, $J = 2.0$ Hz, 1H), 6.93 (s, 1H), 7.03 (d, $J = 8.1$ Hz, 1H), 7.63 (d, $J = 7.8$ Hz, 2H), 12.88 (s, 1H). ESI-MS: 419.1 ($[M + H]^+$). Anal. Calcd for $C_{19}H_{15}BrO_6$: C, 54.43%; H, 3.61%. Found: C, 54.53%; H, 3.71%.

5.4. General experimental procedure for the synthesis of compounds 4–20

To a solution of **3** (0.418 g, 1 mmol) in 20 mL of anhydrous DMF was added different alkylamines and aromatic amines (10 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 ethyl acetate:petroleum ether = 1:8 to afford corresponding products.

5.4.1. Synthesis of 7-(2-(diethylamino)ethoxy)-2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-4H-chromen-4-one (4)

Yellow powder, yield 80%, mp: 147−149 °C. ^{1}H NMR (DMSO-d_6): 1.06 (t, $J = 7.0$ Hz, 6H), 3.17−3.20 (m, 4H), 3.44 (t, $J = 5.0$ Hz, 2H), 4.20 (d, $J = 5.0$ Hz, 4H), 4.50 (t, $J = 8.5$ Hz, 2H), 6.38 (d, $J = 2.0$ Hz, 1H), 6.87 (d, $J = 2.0$ Hz, 1H), 6.94 (s, 1H), 7.04 (d, $J = 8.1$ Hz, 1H), 7.63 (d, $J = 7.8$ Hz, 2H), 12.90 (s, 1H). ESI-MS: 412.1 ($[M + H]^+$). Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14%; H, 6.12%; N, 3.40%. Found: C, 67.28%; H, 6.24%; N, 3.34%.

5.4.2. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]-dioxin-6-yl)-7-(2-(dipropylamino)ethoxy)-5-hydroxy-4H-chromen-4-one (5)

Yellow powder, yield 82%, mp: 156−157 °C. ^{1}H NMR (DMSO-d_6): 0.90 (t, $J = 7.2$ Hz, 6H), 1.42−1.44 (m, 4H), 2.46 (t, $J = 7.1$ Hz, 4H), 3.43 (t, $J = 5.0$ Hz, 2H), 4.32 (d, $J = 5.0$ Hz, 4H), 4.50 (t, $J = 8.5$ Hz, 2H), 6.38 (d, $J = 2.0$ Hz, 1H), 6.86 (d, $J = 2.0$ Hz, 1H), 6.94 (s, 1H), 7.05 (d, $J = 8.1$ Hz, 1H), 7.65 (d, $J = 7.8$ Hz, 2H), 12.87 (s, 1H). ESI-MS: 440.2 ($[M + H]^+$). Anal. Calcd for $C_{25}H_{29}NO_6$: C, 68.32%; H, 6.65%; N, 3.19%. Found: C, 68.45%; H, 6.58%; N, 3.24%.

5.4.3. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]-dioxin-6-yl)-5-hydroxy-7-(2-(propylamino)ethoxy)-4H-chromen-4-one (6)

Yellow powder, yield 81%, mp: 160−162 °C. ^{1}H NMR (DMSO-d_6): 0.97 (t, $J = 7.2$ Hz, 3H), 1.46 (m, 2H), 2.57 (t, $J = 7.1$ Hz, 2H), 3.64 (t, $J = 5.0$ Hz, 2H), 4.13 (d, $J = 5.0$ Hz, 4H), 4.34 (t, $J = 8.5$ Hz, 2H), 6.37 (d, $J = 2.0$ Hz, 1H), 6.83 (d, $J = 2.0$ Hz, 1H), 6.92 (s, 1H), 7.05 (d, $J = 8.1$ Hz, 1H), 7.62 (d, $J = 7.8$ Hz, 2H), 12.88 (s, 1H). ESI-MS: 398.1

($[M+H]^+$). Anal. Calcd for $C_{22}H_{23}NO_6$: C, 66.49%; H, 5.83%; N, 3.52%. Found: C, 66.57%; H, 5.95%; N, 3.47%.

*5.4.4. Synthesis of 7-(2-(butylamino)ethoxy)-2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-4H-chromen-4-one (**7**)*

Yellow powder, yield 83%, mp: 166–167 °C. ^{1}H NMR (DMSO-d_6): 0.90 (t, $J=7.2$ Hz, 3H), 1.30–1.34 (m, 2H), 1.52–1.57 (m, 2H), 2.08 (t, $J=7.1$ Hz, 2H), 3.66 (t, $J=5.0$ Hz, 2H), 4.16 (d, $J=5.0$ Hz, 4H), 4.38 (t, $J=8.5$ Hz, 2H), 6.38 (d, $J=2.0$ Hz, 1H), 6.83 (d, $J=2.0$ Hz, 1H), 6.92 (s, 1H), 7.02 (d, $J=8.1$ Hz, 1H), 7.61 (d, $J=7.8$ Hz, 2H), 12.90 (s, 1H). ESI-MS: 412.1 ($[M+H]^+$). Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14%; H, 6.12%; N, 3.40%. Found: C, 67.25%; H, 6.24%; N, 3.37%.

*5.4.5. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]-dioxin-6-yl)-5-hydroxy-7-(2-(isobutylamino)ethoxy)-4H-chromen-4-one (**8**)*

Yellow powder, yield 83%, mp: 168–170 °C. ^{1}H NMR (DMSO-d_6): 0.91 (t, $J=7.1$ Hz, 6H), 1.62–1.65 (m, 1H), 2.52 (t, $J=7.1$ Hz, 2H), 3.44 (t, $J=5.0$ Hz, 2H), 4.20 (d, $J=5.0$ Hz, 4H), 4.50 (t, $J=8.5$ Hz, 2H), 6.38 (d, $J=2.0$ Hz, 1H), 6.87 (d, $J=2.0$ Hz, 1H), 6.94 (s, 1H), 7.04 (d, $J=8.1$ Hz, 1H), 7.63 (d, $J=7.8$ Hz, 2H), 12.88 (s, 1H). ESI-MS: 412.1 ($[M+H]^+$). Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14%; H, 6.12%; N, 3.40%. Found: C, 67.28%; H, 6.29%; N, 3.32%.

*5.4.6. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]-dioxin-6-yl)-5-hydroxy-7-(2-(2-hydroxyethylamino)ethoxy)-4H-chromen-4-one (**9**)*

Yellow powder, yield 75%, mp: 180–182 °C. ^{1}H NMR (DMSO-d_6): 2.66 (t, $J=6.5$ Hz, 2H); 2.91 (t, $J=6.5$ Hz, 2H), 3.43 (t, $J=5.0$ Hz, 2H), 4.32 (d, $J=5.0$ Hz, 4H), 4.50 (t, $J=8.5$ Hz, 2H), 6.38 (d, $J=2.0$ Hz, 1H), 6.86 (d, $J=2.0$ Hz, 1H), 6.94 (s, 1H), 7.05 (d, $J=8.1$ Hz, 1H), 7.65 (d, $J=7.8$ Hz, 2H), 12.87 (s, 1H). ESI-MS: 400.1 ($[M+H]^+$). Anal. Calcd for $C_{21}H_{21}NO_7$: C, 63.15%; H, 5.30%; N, 3.51%. Found: C, 63.27%; H, 5.42%; N, 3.62%.

*5.4.7. Synthesis of 7-(2-(cyclopentylamino)ethoxy)-2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-4H-chromen-4-one (**10**)*

Yellow powder, yield 84%, mp: 131–132 °C. ^{1}H NMR (DMSO-d_6): 1.46–1.56 (m, 4H), 1.71–1.78 (m, 4H), 2.64 (s, 1H), 3.44 (t, $J=5.0$ Hz, 2H), 4.20 (d, $J=5.0$ Hz, 4H), 4.50 (t, $J=8.5$ Hz, 2H), 6.38 (d, $J=2.0$ Hz, 1H), 6.87 (d, $J=2.0$ Hz, 1H), 6.94 (s, 1H), 7.04 (d, $J=8.1$ Hz, 1H), 7.63 (d, $J=7.8$ Hz, 2H), 12.90 (s, 1H). ESI-MS: 424.1 ($[M+H]^+$). Anal. Calcd for $C_{24}H_{25}NO_6$: C, 68.07%; H, 5.95%; N, 3.31%. Found: C, 68.24%; H, 5.78%; N, 3.45%.

*5.4.8. Synthesis of 7-(2-(cyclohexylamino)ethoxy)-2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-4H-chromen-4-one (**11**)*

Yellow powder, yield 84%, mp: 128–130 °C. ^{1}H NMR (DMSO-d_6): 1.11–1.21 (m, 4H), 1.49–1.60 (m, 6H), 2.56 (s, 1H), 3.44 (t, $J=5.0$ Hz, 2H), 4.20 (d, $J=5.0$ Hz, 4H), 4.50 (t, $J=8.5$ Hz, 2H), 6.38 (d, $J=2.0$ Hz, 1H), 6.87 (d, $J=2.0$ Hz, 1H), 6.94 (s, 1H), 7.04 (d, $J=8.1$ Hz, 1H), 7.63 (d, $J=7.8$ Hz, 2H), 12.88 (s, 1H). ESI-MS: 438.1 ($[M+H]^+$). Anal. Calcd for $C_{25}H_{27}NO_6$: C, 68.63%; H, 6.22%; N, 3.20%. Found: C, 68.75%; H, 6.34%; N, 3.38%.

*5.4.9. Synthesis of 7-(2-(benzylamino)ethoxy)-2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-4H-chromen-4-one (**12**)*

Yellow powder, yield 80%, mp: 137–139 °C. ^{1}H NMR (DMSO-d_6): 3.45 (d, $J=5.0$ Hz, 2H), 3.83 (t, $J=5.0$ Hz, 2H), 4.32 (dd, $J=5.0$ Hz, 8.5 Hz, 4H), 4.45 (t, $J=8.5$ Hz, 2H), 6.39 (d, $J=2.0$ Hz, 1H), 6.86 (d, $J=2.0$ Hz, 1H), 6.93 (s, 1H), 7.03 (d, $J=8.1$ Hz, 1H), 7.23–7.33 (m, 5H), 7.63 (d, $J=7.8$ Hz, 2H), 12.88 (s, 1H). ESI-MS: 446.1 ($[M+H]^+$). Anal. Calcd for $C_{26}H_{23}NO_6$: C, 70.10%; H, 5.20%; N, 3.14%. Found: C, 70.19%; H, 5.41%; N, 3.26%.

*5.4.10. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-7-(2-(4-fluorobenzylamino)ethoxy)-5-hydroxy-4H-chromen-4-one (**13**)*

Yellow powder, yield 81%, mp: 139–140 °C. ^{1}H NMR (DMSO-d_6): 3.43 (d, $J=5.0$ Hz, 2H), 3.82 (t, $J=5.0$ Hz, 2H), 4.29 (dd, $J=5.0$ Hz, 8.5 Hz, 4H), 4.46 (t, $J=8.5$ Hz, 2H) 6.37 (d, $J=2.0$ Hz, 1H), 6.88 (d, $J=2.0$ Hz, 1H), 6.96 (s, 1H), 7.03 (d, $J=8.1$ Hz, 1H), 7.12 (d, $J=2.0$ Hz, 2H), 7.39 (d, $J=8.1$ Hz, 2H), 7.64 (d, $J=7.8$ Hz, 2H), 12.83 (s, 1H). ESI-MS: 464.1 ($[M+H]^+$). Anal. Calcd for $C_{26}H_{22}FNO_6$: C, 67.38%; H, 4.78%; N, 3.02%. Found: C, 67.45%; H, 4.86%; N, 3.16%.

*5.4.11. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-7-(2-(piperidin-1-yl)ethoxy)-4H-chromen-4-one (**14**)*

White powder, yield 82%, mp: 145–146 °C. ^{1}H NMR (DMSO-d_6): 1.46–1.48 (m, 2H), 1.54–1.62 (m, 4H), 2.54 (br s, 4H), 3.83 (t, $J=5.0$ Hz, 2H), 4.32 (dd, $J=5.0$ Hz, 8.5 Hz, 4H), 4.45 (t, $J=8.5$ Hz, 2H) 6.39 (d, $J=2.0$ Hz, 1H), 6.86 (d, $J=2.0$ Hz, 1H), 6.93 (s, 1H), 7.03 (d, $J=8.1$ Hz, 1H), 7.63 (d, $J=7.8$ Hz, 2H), 12.88 (s, 1H). ESI-MS: 424.1 ($[M+H]^+$). Anal. Calcd for $C_{24}H_{25}NO_6$: C, 68.07%; H, 5.95%; N, 3.31%. Found: C, 68.15%; H, 5.89%; N, 3.26%.

*5.4.12. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]-dioxin-6-yl)-5-hydroxy-7-(2-morpholinoethoxy)-4H-chromen-4-one (**15**)*

Yellow powder, yield 84%, mp: 163–164 °C. ^{1}H NMR (DMSO-d_6): 1.91–2.10 (m, 2H), 2.43–2.61 (m, 6H), 3.76 (t, $J=5.0$ Hz, 2H), 4.29 (dd, $J=5.0$ Hz, 8.5 Hz, 4H), 4.49 (t, $J=8.5$ Hz, 2H), 6.38 (d, $J=2.0$ Hz, 1H), 6.84 (d, $J=2.0$ Hz, 1H), 6.93 (s, 1H), 7.03 (d, $J=8.1$ Hz, 1H), 7.63 (d, $J=7.8$ Hz, 2H), 12.91 (s, 1H). ESI-MS: 426.1 ($[M+H]^+$). Anal. Calcd for $C_{23}H_{23}NO_7$: C, 64.93%; H, 5.45%; N, 3.29%. Found: C, 64.85%; H, 5.59%; N, 3.26%.

*5.4.13. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-7-(2-(2-morpholinoethylamino)ethoxy)-4H-chromen-4-one (**16**)*

Yellow powder, yield 84%, mp: 169–170 °C. ^{1}H NMR (DMSO-d_6): 1.98–2.13 (m, 2H), 2.31–2.39 (m, 4H), 2.43–2.61 (m, 6H), 3.82 (t, $J = 5.0$ Hz, 2H), 4.31 (dd, $J = 5.0$ Hz, 8.5 Hz, 4H), 4.45 (t, $J = 8.5$ Hz, 2H), 6.38 (d, $J = 2.0$ Hz, 1H), 6.84 (d, $J = 2.0$ Hz, 1H), 6.93 (s, 1H), 7.03 (d, $J = 8.1$ Hz, 1H), 7.63 (d, $J = 7.8$ Hz, 2H), 12.78 (s, 1H). ESI-MS: 469.1 ($[M + H]^+$). Anal. Calcd for $C_{25}H_{28}N_2O_7$: C, 64.09%; H, 6.02%; N, 5.98%. Found: C, 64.15%; H, 5.99%; N, 5.85%.

*5.4.14. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-7-(2-(3-morpholinopropylamino)ethoxy)-4H-chromen-4-one (**17**)*

Yellow powder, yield 84%, mp: 174–176 °C. ^{1}H NMR (DMSO-d_6): 1.92–2.10 (m, 4H), 2.33–2.41 (m, 6H), 2.46–2.58 (m, 6H), 3.84 (t, $J = 5.0$ Hz, 2H), 4.34 (dd, $J = 5.0$ Hz, 8.5 Hz, 4H), 4.48 (t, $J = 8.5$ Hz, 2H), 6.38 (d, $J = 2.0$ Hz, 1H), 6.84 (d, $J = 2.0$ Hz, 1H), 6.93 (s, 1H), 7.03 (d, $J = 8.1$ Hz, 1H), 7.63 (d, $J = 7.8$ Hz, 2H), 12.87 (s, 1H). ESI-MS: 483.1 ($[M + H]^+$). Anal. Calcd for $C_{26}H_{20}N_2O_7$: C, 64.72%; H, 6.27%; N, 5.81%. Found: C, 64.66%; H, 6.31%; N, 5.87%.

*5.4.15. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-7-(2-(pyrrolidin-1-yl)ethoxy)-4H-chromen-4-one (**18**)*

Yellow powder, yield 77%, mp: 149–150 °C. ^{1}H NMR (DMSO-d_6): 1.62 (br s, 4H), 2.61 (br s, 2H), 2.76 (m, 4H), 3.44 (t, $J = 5.0$ Hz, 2H), 4.20 (d, $J = 5.0$ Hz, 4H), 4.50 (t, $J = 8.5$ Hz, 2H), 6.38 (d, $J = 2.0$ Hz, 1H), 6.87 (d, $J = 2.0$ Hz, 1H), 6.94 (s, 1H), 7.04 (d, $J = 8.1$ Hz, 1H), 7.63 (d, $J = 7.8$ Hz, 2H), 12.88 (s, 1H). ESI-MS: 410.1 ($[M + H]^+$). Anal. Calcd for $C_{23}H_{23}NO_6$: C, 67.47%; H, 5.66%; N, 3.42%. Found: C, 67.56%; H, 5.56%; N, 3.36%.

*5.4.16. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-7-(2-(4-methylpiperazin-1-yl)ethoxy)-4H-chromen-4-one (**19**)*

Yellow powder, yield 79%, mp: 155–156 °C. ^{1}H NMR (DMSO-d_6): 2.28 (s, 3H), 2.36 (br s, 8H), 3.43 (t, $J = 5.0$ Hz, 2H), 4.32 (d, $J = 5.0$ Hz, 4H), 4.50 (t, $J = 8.5$ Hz, 2H), 6.38 (d, $J = 2.0$ Hz, 1H), 6.86 (d, $J = 2.0$ Hz, 1H), 6.94 (s, 1H), 7.05 (d, $J = 8.1$ Hz, 1H), 7.65 (d, $J = 7.8$ Hz, 2H), 12.87 (s, 1H). ESI-MS: 439.1 ($[M + H]^+$). Anal. Calcd for $C_{24}H_{26}N_2O_6$: C, 65.74%; H, 5.98%; N, 6.39%. Found: C, 65.82%; H, 5.56%; N, 6.25%.

*5.4.17. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-hydroxy-7-(2-(piperazin-1-yl)ethoxy)-4H-chromen-4-one (**20**)*

Yellow powder, yield 81%, mp: 147–148 °C. ^{1}H NMR (DMSO-d_6): 2.01 (s, 1H), 2.29 (br s, 4H), 2.65–2.72 (m, 6H), 3.43 (t, $J = 5.0$ Hz, 2H), 4.32 (d, $J = 5.0$ Hz, 4H), 4.50 (t, $J = 8.5$ Hz, 2H), 6.38 (d, $J = 2.0$ Hz, 1H), 6.88 (d, $J = 2.0$ Hz, 1H), 7.01 (s, 1H), 7.14 (d, $J = 8.1$ Hz, 1H), 7.72 (d, $J = 7.8$ Hz, 2H), 12.91 (s, 1H). ESI-MS: 425.1 ($[M + H]^+$). Anal. Calcd for $C_{23}H_{24}N_2O_6$: C, 65.08%; H, 5.70%; N, 6.60%. Found: C, 65.12%; H, 5.66%; N, 6.55%.

5.5. Antibacterial activity

The antibacterial activity of the synthesized compounds was tested against *B. subtilis, E. coli, P. fluorescens* and *S. aureus* using MH medium (Muellere-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs of the test compounds were determined by a colorimetric method using the dye MTT [18]. 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). A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 ml of PBS (phosphate buffered saline 0.01 mol/L, pH 7.4, $Na_2HPO_4 \cdot 12H_2O$ (2.9 g), KH_2PO_4 (0.2 g), NaCl (8.0 g), KCl (0.2 g), distilled water (1000 mL)) 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 570 nm. The observed MICs are presented in Table 1.

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