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Short communication

Synthesis, structure and structure–activity relationship analysis of caffeic acid amides as potential antimicrobials

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ABSTRACT

A series of caffeic acid amides **1**–**23** were synthesized and nine of which (**13**–**17**, **19**–**21** and **23**) were reported for the first time. The chemical structures of these compounds were confirmed by means of ^{1}H NMR, ESI MS and elemental analyses. Compound **15** was determined by single-crystal X-ray diffraction analysis. All of the compounds were assayed for antibacterial (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens* and *Staphylococcus aureus*) and antifungal (*Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*) activities by MTT method. Compounds **10**–**12**, **15**, **18** and **21** showed considerable antibacterial activities against *B. subtilis* with MICs of 7.95, 6.25, 3.89, 1.18, 3.12 and 15.5 μg/mL, respectively. Structure–activity relationship analysis disclosed that caffeic acid anilides with electron-donating groups at *p*-position of benzene ring have better inhibitory activities.

1. Introduction

The rapid incidences of drug resistance of microorganisms to antibiotics were reported [1]. For example, by the late 1950s *Staphylococcus aureus* (*S. aureus*) became resistant to penicillin and infections with these strains caused many deaths [2]. Until recently, antibiotic discovery has kept ahead of microbial resistance, but recent outbreaks of multidrug-resistant *Mycobacterium tuberculosis*, penicillin resistant *Streptococcus pneumoniae*, methicillin-resistant *S. aureus*, vancomycin-resistant enterococci and, more recently, vancomycin-resistant *S. aureus* have prompted an urgent discovery of novel active agents against these pathogens [3,4]. Therefore, a new demand for alternative antibiotics, the structure of which differs from conventional antibiotics is created.

Caffeic acid is one of the most widely distributed hydroxycinnamate and phenylpropanoid metabolites in plant tissues. It is usually found as various simple derivatives including amides, esters, sugar esters, and glycosides [5]. The antimicrobial activity of these derivatives has attracted much attention and has been studied by many research groups in recent years [6–10]. Caffeic acid has potential as a food additive to inhibit growth of *Clostridium botulinum* and reduce thermal processing requirements of heat sensitive foods [6]. Lysozyme–caffeic acid conjugate shows bactericidal effect for *Escherichia coli* (*E. coli*) and *S. aureus* [7]. The dicaffeoylquinic acids and dicaffeoyltartaric acids are potent and selective inhibitors of human immunodeficiency virus type 1 integrase [8]. The caffeic acid phenylethyl esters are demonstrated to have higher antimicrobial activities against *S. aureus*, *B. subtilis* (*B. subtilis*), and *Pseudomonas aeruginosa* than 5-chorogenic acid and caffeic acid [9]. Almajano et al. has studied the effect of pH on the antimicrobial activity and oxidative stability of oil-in-water emulsions containing caffeic acid [10].

Rajan et al. synthesized a series of amides of caffeic acid and evaluated their antioxidant properties as lipid peroxidation inhibitors [11]. Some hydroxycinnamic acid amides and analogues were synthesized and studied antibacterial activity against *S. aureus* [12]. Some caffeoylglycolic and caffeoylamino acid derivatives were synthesized for the purpose of simplifying the structure of L-chicoric acid as new HIV-1 integrase inhibitors [13]. All of these encouraged us to find new caffeic acid amide antimicrobials. Therefore, in this paper, a series of caffeic acid amides **1**–**23** were synthesized and their antimicrobial activities were evaluated. The results were compared with those obtained with the corresponding *p*-coumaric acid amides **24** and **25**, caffeic acid, *p*-coumaric acid, and standard antimicrobials such as kanamycin and penicillin. Based on theses results, structure–activity relationship (SAR) analysis was conducted. The results of this study

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may be useful to researchers attempting to find new potential antimicrobials.

2. Results and discussion

2.1. Chemistry

In order to investigate the antimicrobial activities of caffeic acid amides, a series of caffeic acid amides **1**–**23** were synthesized from the free acid and the amines using benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) as coupling reagent (Scheme 1) [11]. Among these compounds, compounds **13**–**17**, **19**–**21** and **23** are reported for the first time. These compounds gave satisfactory elementary analyses (±0.4%). ^{1}H NMR and ESI MS spectra were consistent with the assigned structures. Compounds **1** and **2** were caffeic acid fatty amides. Compounds **3**–**21** were caffeic acid anilides with a single different substituent (F, Cl, Br, CH_3, OCH_3 or NO_2) in *o*-, *m*-, or *p*-position of benzene ring. Compounds **22** and **23** were caffeic acid secondary amides with phenyl groups. In addition, to demonstrate the importance of caffeoyl group for the activity, two *p*-coumaric acid amides **24** and **25** were synthesized using the same method as for synthesizing caffeic acid amides.

2.2. The crystal structure of compound *15*

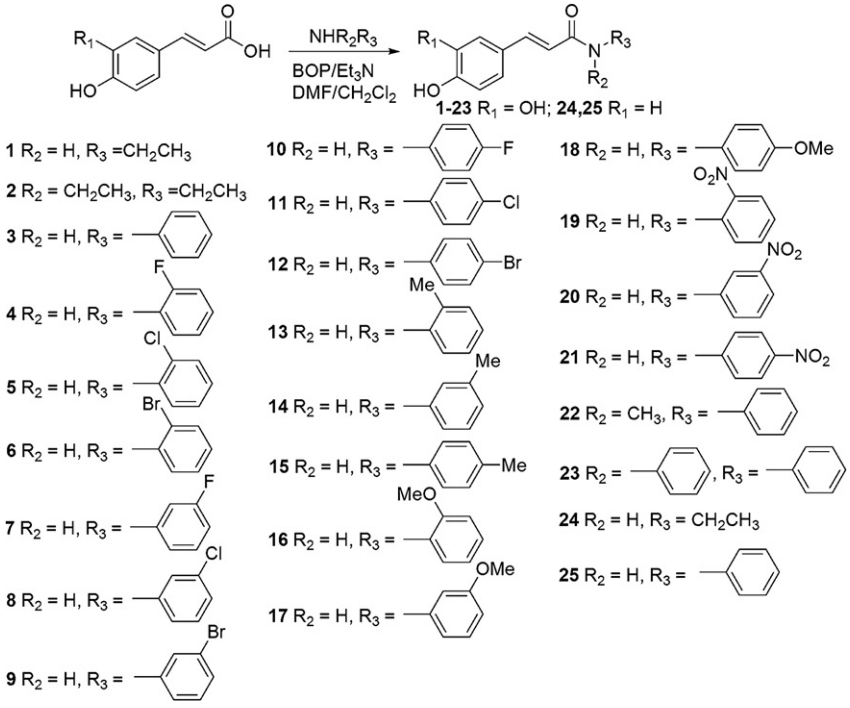
Among these compounds, a crystal structure of compound **15** was determined by X-ray diffraction analysis. The crystal data are presented in Table 1 and Fig. 1 gave perspective views of **15** with the atomic labeling system. It crystallizes in the space group $P\bar{1}$ with the following unit cell parameters: $a = 9.4460\ (19)$ Å, $b = 10.701\ (2)$ Å, $c = 13.812\ (3)$ Å, $\alpha = 78.63\ (3)°$, $\beta = 76.87\ (3)°$, $\gamma = 89.42\ (3)°$, $V = 13{,}332.1\ (5)$ Å^3, $Z = 4$. The selected bond lengths/angles and torsion angles are given in Table 2. The bond lengths and angles are all in normal values. The X-ray data have been deposited at the Cambridge Crystallographic Data Centre, and the CCDC number is 742,862.

2.3. Antimicrobial activity

In this study, attention is focused on the evaluation of the SAR. In screening assay studies, caffeic acid amides **1**–**23** were evaluated for their antimicrobial activities against two Gram-positive bacteria (*B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538), two Gram-negative bacteria (*E. coli* ATCC 35218 and *Pseudomonas fluorescens* ATCC 13525), and three fungi (*Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231, and *Trichophyton rubrum* ATCC 10218) by the 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method [14], taking *p*-coumaric acid amides **24** and **25**, caffeic acid, *p*-coumaric acid, kanamycin and penicillin as comparisons. The results were shown in Table 3 and the minimum inhibitory concentrations (MICs) of the compounds differed greatly, ranging from 1.18 to over 50 μg/mL. These compounds had not significant inhibition activities against *E. coli*, *A. niger*, and *T. rubrum*, supported by the fact that their MICs were all over 50 μg/mL (data not shown). Of these compounds, six (**10**–**12**, **15, 18** and **21**) exhibited potent antibacterial activity against *B. subtilis*, with MICs comparable to kanamycin and penicillin.

Some studies [15,16] reported that Gram-positive bacteria presented higher sensitivity than Gram-negative bacteria to various polyphenols. This higher resistance can be explained on the basis of the different composition of the cell-wall membrane. Our results showed that of the four bacteria, Gram-positive bacterium *B. subtilis* was more sensitive to our compounds than *P. fluorescens*, *S. aureus*, and *E. coli*. However, our compounds had showed weak antibacterial activity against some Gram-positive bacterium such as *S. aureus*.

The data in Table 3 showed that many caffeic acid amides had stronger antimicrobial activities than caffeic acid, suggesting amides groups could enhance the activities. *p*-Coumaric acid amides **24** and **25**, and *p*-coumaric acid also had poorly activities,



Scheme 1. Synthesis of caffeic acid amides and *p*-coumaric acid amides.

Table 1
Crystallographic data for compound **15**.

Crystal data	**15**
Formula	$C_{16}H_{15}NO_3$
Crystal size (mm)	0.30 × 0.20 × 0.10
MW (g mol^{-1})	269.29
Crystal system	Triclinic
Space group	$P\bar{1}$
Cell parameters	
a (Å)	9.4460 (19)
b (Å)	10.701 (2)
c (Å)	13.812 (3)
V (Å^3)	1332.1 (5)
Z	4
μ (mm^{-1})	0.093
ρ calcd (g cm^{-3})	1.343
Data collection	
θ limits (°)	$1.55 < \theta < 25.27$
hkl limits	$0 \le h \le 11$; $-12 \le k \le 12$; $-16 \le l \le 16$
No. collected refl.	5155
No. ind refl. (R_{int})	4835 (0.015)
Refinement	
R [$I > 2$sigma(I)]	0.044
R_w (all data)	0.058
Extinction coefficient	0.056(3)
GOF	1.021
$\Delta\rho_{min}$ (e Å^{-3})	−0.27
$\Delta\rho_{max}$ (e Å^{-3})	0.30

Table 2
Selected bond lengths, bond angles and torsion angles of compound **15**.

Bond lengths (Å)	
O_1–C_8	1.225 (2)
O_2–C_{15}	1.384 (2)
N_1–C_8	1.354 (2)
N_1–C_5	1.423 (2)
C_1–C_2	1.516 (3)
C_2–C_3	1.379 (3)
C_8–C_9	1.480 (2)
C_9–C_{10}	1.327 (3)
C_{10}–C_{11}	1.469 (2)
Bond angles (°)	
H_2–O_2–C_{15}	109.4 (7)
H_1A–N_1–C_8	116.7 (7)
C_5–N_1–C_8	126.5 (2)
H_1B–C_1–C_2	109.4 (7)
C_7–C_2–C_3	117.7 (2)
C_1–C_2–C_3	120.9 (2)
N_1–C_8–O_1	123.6 (2)
C_9–C_8–N_1	113.2 (2)
C_{11}–C_{10}–C_9	125.4 (2)
Selected torsion angles (°)	
C_3–C_4–C_5–N_1	178.5 (2)
C_8–N_1–C_5–C_4	−45.2 (3)
C_8–N_1–C_5–C_6	136.2 (2)
N_1–C_5–C_6–C_7	−178.8 (2)
C_5–N_1–C_8–O_1	1.2 (3)
C_5–N_4–C_8–C_9	−177.8 (2)
O_1–C_8–C_9–C_{10}	−20.8 (3)
N_1–C_8–C_9–C_{10}	158.2 (2)
O_3–C_{14}–C_{15}–O_2	−1.7 (2)

indicating that the presence of adjacent dihydroxy in the caffeoyl group was very important for the antimicrobial activities.

As shown in Table 3, caffeic acid anilides **10**–**12**, **15, 18** and **21** exhibit excellent activities against *B. subtilis*, whereas caffeic acid fatty amides **1** and **2** obviously showed much lower inhibition under the same conditions. This indicated that anilides had better antibacterial activities than fatty amides, and supportive evidence may be found by a comparison of other data listed in Table 3. Caffeic acid secondary amides **2**, **22** and **23** had low activities, proving that H–N is important for the activities.

Although caffeic acid anilides exhibit better antibacterial activity against *B. subtilis*, their MICs differ significantly. One rule could be found: anilides with a single substitute at the *p*-positions of the benzene ring exhibit the most powerful inhibitory activities, whereas anilides with a substituent at *o*- or *m*-positions show less potent activities. From this fact, it is reasoned that the *p*-postion is an essential point for the antibacterial activities of anilides, and substituents at the *o*- or *m*-positions decrease their inhibition. For example, when a methyl is introduced at *p*-position to form **15**, the activity is the most significant. However, when a methyl was introduced at *o*-position (or *m*-position) to form **13** (or **14**), the activity decreases significantly. According to further SAR analysis, replacement of methyl group of (**15**) at *p*-position by a bromide (**12**) resulted in the decreases of activity. Similarly, substitution of a chloride (**11**) at *p*-position for a methoxy group (**18**) also led to a decrease of inhibitory activity. This suggested that compounds with electron-donating groups at *p*-position showed better inhibitory activities than those with electron-withdrawing groups. For more extensive evidences, the substitution of methoxy group at *p*-position (**18**) by a fluoride or nitro (**10** or **21**) produced some decrease in activity.

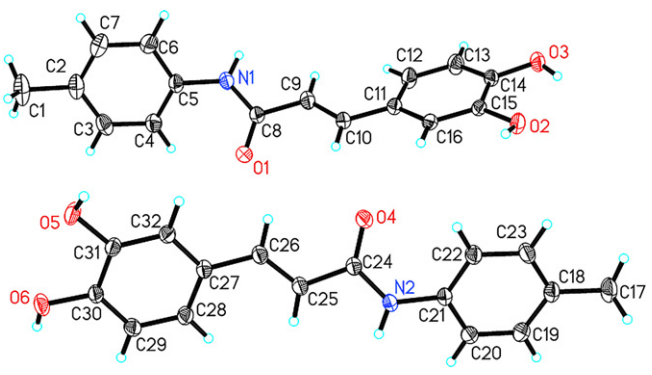


Fig. 1. ORTEP view showing the atom-labeling scheme with thermal ellipsoids drawn at 30% probability for compound **15**.

Table 3
Antimicrobial activities of caffeic acid amides **1**–**25** (MIC, μg/mL).

Compounds	*B. subtilis*	*P. fluorescens*	*S. aureus*	*C. albicans*
1	>50	>50	>50	>50
2	>50	>50	>50	>50
3	28.4	>50	41.5	50
4	50	>50	>50	33.3
5	45.8	41.2	>50	42.8
6	49.5	42.2	>50	27.5
7	46.7	>50	>50	>50
8	50	>50	49.2	>50
9	50	>50	>50	>50
10	7.95	>50	>50	>50
11	6.25	50	46.8	>50
12	3.89	50	>50	>50
13	35.0	42.5	>50	>50
14	40.2	38.8	45.6	>50
15	1.18	42.2	>50	>50
16	39.8	13.2	47.4	>50
17	50	>50	>50	44.7
18	3.12	>50	50	42.8
19	>50	>50	>50	>50
20	>50	>50	>50	>50
21	15.5	>50	>50	49.4
22	>50	>50	>50	>50
23	>50	>50	>50	>50
24	>50	>50	45.4	50
25	>50	>50	>50	>50
Caffeic acid	>50	>50	>50	>50
p-Coumaric acid	>50	>50	>50	>50
Kanamycin	0.39	5.08	1.28	–
Penicillin	0.78	–	1.39	–

3. Conclusions

A series of caffeic acid amides **1**–**23** were synthesized. To study the potential antimicrobial activities of the synthesized amides, screening experiments were performed for four bacteria and three fungi strains. Compounds **10**–**12**, **15, 18** and **21** (MICs = 7.95, 6.25, 3.89, 1.18, 3.12 and 15.5 μg/mL) showed considerable antibacterial activities against *B. subtilis*. According to SAR analysis, it is found that caffeic acid anilides with electron-donating groups at *p*-position of benzene ring have better inhibitory activities. This study shows that caffeic acid anilides are potential antimicrobials. We hope this information might be helpful for the discovery of new antibiotics.

4. Experimental

4.1. Chemistry

4.1.1. Chemistry general

All chemicals (reagent grade) used were purchased from Aldrich (USA). Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM, E. Merck, Germany). The quantity of silica gel used was 50–100 times the weight charged on the column. TLC was run on the silica gel coated aluminum sheets (silica gel 60 GF254, E. Merck, Germany) and visualized in UV light (254 nm). Melting points (uncorrected) were obtained by using an XT4 MP apparatus (Taike Corp. Beijing, China). ^{1}H NMR spectra were recorded at 300 MHz on ^{1}H-Varian-Mercury-300 spectrometers at 25 °C, using TMS as internal standard. ESI MS were recorded with a Mariner System 5304 mass spectrometer. Elementary analyses were performed on a CHN–O-Rapid instrument and were within ±0.4% of the theoretical values.

4.1.2. General synthesis method of caffeic acid amides and p-coumaric acid amides

The hydroxycinnamic acid (5 mmol) is dissolved in 10 mL of DMF and 0.7 mL (5 mmol) of triethylamine. The solution is cooled in an ice-water bath and 5 mmol of the amine are added followed by a solution of 5 mmol of BOP in 10 mL of CH_2Cl_2. The mixture is stirred at 0 °C for 30 min and then at room temperature for 2–5 h CH_2Cl_2 is removed under reduced pressure and the solution is diluted with 80 mL of water. The products are extracted with ethyl acetate. The extract is washed successively with 1 N HCl, water, 1 M $NaHCO_3$, and water, dried over $MgSO_4$, filtered and evaporated. The residue is purified on a silica gel column (eluent: ethyl acetate–petroleum ether).

4.1.3. (E)-3-(3,4-Dihydroxyphenyl)-N-ethylacrylamide (**1**)

Pale green crystal, yield 84%, mp: 117–118 °C, ESI MS: 208.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.36 (s, 2H), 7.47 (d, 1H, J = 15.8 Hz), 7.06 (d, 1H, J = 2.2 Hz), 7.00 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.0 Hz), 6.76 (d, 1H, J = 8.0 Hz), 6.26 (d, 1H, J = 15.9 Hz), 4.16 (q, 2H, J = 6.8 Hz), 1.24 (t, 3H, J = 7.1 Hz). Anal. Calc for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76%. Found: C, 63.36; H, 5.39; N, 6.86%.

4.1.4. (E)-3-(3,4-Dihydroxyphenyl)-N,N-diethylacrylamide (**2**)

White powder, yield 74%, mp: 134–135 °C, ESI MS: 236.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 7.67 (d, 1H, J = 15.4 Hz), 7.24 (d, 1H, J = 1.8 Hz), 6.99 (dd, 1H, J_1 = 8.2 Hz, J_2 = 1.8 Hz), 6.88 (d, 1H, J = 8.2 Hz), 6.65 (d, 1H, J = 15.3 Hz), 3.41 (m, 4H), 1.45 (m, 6H). Anal. Calc for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95%. Found: C, 66.66; H, 7.35; N, 5.61%.

4.1.5. (E)-3-(3,4-Dihydroxyphenyl)-N-phenylacrylamide (**3**)

Pale yellow powder, yield 72%, mp: 178–179 °C, ESI MS: 256.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.96 (s, 1H), 9.42 (s, 1H), 9.16 (s, 1H), 7.57 (dd, 2H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 7.50 (d, 1H, J = 15.6 Hz), 7.12 (m, 2H), 7.08 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.0 Hz), 7.05 (d, 1H, J = 2.0 Hz), 6.82 (dd, 1H, J_1 = 8.3 Hz, J_2 = 1.9 Hz), 6.78 (d, 1H, J = 8.3 Hz), 6.55 (d, 1H, J = 15.6 Hz). Anal. Calc for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49%. Found: C, 70.36; H, 5.32; N, 5.45%.

4.1.6. (E)-3-(3,4-Dihydroxyphenyl)-N-(2-fluorophenyl) acrylamide (**4**)

Pale yellow crystal, yield 62%, mp: 177–178 °C, ESI MS: 274.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.06 (s, 1H), 9.45 (s, 1H), 9.26 (s, 1H), 7.68 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 7.38 (d, 1H, J = 15.6 Hz), 7.12–7.14 (m, 2H), 7.08 (m, 1H), 7.00 (d, 1H, J = 2.0 Hz), 6.90 (dd, 1H, J_1 = 8.3 Hz, J_2 = 1.9 Hz), 6.78 (d, 1H, J = 8.3 Hz), 6.53 (d, 1H, J = 15.5 Hz). Anal. Calc for $C_{15}H_{12}FNO_3$: C, 65.93; H, 4.43; N, 5.13%. Found: C, 65.61; H, 4.36; N, 5.68%.

4.1.7. (E)-N-(2-Chlorophenyl)-3-(3,4-dihydroxyphenyl) acrylamide (**5**)

Pale yellow powder, yield 73%, mp: 188–189 °C, ESI MS: 290.0 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.12 (s, 1H), 9.48 (s, 1H), 9.16 (s, 1H), 7.76 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.2 Hz), 7.56 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.1 Hz), 7.39 (d, 1H, J = 15.5 Hz), 7.25 (m, 1H), 7.16 (m, 1H), 7.00 (d, 1H, J = 2.2 Hz), 6.91 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.2 Hz), 6.79 (d, 1H, J = 8.5 Hz), 6.55 (d, 1H, J = 15.5 Hz). Anal. Calc for $C_{15}H_{12}ClNO_3$: C, 62.19; H, 4.17; N, 4.83%. Found: C, 62.16; H, 4.62; N, 4.61%.

4.1.8. (E)-N-(2-Bromophenyl)-3-(3,4-dihydroxyphenyl) acrylamide (**6**)

Yellow powder, yield 76%, mp: 200–201 °C, ESI MS: 334.0 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.15 (s, 1H), 9.50 (s, 1H), 9.20 (s, 1H), 7.80 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 7.65 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.1 Hz), 7.40 (d, 1H, J = 15.6 Hz), 7.36 (m, 1H), 7.08 (m, 1H), 7.01 (d, 1H, J = 2.1 Hz), 6.92 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.2 Hz), 6.81 (d, 1H, J = 8.5 Hz), 6.57 (d, 1H, J = 15.6 Hz). Anal. Calc for $C_{15}H_{12}BrNO_3$: C, 53.91; H, 3.62; N, 4.19%. Found: C, 53.63; H, 3.72; N, 4.16%.

4.1.9. (E)-3-(3,4-Dihydroxyphenyl)-N-(3-fluorophenyl) acrylamide (**7**)

Pale yellow powder, yield 72%, mp: 197–198 °C, ESI MS: 274.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.06 (s, 1H), 9.45 (s, 1H), 9.26 (s, 1H), 7.58–7.60 (m, 2H), 7.38 (d, 1H, J = 15.6 Hz), 7.32 (dd, H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 7.00 (d, 1H, J = 2.0 Hz), 6.94 (m, 1H), 6.88 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 6.79 (d, 1H, J = 8.2 Hz), 6.51 (d, 1H, J = 15.5 Hz). Anal. Calc for $C_{15}H_{12}FNO_3$: C, 65.93; H, 4.43; N, 5.13%. Found: C, 65.68; H, 4.82; N, 4.91%.

4.1.10. (E)-N-(3-Chlorophenyl)-3-(3,4-dihydroxyphenyl) acrylamide (**8**)

Yellow powder, yield 75%, mp: 205–206 °C, ESI MS: 290.0 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.10 (s, 1H), 9.51 (s, 1H), 9.24 (s, 1H), 7.76 (m, 1H), 7.50 (m, 1H), 7.41 (d, 1H, J = 15.4 Hz), 7.35 (m, 1H), 7.28 (m, 1H), 7.01 (d, 1H, J = 2.1 Hz), 6.90 (dd, 1H, J_1 = 8.3 Hz, J_2 = 2.1 Hz), 6.80 (d, 1H, J = 8.3 Hz), 6.52 (d, 1H, J = 15.4 Hz). Anal. Calc for $C_{15}H_{12}ClNO_3$: C, 62.19; H, 4.17; N, 4.83%. Found: C, 62.70; H, 4.82; N, 4.51%.

4.1.11. (E)-N-(3-Bromophenyl)-3-(3,4-dihydroxyphenyl) acrylamide (**9**)

Yellow powder, yield 61%, mp: 208–209 °C, ESI MS: 334.0 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.14 (s, 1H), 9.52 (s, 1H), 9.25 (s, 1H), 7.81 (m, 1H), 7.55 (m, 1H), 7.42 (d, 1H, J = 15.5 Hz), 7.36 (m, 1H), 7.19 (m, 1H), 7.03 (d, 1H, J = 2.1 Hz), 6.91 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.1 Hz), 6.82 (d, 1H, J = 8.4 Hz), 6.52 (d, 1H, J = 15.5 Hz). Anal. Calc for $C_{15}H_{12}BrNO_3$: C, 53.91; H, 3.62; N, 4.19%. Found: C, 54.29; H, 3.30; N, 4.08%.

*4.1.12. (E)-N-(4-Fluorophenyl)-3-(3,4-dihydroxyphenyl) acrylamide (**10**)*

Pale yellow powder, yield 74%, mp: 199–200 °C, ESI MS: 274.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.96 (s, 1H), 9.42 (s, 1H), 9.12 (s, 1H), 7.66 (d, 2H, $J = 8.0$ Hz), 7.36 (d, 1H, $J = 15.6$ Hz), 7.12 (d, 2H, $J_1 = 8.0$ Hz), 6.98 (d, 1H, $J = 2.0$ Hz), 6.87 (dd, 1H, $J_1 = 8.2$ Hz, $J_2 = 2.0$ Hz), 6.77 (d, 1H, $J = 8.2$ Hz), 6.50 (d, 1H, $J = 15.6$ Hz). Anal. Calc for $C_{15}H_{12}FNO_3$: C, 65.93; H, 4.43; N, 5.13%. Found: C, 65.68; H, 4.80; N, 4.97%.

*4.1.13. (E)-N-(4-Chlorophenyl)-3-(3,4-dihydroxyphenyl) acrylamide (**11**)*

Pale yellow powder, yield 76%, mp: 195–196 °C, ESI MS: 290.0 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.02 (s, 1H), 9.43 (s, 1H), 9.14 (s, 1H), 7.60 (d, 2H, $J = 8.2$ Hz), 7.44 (d, 2H, $J_1 = 8.2$ Hz), 7.37 (d, 1H, $J = 15.4$ Hz), 7.00 (d, 1H, $J = 2.1$ Hz), 6.88 (dd, 1H, $J_1 = 8.2$ Hz, $J_2 = 2.1$ Hz), 6.78 (d, 1H, $J = 8.2$ Hz), 6.51 (d, 1H, $J = 15.4$ Hz). Anal. Calc for $C_{15}H_{12}ClNO_3$: C, 62.19; H, 4.17; N, 4.83%. Found: C, 62.70; H, 4.23; N, 4.35%.

*4.1.14. (E)-N-(4-Bromophenyl)-3-(3,4-dihydroxyphenyl) acrylamide (**12**)*

Yellow powder, yield 75%, mp: 208–209 °C, ESI MS: 334.0 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.11 (s, 1H), 9.45 (s, 1H), 9.16 (s, 1H), 7.56 (d, 2H, $J = 8.3$ Hz), 7.48 (d, 2H, $J = 8.3$ Hz), 7.38 (d, 1H, $J = 15.6$ Hz), 7.02 (d, 1H, $J = 2.2$ Hz), 6.70 (dd, 1H, $J_1 = 8.2$ Hz, $J_2 = 2.2$ Hz), 6.80 (d, 1H, $J = 8.3$ Hz), 6.52 (d, 1H, $J = 15.6$ Hz). Anal. Calc for $C_{15}H_{12}BrNO_3$: C, 53.91; H, 3.62; N, 4.19%. Found: C, 53.36; H, 3.21; N, 4.62%.

*4.1.15. (E)-3-(3,4-Dihydroxyphenyl)-N-o-tolylacrylamide (**13**)*

Yellow powder, yield 73%, mp: 196–197 °C, ESI MS: 270.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.02 (s, 1H), 9.44 (s, 1H), 9.18 (s, 1H), 7.42 (d, 1H, $J = 15.5$ Hz), 7.23–7.35 (m, 3H), 7.07 (m, 1H), 7.00 (d, 1H, $J = 2.0$ Hz), 6.90 (dd, 1H, $J_1 = 8.3$ Hz, $J_2 = 1.9$ Hz), 6.78 (d, 1H, $J = 8.3$ Hz), 6.53 (d, 1H, $J = 15.5$ Hz), 2.16 (s, 3H). Anal. Calc for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20%. Found: C, 71.52; H, 5.31; N, 5.39%.

*4.1.16. (E)-3-(3,4-dihydroxyphenyl)-N-m-tolylacrylamide (**14**)*

White powder, yield 78%, mp: 200–201 °C, ESI MS: 270.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.12 (s, 1H), 9.50 (s, 1H), 9.22 (s, 1H), 7.60 (m, 1H), 7.48 (m, 1H), 7.38 (d, 1H, $J = 15.6$ Hz), 7.25 (m, 1H), 6.98 (d, 1H, $J = 2.1$ Hz), 6.95 (m, 1H), 6.90 (dd, 1H, $J_1 = 8.3$ Hz, $J_2 = 2.1$ Hz), 6.79 (d, 1H, $J = 8.3$ Hz), 6.53 (d, 1H, $J = 15.6$ Hz), 2.28 (s, 3H). Anal. Calc for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20%. Found: C, 71.62; H, 5.52; N, 5.25%.

*4.1.17. (E)-3-(3,4-Dihydroxyphenyl)-N-p-tolylacrylamide (**15**)*

Pale yellow crystal, yield 77%, mp: 199–200 °C, ESI MS: 270.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.96 (s, 1H), 9.42 (s, 1H), 9.16 (s, 1H), 7.57 (d, 2H, $J = 8.2$ Hz), 7.38 (d, 1H, $J = 15.6$ Hz), 7.12 (d, 2H, $J = 8.0$ Hz), 7.00 (d, 1H, $J = 2.1$ Hz), 6.90 (dd, 1H, $J_1 = 8.2$ Hz, $J_2 = 2.1$ Hz), 6.78 (d, 1H, $J = 8.2$ Hz), 6.53 (d, 1H, $J = 15.5$ Hz), 2.26 (s, 3H). Anal. Calc for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20%. Found: C, 71.61; H, 5.41; N, 5.02%.

*4.1.18. (E)-3-(3,4-Dihydroxyphenyl)-N-(2-methoxyphenyl) acrylamide (**16**)*

Pale yellow powder, yield 75%, mp: 205–206 °C, ESI MS: 286.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.14 (s, 1H), 9.52 (s, 1H), 9.26 (s, 1H), 7.56 (dd, 1H, $J_1 = 8.5$ Hz, $J_2 = 2.3$ Hz), 7.40 (d, 1H, $J = 15.6$ Hz), 7.06 (d, 1H, $J = 2.0$ Hz), 6.99–7.05 (m, 3H), 6.90 (dd, 1H, $J_1 = 8.3$ Hz, $J_2 = 2.0$ Hz), 6.78 (d, 1H, $J = 8.3$ Hz), 6.54 (d, 1H, $J = 15.6$ Hz), 3.96 (s, 3H). Anal. Calc for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91%. Found: C, 67.57; H, 5.32; N, 5.09%.

*4.1.19. (E)-3-(3,4-Dihydroxyphenyl)-N-(3-methoxyphenyl) acrylamidez (**17**)*

White powder, yield 86%, mp: 199–200 °C, ESI MS: 286.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.06 (s, 1H), 9.46 (s, 1H), 9.15 (s, 1H), 7.40 (d, 1H, $J = 15.6$ Hz), 7.30–7.35 (m, 2H), 7.20 (m, 1H), 7.12 (d, 1H, $J = 2.1$ Hz), 6.88 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 2.1$ Hz), 6.76 (d, 1H, $J = 8.4$ Hz), 6.66 (m, 1H), 6.52 (d, 1H, $J = 15.6$ Hz), 3.92 (s, 3H). Anal. Calc for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91%. Found: C, 67.02; H, 5.61; N, 5.01%.

*4.1.20. (E)-3-(3,4-Dihydroxyphenyl)-N-(4-methoxyphenyl) acrylamide (**18**)*

Yellow powder, yield 77%, mp: 201–202 °C, ESI MS: 286.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.98 (s, 1H), 9.39 (s, 1H), 9.02 (s, 1H), 7.52 (d, 2H, $J = 8.2$ Hz), 7.38 (d, 1H, $J = 15.6$ Hz), 7.06 (d, 1H, $J = 2.1$ Hz), 6.96 (d, 2H, $J = 8.2$ Hz), 6.83 (dd, 1H, $J_1 = 8.5$ Hz, $J_2 = 2.3$ Hz), 6.72 (d, 1H, $J = 8.5$ Hz), 6.48 (d, 1H, $J = 15.6$ Hz), 3.86 (s, 3H). Anal. Calc for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91%. Found: C, 67.54; H, 5.62; N, 4.72%.

*4.1.21. (E)-3-(3,4-Dihydroxyphenyl)-N-(2-nitrophenyl) acrylamide (**19**)*

Yellow powder, yield 74%, mp: 200–201 °C, ESI MS: 301.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.16 (s, 1H), 9.54 (s, 1H), 9.28 (s, 1H), 8.46 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 2.2$ Hz), 8.18 (dd, 1H, $J_1 = 8.3$ Hz, $J_2 = 2.1$ Hz), 7.68–7.74 (m, 2H), 7.38 (d, 1H, $J = 15.5$ Hz), 7.05 (d, 1H, $J = 2.0$ Hz), 6.88 (dd, 1H, $J_1 = 8.2$ Hz, $J_2 = 2.0$ Hz), 6.78 (d, 1H, $J = 8.2$ Hz), 6.52 (d, 1H, $J = 15.5$ Hz). Anal. Calc for $C_{15}H_{12}N_2O_5$: C, 60.00; H, 4.03; N, 9.33%. Found: C, 60.34; H, 4.38; N, 9.01%.

*4.1.22. (E)-3-(3,4-Dihydroxyphenyl)-N-(3-nitrophenyl) acrylamide (**20**)*

White powder, yield 72%, mp: 201–202 °C, ESI MS: 301.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.12 (s, 1H), 9.51 (s, 1H), 9.14 (s, 1H), 8.66 (m, 1H), 7.88–7.90 (m, 2H), 7.58 (m, 1H), 7.39 (d, 1H, $J = 15.4$ Hz), 7.12 (d, 1H, $J = 2.1$ Hz), 6.85 (dd, 1H, $J_1 = 8.2$ Hz, $J_2 = 2.0$ Hz), 6.76 (d, 1H, $J = 8.2$ Hz), 6.49 (d, 1H, $J = 15.5$ Hz). Anal. Calc for $C_{15}H_{12}N_2O_5$: C, 60.00; H, 4.03; N, 9.33%. Found: C, 60.25; H, 4.32; N, 9.25%.

*4.1.23. (E)-3-(3,4-Dihydroxyphenyl)-N-(4-nitrophenyl) acrylamide (**21**)*

Colorless needle, yield 71%, mp: 199–200 °C, ESI MS: 301.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.02 (s, 1H), 9.48 (s, 1H), 9.24 (s, 1H), 8.16 (d, 2H, $J = 8.3$ Hz), 7.78 (d, 2H, $J = 8.3$ Hz), 7.34 (d, 1H, $J = 15.3$ Hz), 7.08 (d, 1H, $J = 2.1$ Hz), 6.85 (dd, 1H, $J_1 = 8.3$ Hz, $J_2 = 2.1$ Hz), 6.72 (d, 1H, $J = 8.3$ Hz), 6.44 (d, 1H, $J = 15.3$ Hz). Anal. Calc for $C_{15}H_{12}N_2O_5$: C, 60.00; H, 4.03; N, 9.33%. Found: C, 60.41; H, 3.93; N, 9.45%.

*4.1.24. (E)-3-(3,4-Dihydroxyphenyl)-N-methyl-N-phenylacrylamide (**22**)*

White powder, yield 80%, mp: 190–191 °C, ESI MS: 270.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.42 (s, 1H), 9.16 (s, 1H), 7.77 (dd, 2H, $J_1 = 8.3$ Hz, $J_2 = 2.0$ Hz), 7.50 (d, 1H, $J = 15.6$ Hz), 7.32 (m, 2H), 7.10 (dd, 1H, $J_1 = 8.3$ Hz, $J_2 = 2.0$ Hz), 7.04 (d, 1H, $J = 2.0$ Hz), 6.80 (dd, 1H, $J_1 = 8.3$ Hz, $J_2 = 1.9$ Hz), 6.77 (d, 1H, $J = 8.3$ Hz), 6.50 (d, 1H, $J = 15.6$ Hz), 3.42 (s, 3H). Anal. Calc for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20%. Found: C, 71.50; H, 5.18; N, 5.14%.

*4.1.25. (E)-3-(3,4-Dihydroxyphenyl)-N,N-diphenylacrylamide (**23**)*

White powder, yield 83%, mp: 191–192 °C, ESI MS: 332.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.48 (s, 1H), 9.18 (s,

1H), 7.52–7.58 (m, 8H), 7.46 (d, 1H, J = 15.6 Hz), 7.18 (dd, 2H, J_1 = 8.3 Hz, J_2 = 2.2 Hz), 7.02 (d, 1H, J = 2.2 Hz), 6.80 (dd, 1H, J_1 = 8.3 Hz, J_2 = 2.2 Hz), 6.76 (d, 1H, J = 8.3 Hz), 6.52 (d, 1H, J = 15.6 Hz). Anal. Calc for $C_{21}H_{17}NO_3$: C, 76.12; H, 5.17; N, 4.23%. Found: C, 76.06; H, 5.24; N, 4.61%.

*4.1.26. (E)-N-Ethyl-3-(4-hydroxyphenyl)acrylamide (**24**)*

White powder, yield 75%, mp: 139–140 °C, ESI MS: 192.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.32 (s, 1H), 8.91 (s, 1H), 7.55 (d, 2H, J = 8.2 Hz), 7.47 (d, 1H, J = 15.8 Hz), 6.67 (d, 1H, J = 8.2 Hz), 6.36 (d, 1H, J = 15.9 Hz), 4.02 (q, 2H, J = 6.8 Hz), 1.18 (t, 3H, J = 7.0 Hz). Anal. Calc for $C_{11}H_{13}NO_2$: C, 65.09; H, 6.85; N, 7.32%. Found: C, 65.42; H, 8.55; N, 7.42%.

*4.1.27. (E)-3-(4-Hydroxyphenyl)-N-phenylacrylamide (**25**)*

Pale yellow crystal, yield 75%, mp: 197–198 °C, ESI MS: 240.1 $[M + H]^+$, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.44 (s, 1H), 9.24 (s, 1H), 7.61 (d, 2H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 7.55 (d, J = 8.2 Hz), 7.48 (d, 1H, J = 15.6 Hz), 7.32 (m, 2H), 7.19 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 6.68 (d, 1H, J = 8.3 Hz), 6.54 (d, 1H, J = 15.6 Hz). Anal. Calc for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85%. Found: C, 75.65; H, 5.62; N, 5.64%.

4.2. Crystallographic studies

X-ray single-crystal diffraction data for compound **15** were collected on a Bruker SMART APEX CCD diffractometer at 293 (2) K using Mo Kα radiation (λ = 0.71073 Å) by the ω scan mode. The program SAINT was used for integration of the diffraction profiles. Structure was solved by direct methods using the SHELXS program of the SHELXTL package and refined by full-matrix least-squares methods with SHELXL [17]. All non-hydrogen atoms of compound **15** were refined with anisotropic thermal parameters. All hydrogen atoms were placed in geometrically idealized positions and constrained to ride on their parent atoms.

4.3. Antimicrobial activity assay

The antibacterial activities of the synthesized compounds were tested against *B. subtilis* ATCC 6633, *E. coli* ATCC 35218, *P. fluorescens* ATCC 13525 and *S. aureus* ATCC 6538 using Mueller–Hinton (MH) medium. The antifungal activities of the compounds were tested against *A. niger* ATCC 16404, *C. albicans* ATCC 10231, and *T. rubrum* ATCC 16404, using RPMI-1640 medium. The MICs of the test compounds were determined by a colorimetric method using the dye MTT [14]. A stock solution of the synthesized compounds (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 medium containing the test compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtimicrotitration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h and 48 h for bacteria and fungi, 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.

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