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Synthesis, structure, and structure–activity relationship analysis of enamines as potential antibacterials

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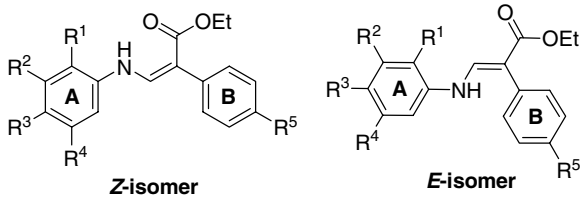
Abstract—Twenty-four enamines were synthesized and reported for the first time. Their chemical structures were confirmed by means of ^{1}H NMR, ESI mass spectra, and elemental analyses, and four of them were determined by single crystal X-ray diffraction analysis. All of the compounds were assayed for antibacterial (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *Pseudomonas fluorescens* ATCC 13525, and *Staphylococcus aureus* ATCC 6538) and antifungal (*Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231, and *Trichophyton rubrum* ATCC 10218) activities by MTT method. Compounds (*E*)-ethyl 3-(4-hydroxyphenylamino)-2-(4-methoxyphenyl)acrylate (**9b**), (*E*)-ethyl 3-(3,5-difluorophenylamino)-2-(4-chlorophenyl)acrylate (**11b**), (*E*)-ethyl 3-(3,5-dichlorophenylamino)-2-(4-chlorophenyl)acrylate (**12b**), and (*E*)-ethyl 3-(4-methylphenylamino)-2-(4-chlorophenyl)acrylate (**15b**) showed considerable antibacterial activities against *S. aureus* ATCC 6538 with MICs of 3.8, 1.9, 1.1, and 0.9 μg/mL, respectively. Structure–activity relationship (SAR) analysis disclosed, generally, an *E*-isomer exhibited higher antibacterial activity than the corresponding *Z*-isomer. An electron-withdrawing group on A-ring led to some decrease in activity, while on B-ring, a similar substitution provided higher activity.



1. Introduction

Since the first Schiff base metal complex was synthesized by H. Schiff in 1869, the study of antibacterial, antifungal, and antitumor activities of Schiff base compounds and their metal complexes has been widely discussed.[1–3] To our knowledge, no researchers have reported biological properties of enamines, which are the tautomers of Schiff bases. From the comparison of the chemical structure of a Schiff base to the corresponding enamine, it turns out that a Schiff base shows a high similarity to an enamine, which leads to the conception that a stable enamine may possess similar biological properties as a Schiff base does. This hypothesis is supported by the fact that isoflavones possess similar biological activities to those of flavones.[4–7] In our recent work, we reported the antibacterial activities of Schiff bases synthesized from 5-chloro-salicylaldehyde.[8] As the continuation of this work and together with above-mentioned hypothesis, we directed our attention

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to the investigation of enamines. As expected, the results indicated that enamines exhibited antibacterial activities as good as penicillin against some bacterial strains such as *Staphylococcus aureus* ATCC 6538. This is the first report on the antibacterial activities of enamines.

2. Results and discussion

2.1. Chemistry

The general method for preparing the final compounds **4a**–**15a** and **4b**–**15b** is outlined in Scheme 1. The designed enamines were prepared by a dehydration reaction of aldehydes (**2a**–**2b**) with different arylamines (**3a**–**3i**). Aldehydes (**2a**–**2b**), which were not commercially available, were synthesized using modified procedures of Beccalh.[9] The crude products consisted of the mixture of *Z*- and *E*-isomers (**4a**–**15a** and **4b**–**15b**). Subsequent purification with flash chromatography, **4a**–**15a** and **4b**–**15b** were obtained as pure isomer with high purity. The total yields of *Z*- and *E*-isomers and uncorrected melting points are summarized in Table 1. It is clear that an *E*-isomer had higher melting points than the corresponding *Z*-isomer. The structures of the enamines (**4a**–**15a** and **4b**–**15b**) were fully characterized by spectroscopic methods and elemental analysis. Out of the compounds, four enamines were characterized by single crystal X-ray determinations. The crystal structures may be helpful to account for the structure–activity relationships (SAR).

2.2. Description of the crystal structure

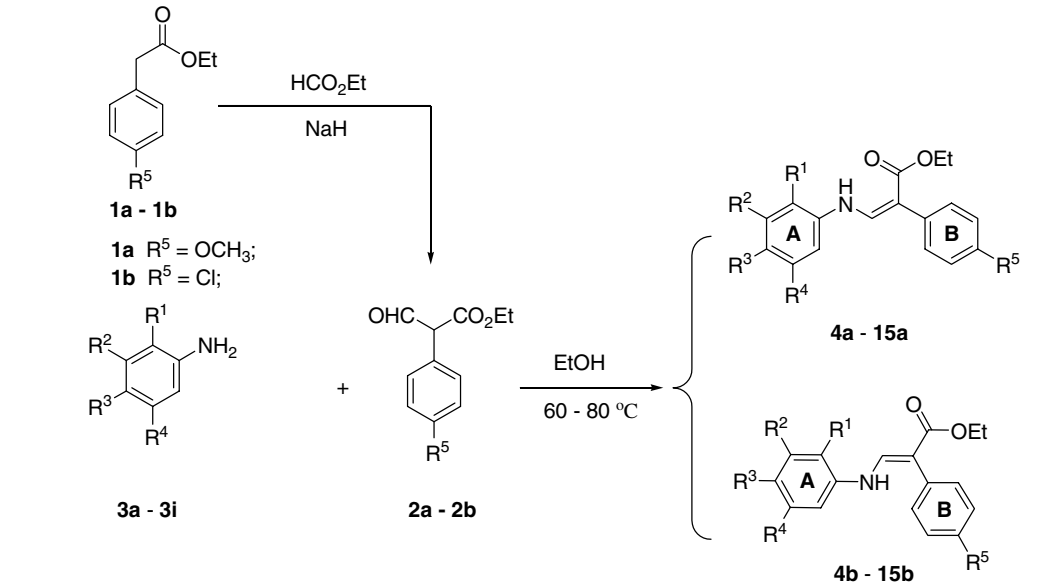
Among the compounds, the molecular structures of four compounds (**9a**, **9b**, **11a**, and **12b**) were determined by X-ray diffraction analysis. The crystal data are presented in Table 2, and Figure 1 gave perspective views of compounds **9a** and **9b** with the atomic labeling system. Obviously, **9a** is a *Z*-isomer and **9b** is an *E*-isomer. Based on the result and data of ^{1}H NMR, the *Z*/*E*-configurations of all new compounds were determined. Perspective view of compound **11a** was not given, because **9a** can account for the molecular structure of *Z*-isomer as a representative. Based on the same consideration, perspective view of compound **12b** was also not given. As for **9a**, C(1), C(2), C(3), C(4), C(5), and C(6) formed a plane with the mean deviation of 0.0072 Å, defined as plane I (A-ring); N(1), C(13), C(14), C(15), O(1), and O(2) were nearly coplanar with the mean deviation of 0.0326 Å, defined as plane II; C(7), C(8), C(9), C(10), C(11), and C(12) formed a plane (B-ring) with the mean deviation of 0.0072 Å, defined as plane III (Fig. 1 and Scheme 1). Plane II and plane III make a dihedral angle with plane I of 22.0 and 42.6°, and the dihedral angle between plane II and plane III is 55.2°. The molecular structures of **9b**, **11a**, and **12b** could be described as the above and the related data was shown in Table 3. The different dihedral angles for *Z*-isomer and *E*-isomers, as well as the different directions of A-rings compared with carbonyl moieties may be helpful to explain the different antibacterial activities of *Z*- and *E*-isomers. Some important bond lengths are given in Table 4. For compound **9a**, C13–C14 bond distance (1.371(4) Å) is followed in the range of a typical double bond, and C13-N1 (1.340(4) Å) bond has shorter bond distance than the standard C–N single bond (1.48 Å), but longer than C–N double bond (1.28 Å). This clearly indicated that the *p* orbital of N1 seems to be conjugated with the π molecular orbital of C13–C14 double bond. But out of our expectation, the *p* orbit of N1 seemed not to be conjugated with the π molecular orbital of the A-ring, which was explained by the bond length of C1–N1 (1.417(3) Å). We also speculated that the π molecular orbital of C13–C14 double bond was not conjugated with the π molecular orbital of B-ring, because the bond length of C14–C7 is 1.488(4) Å which follows in the normal range of single C–C bond length. The crystal structures of **9b**, **11a**, and **12b** gave the similar conclusions as described for **9a**.

2.3. Biological activity

New compounds (**4a**–**15a**, and **4b**–**15b**) were evaluated for their antimicrobial activities against four bacteria (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *Pseudomonas fluorescens* ATCC 13525 and *S. aureus* ATCC 6538) and three fungi (*Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231, and *Trichophyton rubrum* ATCC 10218), and the results are shown in Table 5. The MIC values of the compounds differed greatly, ranging from 0.9 to 50 μg/mL. Out of the compounds, four (**9b**, **11b**, **12b**, and **15b**) exhibited potent antibacterial activities against *S. aureus* ATCC 6538, with their MIC values at the same fold as penicillin and kanamycin. And of the four bacteria stains, *S. aureus* ATCC 6538 was more sensitive to the compounds than *E. coli* ATCC 35218, *P. fluorescens* ATCC 13525, or *B. subtilis* ATCC 6633. However, no compound showed significant inhibition activity against fungi, supported by the fact that their MICs were all over 50 μg/mL (data not shown).

As shown in Table 5, compounds **9b**, **11b**, **12b**, and **15b** exhibited excellent activities against *S. aureus* ATCC 6538, whereas obviously their *Z*-isomers (compounds **9a**, **11a**, **12a**, and **15a**) showed much lower inhibitions under the same conditions. This indicated that *E*-isomer was more active than *Z*-isomer, and we could find support from other data listed in Table 5, like compounds **6a**, **6b**, **9a**, and **9b**, and so on.

Inspection of the chemical structure of the final compound (Scheme 1) suggested that it could be divided into two subunits: A-ring and B-ring. Initial SAR studies were performed by modification of the parent compound to determine how the substituents of the subunits affected the antibacterial activities. Replacement of a methyl group (compound **15b**) at 4-position on A-ring by a bromide resulted in the complete loss of its antibacterial activity (compound **10b**). Similarly, substitution of a bromide (compound **8b**) for a hydroxyl group (compound **9b**) also led to a decrease of inhibitory activity. This suggested that compounds with electron-donating groups on A-ring showed better inhibitory activities



3a R^1 = H, R^2 = R^4 = Br, R^3 = OCH_3;
3b R^1 = R^3 =H, R^2 = R^4 = OCH_3;
3c R^1 = R^3 =H, R^2 = R^4 = Cl;
3d R^1 = R^3 =H, R^2 = R^4 = F;
3e R^1 = R^2 = R^4 = H, R^3 = Br;
3f R^1 = R^2 = R^4 =H, R^3 = OH;
3g R^1 = Cl, R^2 =R^3 = R^4 =H;
3h R^1 = R^2 = R^4 = H, R^3 = CH_3;
3i R^1 = R^2 = R^4 = H, R^3 = Cl;

2a R^5 = OCH_3;
2b R^5 = Cl;

4 R^1 = H, R^2 = R^4 = Br, R^3 = R^5 = OCH_3;
5 R^1 = R^3 =H, R^2 = R^4 = R^5 = OCH_3;
6 R^1 = R^3 =H, R^2 = R^4 = Cl, R^5 = OCH_3;
7 R^1 = R^3 =H, R^2 = R^4 = F, R^5 = OCH_3;
8 R^1 = R^2 = R^4 = H, R^3 = Br, R^5 = OCH_3;
9 R^1 = R^2 = R^4 =H, R^3 = OH, R^5 = OCH_3;
10 R^1 = R^2 = R^4 = H, R^3 = Br, R^5 = Cl;
11 R^1 = R^3 =H, R^2 = R^4 = F, R^5 = Cl;
12 R^1 = R^3 =H, R^2 = R^4 = Cl, R^5 = Cl;
13 R^1 = Cl, R^2 =R^3 = R^4 =H, R^5 = Cl;
14 R^1 = R^2 = R^4 = H, R^3 = Cl, R^5 = Cl;
15 R^1 = R^2 = R^4 = H, R^3 = CH_3, R^5 = Cl;

An "**a**" is for a *Z*-isomer and a "**b**" is for an *E*-isomer. For example, **4a** is a *Z*-isomer and **4b** is an *E*-isomer.

Scheme 1.

than those with electron-withdrawing groups. For more extensive evidences, the substitution of methoxy groups at 3,5-positions on A-ring (compound**5b**) by halogens (compounds **6b** and **7b**) produced some decrease in activity. On the contrary, the effect of a substitution on the B-ring was reverse compared with that on A-ring. For instance, changing the methoxy group at 4-position on B-ring (compounds **6b** and **7b**) for a chloride (compounds **12b** and **11b**) significantly increased activity. In summary, an electron-donating group on A-ring led to some increase in activity, but as for B-ring, a similar substitution provided a significant decrease in activity.

3. Conclusions

Twenty-four enamines (**4a**–**15a** and **4b**–**15b**) were synthesized and four (**9a**, **9b**, **11a**, and **12b**) were determined by X-ray diffraction analysis for the molecular structures. To study the potential antibacterial activities of the synthesized compounds, screening experiments were performed for four bacteria and three fungi strains. In general, these synthesized compounds were shown more effective to inhibiting growth of bacteria than fungi. Compounds **9b**, **11b**, **12b**, and **15b** (MIC = 3.8, 1.9, 1.1, and 0.9 μg/mL) showed considerable antibacterial activities against *S. aureus* ATCC 6538. Generally, an *E*-isomer exhibited higher antibacterial activity than the corresponding *Z*-isomer, and for compounds with the same configuration, electron-donating groups on A-ring helped to enhance the activities, whereas electron-withdrawing group on B-ring did the same thing.

4. Experiments

4.1. Antimicrobial activity

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 MH medium, The antifungal activities of the compounds were tested against *A. niger* ATCC 16404, *C. albicans* ATCC 10231, and *T. rubrum* ATCC 10218 using RPMI-1640 medium. The MICs of the test compounds were determined by a colorimetric method using the dye MTT.[10] A stock solution of the synthesized compounds (50 μg/mL) in

Table 1. Percent yields and melting points of enamines

Compound	Formula	Yield (%)	Mp (°C) (uncorrected)
4a	$C_{19}H_{19}Br_2NO_4$	80	102–104
4b	$C_{19}H_{19}Br_2NO_4$		101–102
5a	$C_{20}H_{23}NO_5$	33	87–89
5b	$C_{20}H_{23}NO_5$		110–111.5
6a	$C_{18}H_{17}Cl_2NO_3$	90	101–102
6b	$C_{18}H_{17}Cl_2NO_3$		108–109
7a	$C_{18}H_{17}F_2NO_3$	98	82–84
7b	$C_{18}H_{17}F_2NO_3$		93–95
8a	$C_{18}H_{18}BrNO_3$	83	58–59.5
8b	$C_{18}H_{18}BrNO_3$		124–125
9a	$C_{18}H_{19}NO_4$	79	104–105
9b	$C_{18}H_{19}NO_4$		136–137
10a	$C_{17}H_{15}BrClNO_2$	74	105–106
10b	$C_{17}H_{15}BrClNO_2$		146–147.5
11a	$C_{17}H_{14}ClF_2NO_2$	83	106–107
11b	$C_{17}H_{14}ClF_2NO_2$		118–120
12a	$C_{17}H_{14}Cl_3NO_2$	78	115–116
12b	$C_{17}H_{14}Cl_3NO_2$		126–128
13a	$C_{17}H_{15}Cl_2NO_2$	78	90–91
13b	$C_{17}H_{15}Cl_2NO_2$		97–98
14a	$C_{17}H_{15}Cl_2NO_2$	59	84–85
14b	$C_{17}H_{15}Cl_2NO_2$		135–137
15a	$C_{17}H_{18}ClNO_2$	71	71–72
15b	$C_{17}H_{18}ClNO_2$		121–122

Table 2. Crystal structure data for **9a**, **9b**, **11a**, and **12b**

Compound	**9a**	**9b**	**11a**	**12b**
Formula	$C_{18}H_{19}NO_4$	$C_{18}H_{19}NO_4$	$C_{17}H_{14}ClF_2NO_2$	$C_{17}H_{14}Cl_3NO_2$
M_r	313.34	313.34	337.74	370.64
Crystal size (mm^3)	$0.40 \times 0.30 \times 0.20$	$0.40 \times 0.20 \times 0.20$	$0.30 \times 0.20 \times 0.10$	$0.40 \times 0.30 \times 0.30$
Crystal system	Orthorhombic	Triclinic	Monoclinic	Monoclinic
Space group	$P2(1)2(1)2(1)$	$P\bar{1}$	$P121/c$ 1	$P2(1)/n$
a (Å)	7.4770(15)	9.1510(18)	9.491	10.698(2)
b (Å)	11.662(2)	9.4750(19)	7.637	7.7440(15)
c (Å)	18.417(4)	11.509(2)	21.501	21.348(4)
α (°C)	90	110.39(3)	90	90
β (°C)	90	96.53(3)	91.38	101.46(3)
γ (°C)	90	113.89(3)	90	90
V ($Å^3$)	1605.9(6)	816.5(3)	1558.0	1733.3(6)
Z	4	2	4	4
D_c (g/cm^{-3})	1.159	1.275	1.440	1.420
μ (mm^{-1})	0.072	0.090	0.275	0.536
$F(000)$	580	332	696	760
θ range (°C)	2.07/25.96	1.98/25.97	1.89/25.98	1.95/25.98
Index range (h,k,l)	0/9, 0/14, 0/22	−11/0, −10/11, −14/14	0/11, 0/9, −26/26	0/13, 0/9, −26/25
Measured reflections	1882	3398	3201	3579
Observed reflections $I > 2\sigma(I)$	1822	3186	3017	3392
Min. and max. transmission	0.9858/0.9718	0.9822/0.9648	0.9731/0.9221	0.8557/0.8142
Data/restraints/parameters	1822/0/215	3186/0/215	3017/0/213	3392/0/213
Goodness-of-fit on F^2	1.074	0.932	0.977	1.000
R_1, wR_2 $[I > 2\sigma(I)]$	0.0450/0.0969	0.0664/0.1861	0.0738/0.1206	0.0674/0.1710
R_1, wR_2	0.0598/0.1061	0.1036/0.2276	0.1520/0.1523	0.1028/0.1991
Large diff. peak and hole (e $Å^{-3}$)	0.158/−0.234	0.217/−0.298	0.234/−0.235	0.403/−0.666

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 microtitration 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%

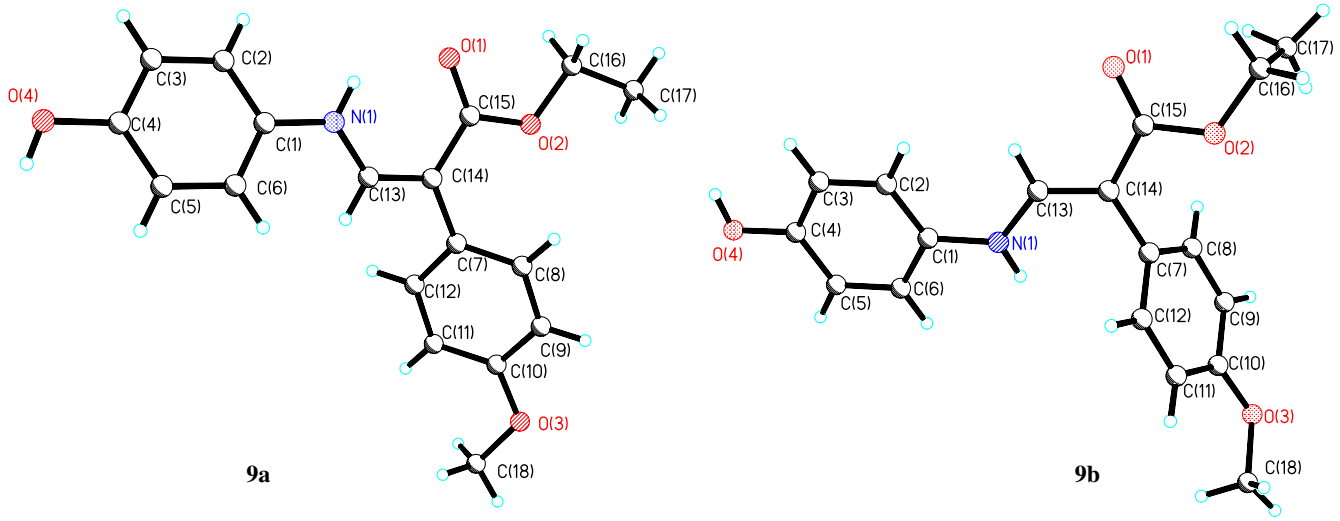


Figure 1. Molecular structures of **9a** and **9b**. Displacement ellipsoids are drawn at the 30% probability level.

Table 3. The dihedral angles and the mean deviation of the planes in compounds **9a**, **9b**, **11a**, and **12b**

	9a	9b	11a	12b
Dihedral angles (°)				
Plane I and plane II	22.0	16.3	19.6	42.5
Plane II and plane III	42.6	117.5	45.7	49.3
Plane I and plane III	55.2	107.6	51.1	74.2
The mean deviation of the plane (Å)				
Plane I	0.0072	0.0118	0.0099	0.0075
Plane II	0.0326	0.0373	0.019	0.0279
Plane III	0.0072	0.0023	0.0064	0.0102

Note: defined C1–C6 as plane I, defined C13, C14, C15, O1, and O2 as plane II, defined C7–C12 as plane III.

Table 4. Important bond lengths (Å) of compounds **9a**, **9b**, **11a**, and **12b**

	9a	9b	11a	12b
C1–N1	1.417(3)	1.414(3)	1.398(5)	1.404(4)
C7–C14	1.488(4)	1.491(4)	1.488(5)	1.479(4)
C13–N1	1.340(4)	1.337(4)	1.344(5)	1.352(4)
C13–C14	1.371(4)	1.354(4)	1.350(5)	1.352(4)
C14–C15	1.447(4)	1.447(4)	1.455(5)	1.470(5)
C15–O1	1.231(3)	1.222(3)	1.207(4)	1.214(4)
C15–O2	1.337(3)	1.335(3)	1.347(4)	1.342(4)

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 5.

4.2. Chemistry

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). The quantity of silica gel used was 50–100 times the weight charged on the column. Then, the eluates were monitored using TLC. 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 on a Bruker PX500 or DPX300 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN–O-Rapid instrument and were within ±0.4% of the theoretical values.

4.2.1. General synthesis method of enamines. The starting materials (aldehyde **2a**–**2b**) for the synthesis of enamines have been previously published.[11–13] Equimolar quantities (6 mmol) of the appropriate substituted aromatic amines and the aldehyde (**2a**–**2b**) in absolute alcohol (18 mL) were heated at 70–80 °C for 1–4 h. The excess solvent was removed under reduced pressure. The residue was purified by a flash chromatography with EtOAc–petroleum ether to afford two fractions. The first fraction gave a *Z*-isomer, and the second fraction gave an *E*-isomer.

Table 5. MICs of the synthesized compounds

Compound	Minimum inhibitory concentrations (μg/mL)			
	A	B	C	D
4a	>50	>50	>50	>50
4b	>50	>50	33	>50
5a	>50	>50	30	43.5
5b	>50	41.5	25.2	15.8
6a	>50	>50	33.8	>50
6b	42.2	42.2	27.8	20.2
7a	>50	>50	>50	>50
7b	>50	>50	31.5	22.8
8a	>50	>50	>50	>50
8b	>50	>50	31.7	>50
9a	>50	>50	>50	7.9
9b	>50	>50	>50	3.8
10a	>50	>50	>50	>50
10b	>50	>50	>50	>50
11a	>50	>50	>50	>50
11b	48.2	13.5	31.5	1.9
12a	>50	>50	>50	>50
12b	>50	>50	25.7	1.1
13a	>50	42.5	>50	>50
13b	43.8	38.8	35.3	>50
14a	>50	>50	32.2	>50
14b	42	42.5	29.5	>50
15a	>50	>50	>50	>50
15b	>50	>50	>50	0.9
Kanamycin	0.39	3.9	3.9	1
Penicillin	0.78	\	\	2

Note: A, *Bacillus subtilis* ATCC 6633; B, *Escherichia coli* ATCC 35218; C, *Pseudomonas fluorescens* ATCC 13525; D, *Staphylococcus aureus* ATCC 6538.

4.2.2. (*Z*)-Ethyl 3-(3,5-dibromo-4-methoxyphenylamino)-2-(4-methoxyphenyl)acrylate (4a). Shallow yellow crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 1.20 (t, J = 7.5 Hz, 3H); 3.74 (s, 3H); 3.75 (s, 3H); 4.18 (q, J = 7.1 Hz, 2H); 6.88 (d, J = 8.7 Hz, 2H); 7.27 (d, J = 8.8 Hz, 2H); 7.55 (d, J = 12.5 Hz, 1H); 7.70 (s, 2H); 10.11 (d, J = 12.2 Hz, 1H); MS (ESI): 484.0 ($C_{19}H_{20}Br_2NO_4$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{19}Br_2NO_4$: C, 47.04; H, 3.95; N, 2.89; Found: C, 47.04; H, 3.96; N, 2.87.

4.2.3. (*E*)-Ethyl 3-(3,5-dibromo-4-methoxyphenylamino)-2-(4-methoxyphenyl)acrylate (4b). Shallow yellow crystal, ^{1}H NMR (500 MHz, d_6-DMSO): 1.18 (t, J = 7.1 Hz, 3H); 3.72 (s, 3H); 3.78 (s, 3H); 4.09 (q, J = 7.0 Hz, 2H); 6.96 (d, J = 8.5 Hz, 2H); 7.13 (d, J = 8.5 Hz, 2H); 7.48 (s, 2H); 7.86 (d, J = 12.9 Hz, 1H); 8.51 (d, J = 13.0 Hz, 1H); MS (ESI): 484.0 ($C_{19}H_{20}Br_2NO_4$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{19}Br_2NO_4$: C, 47.04; H, 3.95; N, 2.89; Found: C, 47.02; H, 3.97; N, 2.86.

4.2.4. (*Z*)-Ethyl 3-(3,5-dimethoxyphenylamino)-2-(4-methoxyphenyl)acrylate (5a). White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 1.20 (t, J = 7.5 Hz, 3H); 3.73 (s, 6H); 3.75 (s, 3H); 4.17 (q, J = 7.0 Hz, 2H); 6.12 (d, J = 1.1 Hz, 1H); 6.44 (d, J = 1.3 Hz, 2H); 6.87 (d, J = 8.8 Hz, 2H); 7.24 (d, J = 8.8 Hz, 2H); 7.59 (d, J = 12.7 Hz, 1H); 10.19 (d, J = 12.6 Hz, 1H); MS (ESI): 358.2 ($C_{20}H_{24}NO_5$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{23}NO_5$: C, 67.24; H, 6.47; N, 3.91; Found: C, 67.21; H, 6.49; N, 3.92.

4.2.5. (*E*)-Ethyl 3-(3,5-dimethoxyphenylamino)-2-(4-methoxyphenyl)acrylate (5b). Colorless crystal, ^{1}H NMR (500 MHz, d_6-acetone): 1.21 (t, J = 7.5 Hz, 3H); 3.74 (s, 6H); 3.82 (s, 3H); 4.12 (q, J = 7.0 Hz, 2H); 6.11 (d, J = 1.8 Hz, 1H); 6.29 (d, J = 1.9 Hz, 2H); 6.92 (d, J = 8.6 Hz, 2H); 7.18 (d, J = 8.5 Hz, 2H); 7.69 (d, J = 12.6 Hz, 1H); 8.10 (d, J = 12.9 Hz, 1H); MS (ESI): 358.1 ($C_{20}H_{24}NO_5$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{23}NO_5$: C, 67.24; H, 6.47; N, 3.91; Found: C, 67.22; H, 6.50; N, 3.90.

4.2.6. (*Z*)-Ethyl 3-(3,5-dichlorophenylamino)-2-(4-methoxyphenyl)acrylate (6a). White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 1.19 (t, J = 7.2 Hz, 3H); 3.72 (s, 3H); 4.15 (q, J = 7.3 Hz, 2H); 6.71 (d, J = 1.5 Hz, 1H); 6.84 (d, J = 8.7 Hz, 2H); 7.13 (d, J = 1.6 Hz, 2H); 7.25 (d, J = 8.6 Hz, 2H); 7.55 (d, J = 12.4 Hz, 1H); 10.18 (d, J = 12.6 Hz, 1H); MS (ESI): 366.2 ($C_{18}H_{18}Cl_2NO_3$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{17}Cl_2NO_3$: C, 59.03; H, 4.68; N, 3.82; Found: C, 59.07; H, 4.67; N, 3.83.

4.2.7. (*E*)-Ethyl 3-(3,5-dichlorophenylamino)-2-(4-methoxyphenyl)acrylate (6b). Colorless crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 1.18 (t, J = 7.1 Hz, 3H); 3.74 (s, 3H); 4.06 (q, J = 7.3 Hz, 2H); 6.69 (d, J = 1.7 Hz, 1H); 6.88 (d, J = 8.8 Hz, 2H); 6.94 (d, J = 1.9 Hz, 2H);

7.15 (s, J = 8.7 Hz, 2H); 7.79 (s, J = 12.2 Hz, 1H); 8.54 (d, J = 12.5 Hz, 1H); MS (ESI): 366.1 ($C_{18}H_{18}Cl_2NO_3$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{17}Cl_2NO_3$: C, 59.03; H, 4.68; N, 3.82; Found: C, 59.01; H, 4.69; N, 3.85.

4.2.8. (*Z*)-Ethyl 3-(3,5-difluorophenylamino)-2-(4-methoxyphenyl)acrylate (7a). White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 1.18 (t, J = 7.1 Hz, 3H); 3.73 (s, 3H); 4.16 (q, J = 7.1 Hz, 2H); 6.73 (t, J = 9.5 Hz, 1H); 6.85 (d, J = 8.7 Hz, 2H); 7.12 (d, J = 7.8 Hz, 2H); 7.25 (d, J = 8.7 Hz, 2H); 7.54 (d, J = 12.2 Hz, 1H); 10.23 (d, J = 12.7 Hz, 1H); MS (ESI): 334.0 ($C_{18}H_{18}F_2NO_3$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{17}F_2NO_3$: C, 64.86; H, 5.14; N, 4.20; Found: C, 64.88; H, 5.12; N, 4.19.

4.2.9. (*E*)-Ethyl 3-(3,5-difluorophenylamino)-2-(4-methoxyphenyl)acrylate (7b). Colorless crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 1.18 (t, J = 7.1 Hz, 3H); 3.77 (s, 3H); 4.08 (q, J = 7.1 Hz, 2H); 6.70 (t, J = 9.4 Hz, 1H); 6.90 (d, J = 9.7 Hz, 2H); 6.95 (d, J = 8.7 Hz, 2H); 7.14 (s, J = 8.7 Hz, 2H); 7.90 (s, J = 12.8 Hz, 1H); 8.70 (d, J = 12.9 Hz, 1H); MS (ESI): 334.1 ($C_{18}H_{18}F_2NO_3$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{17}F_2NO_3$: C, 64.86; H, 5.14; N, 4.20; Found: C, 64.87; H, 5.13; N, 4.19.

4.2.10. (*Z*)-Ethyl 3-(4-bromophenylamino)-2-(4-methoxyphenyl)acrylate (8a). White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 1.19 (t, J = 7.1 Hz, 3H); 3.74 (s, 3H); 4.16 (q, J = 7.1 Hz, 2H); 6.87 (d, J = 8.6 Hz, 2H); 7.24 (d, J = 8.8 Hz, 2H); 7.25 (d, J = 8.5 Hz, 2H); 7.44 (d, J = 8.9 Hz, 2H); 7.51 (d, J = 12.7 Hz, 1H); 10.19 (d, J = 12.4 Hz, 1H); MS (ESI): 376.1 ($C_{18}H_{19}BrNO_3$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}BrNO_3$: C, 57.46; H, 4.82; N, 3.72; Found: C, 57.44; H, 4.80; N, 3.73.

4.2.11. (*E*)-Ethyl 3-(4-bromophenylamino)-2-(4-methoxyphenyl)acrylate (8b). Colorless crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 1.17 (t, J = 7.1 Hz, 3H); 3.77 (s, 3H); 4.07 (q, J = 6.9 Hz, 2H); 6.95 (d, J = 8.6 Hz, 2H); 7.11 (d, J = 8.3 Hz, 2H); 7.14 (d, J = 8.4 Hz, 2H); 7.41 (d, J = 8.7 Hz, 2H); 7.94 (d, J = 12.8 Hz, 1H); 8.55 (d, J = 13.0 Hz, 1H); MS (ESI): 376.0 ($C_{18}H_{19}BrNO_3$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}BrNO_3$: C, 57.46; H, 4.82; N, 3.72; Found: C, 57.47; H, 4.80; N, 3.71.

4.2.12. (*Z*)-Ethyl 3-(4-hydroxyphenylamino)-2-(4-methoxyphenyl)acrylate (9a). Colorless crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 1.18 (t, J = 7.1 Hz, 3H); 3.73 (s, 3H); 4.14 (q, J = 7.0 Hz, 2H); 6.71 (d, J = 8.7 Hz, 2H); 6.85 (d, J = 8.7 Hz, 2H); 7.06 (d, J = 8.7 Hz, 2H); 7.23 (d, J = 8.4 Hz, 2H); 7.40 (d, J = 13.0 Hz, 1H); 10.09 (d, J = 12.8 Hz, 1H); MS (ESI): 314.1 ($C_{18}H_{20}NO_4$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{19}NO_4$: C, 68.99; H, 6.11; N, 4.47; Found: C, 69.01; H, 6.09; N, 4.48.

4.2.13. (*E*)-Ethyl 3-(4-hydroxyphenylamino)-2-(4-methoxyphenyl)acrylate (9b). Colorless crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 1.15 (t, J = 7.1 Hz, 3H); 3.76 (s, 3H); 4.04 (q, J = 7.1 Hz, 2H); 6.67 (d, J = 8.8 Hz, 2H); 6.93 (d, J = 8.7 Hz, 2H); 6.96 (d, J = 8.6 Hz, 2H); 7.13 (d, J = 8.7 Hz, 2H); 7.87 (d, J = 13.6 Hz, 1H); 8.23 (d, J = 13.5 Hz, 1H); MS (ESI): 314.2 ($C_{18}H_{20}NO_4$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{19}NO_4$: C, 68.99; H, 6.11; N, 4.47; Found: C, 68.98; H, 6.12; N, 4.46.

4.2.14. (*Z*)-Ethyl 3-(4-bromophenylamino)-2-(4-chlorophenyl)acrylate (10a). White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 1.20 (t, J = 7.1 Hz, 3H); 4.17 (q, J = 7.1 Hz, 2H); 7.28 (d, J = 9.0 Hz, 2H); 7.34 (d, J = 9.0 Hz, 2H); 7.38 (d, J = 8.9 Hz, 2H); 7.45 (d, J = 8.9 Hz, 2H); 7.63 (d, J = 12.7 Hz, 1H); 10.27 (d, J = 12.9 Hz, 1H); MS (ESI): 380.0 ($C_{17}H_{16}BrClNO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}BrClNO_2$: C, 53.64; H, 3.97; N, 3.68; Found: C, 53.66; H, 3.98; N, 3.67.

4.2.15. (*E*)-Ethyl 3-(4-bromophenylamino)-2-(4-chlorophenyl)acrylate (10b). Colorless crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 1.17 (t, J = 7.1 Hz, 3H); 4.08 (q, J = 7.1 Hz, 2H); 7.12 (d, J = 8.6 Hz, 2H); 7.24 (d, J = 8.5 Hz, 2H); 7.43 (d, J = 8.4 Hz, 4H); 7.98 (d, J = 13.0 Hz, 1H); 8.76 (d, J = 13.2 Hz, 1H); MS (ESI): 380.1 ($C_{17}H_{16}BrClNO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}BrClNO_2$: C, 53.64; H, 3.97; N, 3.68; Found: C, 53.63; H, 3.98; N, 3.66.

4.2.16. (*Z*)-Ethyl 3-(3,5-difluorophenylamino)-2-(4-chlorophenyl)acrylate (11a). Colorless crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 1.19 (t, J = 7.1 Hz, 3H); 4.18 (q, J = 7.1 Hz, 2H); 6.77 (d, J = 9.3 Hz, 1H); 7.17 (d, J = 8.1 Hz, 2H); 7.34 (d, J = 8.9 Hz, 2H); 7.38 (d, J = 8.4 Hz, 2H); 7.65 (d, J = 12.5 Hz, 1H); 10.30 (d, J = 12.8 Hz, 1H); MS (ESI): 338.1 ($C_{17}H_{15}ClF_2NO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}ClF_2NO_2$: C, 60.45; H, 4.18; N, 4.15; Found: C, 60.48; H, 4.19; N, 4.16.

4.2.17. (*E*)-Ethyl 3-(3,5-difluorophenylamino)-2-(4-chlorophenyl)acrylate (11b). Colorless crystal, ^{1}H NMR (500 MHz, d_6-DMSO): 1.19 (t, J = 7.2 Hz, 3H); 4.11 (q, J = 7.2 Hz, 2H); 6.75 (d, J = 9.1 Hz, 1H); 6.94 (d, J = 9.1 Hz, 2H); 7.26 (d, J = 8.3 Hz, 2H); 7.46 (d, J = 8.3 Hz, 2H); 7.97 (d, J = 12.9 Hz, 1H); 8.87 (d, J = 13.0 Hz, 1H); MS (ESI): 338.0 ($C_{17}H_{15}ClF_2NO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}ClF_2NO_2$: C, 60.45; H, 4.18; N, 4.15; Found: C, 60.42; H, 4.18; N, 4.17.

4.2.18. (*Z*)-Ethyl 3-(3,5-dichlorophenylamino)-2-(4-chlorophenyl)acrylate (12a). White powder, ^{1}H NMR (500 MHz, d_6-DMSO): 1.22 (t, J = 7.1 Hz, 3H); 4.20 (q, J = 7.1 Hz, 2H); 7.15 (s, 1H); 7.37 (d, J = 8.4 Hz, 2H); 7.41 (d, J = 8.4 Hz, 2H); 7.52 (s, 2H); 7.71 (d, J = 12.6 Hz, 1H); 10.28 (d, J = 12.5 Hz, 1H); MS (ESI): 370.0 ($C_{17}H_{15}Cl_3NO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Cl_3NO_2$: C, 55.09; H, 3.81; N, 3.78; Found: C, 55.13; H, 3.80; N, 3.75.

4.2.19. (*E*)-Ethyl 3-(3,5-dichlorophenylamino)-2-(4-chlorophenyl)acrylate (12b). Colorless crystal, ^{1}H NMR (500 MHz, d_6-DMSO): 1.19 (t, J = 6.9 Hz, 3H); 4.12 (q, J = 7.0 Hz, 2H); 7.11 (s, 1H); 7.26 (d, J = 7.9 Hz, 2H); 7.27 (s, 2H); 7.46 (d, J = 8.2 Hz, 2H); 7.97 (d, J = 12.9 Hz, 1H); 8.81 (d, J = 12.9 Hz, 1H); MS (ESI): 370.1 ($C_{17}H_{15}Cl_3NO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Cl_3NO_2$: C, 55.09; H, 3.81; N, 3.78; Found: C, 55.12; H, 3.82; N, 3.80.

4.2.20. (*Z*)-Ethyl 3-(2-chlorophenylamino)-2-(4-chlorophenyl)acrylate (13a). White powder, ^{1}H NMR (500 MHz, $CDCl_3$): 1.29 (t, J = 7.1 Hz, 3H); 4.28 (q, J = 7.0 Hz, 2H); 6.92 (t, J = 8.2 Hz, 1H); 7.11 (d, J = 8.2 Hz, 1H); 7.23 (t, J = 8.2 Hz, 1H); 7.26 (d, J = 8.0 Hz, 1H); 7.29 (d, J = 7.6 Hz, 4H); 7.36 (d, J = 12.5 Hz, 1H); 10.68 (d, J = 12.4 Hz, 1H); MS (ESI): 370.0 ($C_{17}H_{15}Cl_3NO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Cl_3NO_2$: C, 55.09; H, 3.81; N, 3.78; Found: C, 55.13; H, 3.80; N, 3.80.

4.2.21. (*E*)-Ethyl 3-(2-chlorophenylamino)-2-(4-chlorophenyl)acrylate (13b). Colorless crystal, ^{1}H NMR (500 MHz, $CDCl_3$): 1.26 (t, J = 7.1 Hz, 3H); 4.18 (q, J = 7.2 Hz, 2H); 6.90 (t, J = 8.1 Hz, 1H); 7.05 (d, J = 8.1 Hz, 1H); 7.20 (t, J = 8.2 Hz, 1H); 7.18 (d, J = 8.1 Hz, 1H); 7.26 (d, J = 7.6 Hz, 4H); 7.43 (d, J = 12.5 Hz, 1H); 80.13 (d, J = 12.9 Hz, 1H);MS (ESI): 370.1 ($C_{17}H_{15}Cl_3NO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Cl_3NO_2$: C, 55.09; H, 3.81; N, 3.78; Found: C, 55.12; H, 3.82; N, 3.80.

4.2.22. (*Z*)-Ethyl 3-(4-chlorophenylamino)-2-(4-chlorophenyl)acrylate (14a). White powder, ^{1}H NMR (300 MHz, d_6-acetone): 1.25 (t, J = 7.1 Hz, 3H); 4.23 (q, J = 7.1 Hz, 2H); 7.28–7.43 (m, 8H); 7.66 (d, J = 12.6 Hz, 1H); 10.45 (d, J = 12.4 Hz, 1H); MS (ESI): 336.1 ($C_{17}H_{16}Cl_2NO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}Cl_2NO_2$: C, 60.73; H, 4.50; N, 4.17; Found: C, 60.75; H, 4.51; N, 4.18.

4.2.23. (*E*)-Ethyl 3-(4-chlorophenylamino)-2-(4-chlorophenyl)acrylate (14b). Colorless crystal, ^{1}H NMR (300 MHz, d_6-acetone): 1.21 (t, J = 7.1 Hz, 3H); 4.13 (q, J = 7.1 Hz, 2H); 7.16 (d, J = 8.9 Hz, 2H); 7.30 (d, J = 8.8 Hz, 4H); 7.39 (d, J = 8.6 Hz, 2H); 8.15 (bs, 2H); MS (ESI): 336.0 ($C_{17}H_{16}Cl_2NO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}Cl_2NO_2$: C, 60.73; H, 4.50; N, 4.17; Found: C, 60.78; H, 4.48; N, 4.19.

4.2.24. (*Z*)-Ethyl 3-(4-methyphenylamino)-2-(4-chlorophenyl)acrylate (15a). White powder, ^{1}H NMR (500 MHz, $CDCl_3$): 1.28 (t, J = 7.1 Hz, 3H); 2.30 (s, 3H); 4.25 (q, J = 7.0 Hz, 2H); 6.91 (d, J = 8.4 Hz, 2H); 7.11 (d, J = 8.3 Hz, 2H); 7.27 (d, J = 7.9 Hz, 4H); 7.35 (d, J = 12.8 Hz, 1H); 10.28 (d, J = 12.6 Hz, 1H); MS (ESI): 315.8 ($C_{18}H_{19}ClNO_2$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}ClNO_2$: C, 68.46; H, 5.75; Cl, 11.23; N, 4.44; Found: C, 68.51; H, 5.74; Cl, 11.20; N, 4.45.

4.2.25. (*E*)-Ethyl 3-(4-methyphenylamino)-2-(4-chlorophenyl)acrylate (15b). Colorless crystal, ^{1}H NMR (500 MHz, $CDCl_3$): 1.26 (t, J = 7.1 Hz, 3H); 2.28 (s, 3H); 4.22 (q, J = 7.0 Hz, 2H); 6.81 (d, J = 8.4 Hz, 2H); 7.10 (d, J = 8.3 Hz, 2H); 7.25 (d, J = 8.7 Hz, 2H); 7.27 (d, J = 8.6 Hz, 2H); 7.40 (d, J = 12.3 Hz, 1H); 8.12 (d, J = 12.4 Hz, 1H); MS (ESI): 315.7 ($C_{18}H_{19}ClNO_2$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}ClNO_2$: C, 68.46; H, 5.75; Cl, 11.23; N, 4.44; Found: C, 68.50; H, 5.76; Cl, 11.24; N, 4.43.

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