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Original article

Enamines as novel antibacterials and their structure–activity relationships

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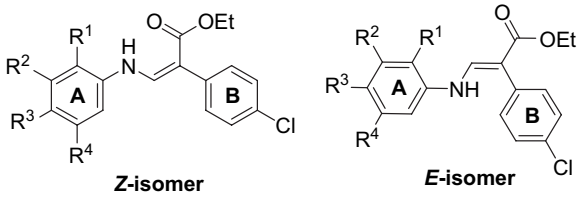
Abstract

Twenty-six enamines were synthesized to screen for the antimicrobial activity. Out of the compounds, 22 were reported for the first time. Their chemical structures including *E*/*Z*-configurations were clearly determined by ^{1}H NMR, ESI mass spectra and elemental analyses, coupled with three selected single-crystal structures. In general, these synthetic compounds were shown to be more effective to inhibit growth of bacteria than fungi. The most active compound, (*E*)-ethyl 3-(4-hydroxyphenylamino)-2-(4-chlorophenyl)acrylate (**1b**), showed considerable antibacterial activities against *Staphylococcus aureus* ATCC 6538 with MIC of 0.5 μg/mL and against *Pseudomonas fluorescens* ATCC 13525 with MIC of 1.5 μg/mL, which was superior to the positive controls penicillin and kanamycin, respectively. Structure–activity relationship analysis revealed: as for A-ring, the compounds substituted at 3,5-positions were more active than 2,4-position-substituted derivatives, and halo-substituted analogs at 2-position had essentially same activities as the 4-position-substituted derivatives. Increase of steric hindrance around the nitrogen atom led to an inactive compound.



Keywords: Enamine; Antibacterial; Crystal structure; Structure–activity relationship

1. Introduction

Since the first Schiff base metal complex was synthesized by Schiff in 1869, the study of antibacterial, antifungal and antitumor activities of Schiff bases and their metal complexes has been widely discussed [1–7]. An enamine, a tautomer of a Schiff base, shows a high similarity to the corresponding Schiff base in chemical structure, which leads to the conception that a stable enamine may possess similar biological properties as a Schiff base does. Our recent work [8] affirmed their activities and disclosed early structure–activity relationships (SARs). Most significantly, an *E*-isomer exhibited higher antibacterial activity than the corresponding *Z*-isomer. Secondly, electron-donating groups on A-ring helped to enhance the activities, whereas electron-withdrawing group on B-ring did the same thing. In light of these observations, we were

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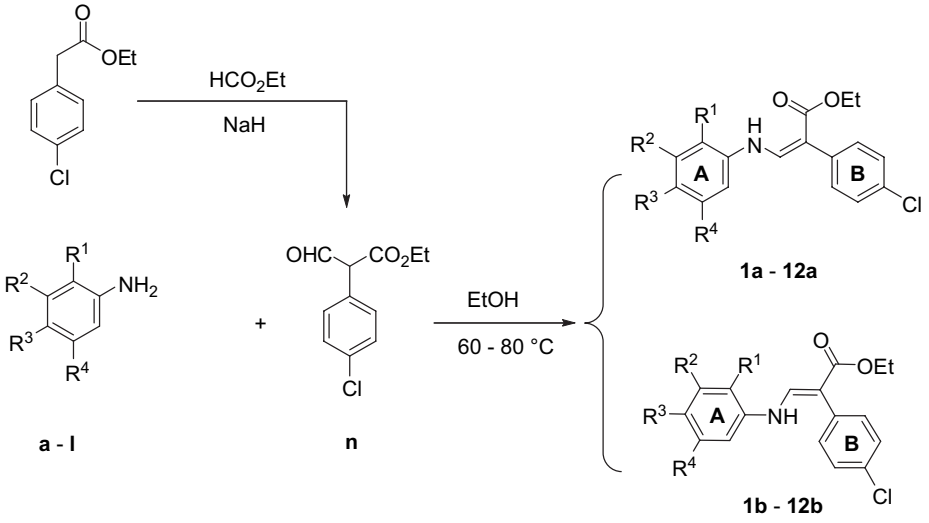


a $R^1 = R^2 = R^4 = H$, $R^3 = OH$;
b $R^1 = R^3 = H$, $R^2 = R^4 = OCH_3$;
c $R^1 = H$, $R^3 = OBn$, $R^2 = R^4 = Br$;
d $R^1 = H$, $R^3 = OMe$, $R^2 = R^4 = Br$;
e $R^1 = H$, $R^3 = OH$, $R^2 = R^4 = Br$;
f $R^1 = R^2 = R^4 = H$, $R^3 = F$;
g $R^1 = F$, $R^2 = R^3 = R^4 = H$;
h $R^1 = R^3 = Cl$, $R^2 = R^4 = H$;
i $R^1 = R^3 = F$, $R^2 = R^4 = H$;
j $R^1 = R^3 = Br$, $R^2 = R^4 = H$;
k $R^1 = R^3 = H$, $R^2 = R^4 = Cl$;
l $R^1 = R^3 = H$, $R^2 = R^4 = F$;

1 $R^1 = R^2 = R^4 = H$, $R^3 = OH$;
2 $R^1 = R^3 = H$, $R^2 = R^4 = OCH_3$;
3 $R^1 = H$, $R^3 = OBn$, $R^2 = R^4 = Br$;
4 $R^1 = H$, $R^3 = OMe$, $R^2 = R^4 = Br$;
5 $R^1 = H$, $R^3 = OH$, $R^2 = R^4 = Br$;
6 $R^1 = R^2 = R^4 = H$, $R^3 = F$;
7 $R^1 = F$, $R^2 = R^3 = R^4 = H$;
8 $R^1 = R^3 = Cl$, $R^2 = R^4 = H$;
9 $R^1 = R^3 = F$, $R^2 = R^4 = H$;
10 $R^1 = R^3 = Br$, $R^2 = R^4 = H$;
11 $R^1 = R^3 = H$, $R^2 = R^4 = Cl$;
12 $R^1 = R^3 = H$, $R^2 = R^4 = F$;

An "**a**" is for a *Z*-isomer and a "**b**" is for an *E*-isomer. For example, **1a** is a *Z*-isomer and **1b** is an *E*-isomer.

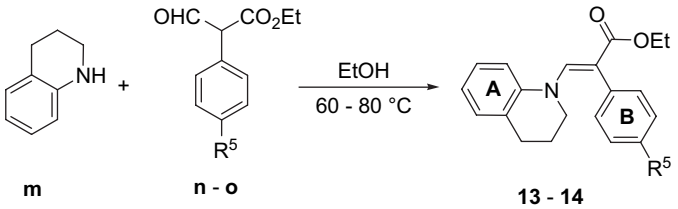
Scheme 1.

encouraged to use the most active enamine ((*E*)-ethyl 3-(3,5-dichlorophenylamino)-2-(4-chlorophenyl)acrylate (**11b**) with MIC of 1.1 μg/mL against *Staphylococcus aureus* ATCC 6538 and of 25.7 μg/mL against *Pseudomonas fluorescens* ATCC 13525) as a precursor compound for further optimization and to undertake more extensive study into the SAR, the results of which are described below.

2. Results and discussion

2.1. Chemistry

In our recent paper [8], we reported that some enamines were comparable or superior to penicillin and kanamycin in vitro antimicrobial activity. For the optimization of antibacterial activity, we subsequently shifted our focus to the modification of the A-ring and amino group moiety as a means of increasing potency (Scheme 1). Twenty-six compounds were synthesized for this consideration, and 22 of them were reported for the first time. The synthesis of compounds **1a**–**12a** and **1b**–**12b** followed the general pathway outlined in Scheme 1, while that of compounds **13** and **14** is shown in Scheme 2. The designed enamines were prepared by a dehydration reaction of aldehydes (**n**–**o**) with different arylamines (**a**–**m**). The crude products consisted of the mixture of *Z*- and *E*-isomer (**1a**–**12a** and **1b**–**12b**). Subsequent purification by flash chromatography provided **1a**–**12a** and **1b**–**12b** as pure isomers. It is noted that **13** and **14** were only given as *E*-isomers, for which steric hindrance around the nitrogen atom may be responsible. The total yields of *Z*- and *E*-isomer and uncorrected melting points are summarized in Table 1. An *E*-isomer had higher melting points than that of the corresponding *Z*-isomer, but melting points of **9a** and **9b** was reversed, which is difficult to explain. The stereochemistry



n $R^5 = Cl$;
o $R^5 = OCH_3$;

13 $R^5 = Cl$;
14 $R^5 = OCH_3$;

Scheme 2.

Table 1
Percent yields and melting points of enamines

Compound	Formula	Yield, %	Mp., °C (uncorrected)
1a	$C_{17}H_{16}ClNO_3$	86.3	149–150
1b	$C_{17}H_{16}ClNO_3$		152–153
2a	$C_{19}H_{20}ClNO_4$	68	101–103
2b	$C_{19}H_{20}ClNO_4$		108–109
3a	$C_{24}H_{20}Br_2ClNO_3$	67	119–120
3b	$C_{24}H_{20}Br_2ClNO_3$		122–124
4a	$C_{18}H_{16}Br_2ClNO_3$	76	138–139
4b	$C_{18}H_{16}Br_2ClNO_3$		154–156
5a	$C_{17}H_{14}Br_2ClNO_3$	92	175–176
5b	$C_{17}H_{14}Br_2ClNO_3$		181–183
6a	$C_{17}H_{15}ClFNO_2$	94	68–70
6b	$C_{17}H_{15}ClFNO_2$		118–119
7a	$C_{17}H_{15}ClFNO_2$	96	85–86
7b	$C_{17}H_{15}ClFNO_2$		92–93
8a	$C_{17}H_{14}Cl_3NO_2$	97	101–102
8b	$C_{17}H_{14}Cl_3NO_2$		117–118
9a	$C_{17}H_{14}ClF_2NO_2$	64	131–132
9b	$C_{17}H_{14}ClF_2NO_2$		54–55
10a	$C_{17}H_{14}Br_2ClNO_2$	97	92–94
10b	$C_{17}H_{14}Br_2ClNO_2$		108–110
11a	$C_{17}H_{14}Cl_3NO_2$	78	115–116
11b	$C_{17}H_{14}Cl_3NO_2$		126–128
12a	$C_{17}H_{14}ClF_2NO_2$	83	106–107
12b	$C_{17}H_{14}ClF_2NO_2$		118–120
13	$C_{20}H_{20}ClNO_2$	93	93–94
14	$C_{21}H_{23}NO_3$	94	88–89

of all newly reported enamines (**1a**–**10a**, **1b**–**10b**, **13** and **14**) was determined by their ^{1}H NMR spectra together with the crystal structures of the selected compounds.

2.2. Description of the crystal structure and determination of stereochemistry

Among these compounds, the crystal structures of compounds **2b**, **6a** and **14** were determined by X-ray diffraction analysis. The crystallographic data of them are presented in Table 2, and their selected bond lengths/angles and torsion angles are given in Table 3. The bond angles related to atoms C13 and C14 are all in normal values. The bond distances of C13–C14 (1.343(8)–1.358(3) Å) conformed to the value for a C=C double bond, while the bond lengths of C13–N1 (1.347(7)–1.361(3) Å) were shorter than standard C–N single bond (1.48 Å) but longer than C=N double bond (1.28 Å), which might be induced by the conjugation of the p orbital of N1 and the π molecular orbital of C13–C14 double bond. There was another evidence to prove that C13–C14 were double bonds. The proof is that the torsion angles of N1–C13–C14–C15 and N1–C13–C14–C7 were close to 0° or 180° (Table 3). Namely, N1–C13–C14–C15–C7 was coplanar. In fact, the N1, H13, C13, C14, C15 and C7 were in a least-square plane with the mean deviations of 0.014, 0.015 and 0.0596 Å for **2b**, **6a** and **14**, respectively. Compounds **2b**, **6a** and **14** were therefore identified as enamines. By comparing their ^{1}H NMR spectra (**2b**, **6a** and **14**) with others all synthetic compounds were determined as enamines.

Fig. 1 gives perspective views of compounds **2b**, **6a** and **14** with the atomic labeling system. The dihedral angles between two phenyl planes are 20.33(16)°, 74.60(23)° and 89.91(08)° for compounds **2b**, **6a** and **14**, respectively. Although the ethyl group of **6a** and **14** was disordered, the configurations of the enamines could be identified obviously. Compound **6a** was a *Z*-isomer and the compounds **2b** and **14** were both *E*-isomers. As shown in Table 4, there are intramolecular C–H ···O hydrogen bonds in all three crystal structures, intermolecular N–H···Cl hydrogen bond in **2b** and intramolecular N–H···O hydrogen bond in **6a**. Due to the intramolecular N1–H1··· O1 hydrogen bond (in **6a**), the ^{1}H NMR signal of H1 ($\delta = 10.31$ ppm) was shifted downfield compared to the corresponding resonance in compound **6b** ($\delta = 8.53$ ppm). The specific hydrogen resonance (δ at about 10 ppm) helped to assign compounds **1a**–**12a** as *Z*-isomers. Similarly, based on the crystal structure of **2b** and data of ^{1}H NMR, **1b**–**12b** were determined as *E*-configuration. As for compounds **13** and **14**, their configurations were not determined by the aforementioned method because the specific hydrogen signal (bonded with nitrogen) did not exist. Fortunately, the configuration of compound **14** was determined by X-ray crystal structure. The similarity of the ^{1}H NMR spectra of compounds **13** and **14** suggested that compound **13** was also of the *E*-configuration.

2.3. Biological activity

In order to determine whether the geometrical isomers are configurationally stable in aqueous media, specified quantities of the test compounds (**1a**–**12a** and **1b**–**12b**) were added to the RPMI-1640 media and heated at 37 °C for 24 h. TLC monitoring indicated that the geometrical isomers are configurationally stable in the test media. The minimum inhibitory concentration (MIC) was determined for each compound along with penicillin and kanamycin as standard controls. All of the synthetic compounds were evaluated for their antimicrobial activities against four bacteria (*Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *P. 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 presented in Table 5. These compounds displayed very poor activity (MICs > 50 μg/mL) against fungal strains, but they showed good activity against bacterial strains, especially against *S. aureus* ATCC 6538 and *P. fluorescens* ATCC 13525. The most active agents against the two bacterial strains were compounds **1b**, **2b** and **5b** (with MICs of 0.5, 0.9 and 1.0 against *S. aureus* ATCC 6538; 1.5, 1.6 and 3.7 against *P. fluorescens* ATCC 13525). As expected, their *Z*-isomers (compounds **1a**, **2a** and **5a**) showed much lower inhibitions under the same conditions. This indicated that *E*-isomer was more active than *Z*-isomer, which agreed with the published observations [8]. Modifications of the A-ring moiety resulted in three compounds (**1b**, **2b** and **5b**) which showed a significant increase in activity against *P. fluorescens* ATCC 13525 and a slight increase against *S. aureus* ATCC 6538 compared to the lead compound **11b**.

Table 2
Crystal structure data for **2b**, **6a** and **14**

Compounds	**2b**	**6a**	**14**
Formula	$C_{19}H_{20}ClNO_4$	$C_{17}H_{15}ClFNO_2$	$C_{21}H_{23}NO_3$
M_r	361.81	319.75	337.40
Crystal size/mm^3	0.30 × 0.20 × 0.10	0.30 × 0.20 × 0.10	0.30 × 0.20 × 0.20
Crystal system	Triclinic	Orthorhombic	Triclinic
Space group	*P*-1	$P2_12_12_1$	*P*-1
a/Å	9.6010(19)	8.2330(16)	8.9720(18)
b/Å	9.6071(19)	10.618(2)	9.6660(19)
c/Å	10.368(2)	17.944(4)	11.696(2)
α/°	77.84(3)	90	107.16(3)
β/°	75.42(3)	90	100.58(3)
γ/°	113.89(3)	90	99.09(3)
V/Å^3	904.7(3)	1568.6(5)	928.1(3)
Z	2	4	2
D_c/(g/cm^3)	1.328	1.354	1.207
μ/mm^{-1}	0.234	0.260	0.08
F(000)	380	664	360
Max. and min. trans.	0.9770 and 0.9331	0.9745 and 0.9261	0.9841 and 0.9763
θ Range/°	2.07/25.25	2.23/26.02	1.88/25.00
Index range (*h*, *k*, *l*)	0/11, −11/11, −12/12	0/10, −13/13, −22/0	0/10, −11/11, −13/13
Reflections collected/unique	3493/3280	3368/3060	3481/3251
Data/restraints/parameters	3280/0/234	3060/41/203	3251/24/231
R_{int}	0.0280	0.0900	0.0311
Goodness-of-fit on F^2	1.017	1.051	1.062
R_1, wR_2 [$I > 2\sigma(I)$]	0.0514/0.1096	0.0739/0.1601	0.0606/0.1598
R_1, wR_2	0.0891/0.1247	0.1587/0.2116	0.1053/0.1826
Extinction coefficient	0.017(2)		
$(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$/(e/Å^3)	0.210/−0.203	0.346/−0.233	0.237/−0.239

$R_1 = \sum(|F_o| - |F_c|)/\sum|F_o|$.
$wR_2 = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$, $w = [\sigma^2(F_o)^2 + (0.1(\max(0, F_o^2) + 2F_c^2)/3)^2]^{-1}$.

Scanning Table 5, we found that there is no clear SAR against *P. fluorescens* ATCC 13525, so the SAR analysis is based on the results of growth inhibition against *S. aureus* ATCC 6538. 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. A series of compounds substituted at nitrogen or on A-ring in different positions were synthesized to determine how the substituents affected the antibacterial activities. A comparison of the substitution pattern on A-ring demonstrated that the 2-position-substituted analogs were comparable to the activities of the 4-position-substituted derivatives such as **6a**, **7a**, **6b** and **7b**. However, introduction of groups at 3,5-positions afforded much more potent compounds (**11a**, **11b**, **12a** and **12**b) than 2,4-position-substituted analogs (**8a**, **8b**, **9a** and **9b**). Most significantly, compounds (**1b**, **6b**, **11b**, **2b** and **12b**) with strong electron-donating substituents on the A-ring had greater antibacterial activity, which was illustrated by the potency order $CH_3O > Cl > F$. Replacement of a hydroxy group (**5b**) at 4-position on A-ring by a methoxy or benzoxy group (**3b** and **4b**) led to a slight decrease in antibacterial activity. Inactive compounds **13** and **14** disclose that increase of the steric hindrance around N atom significantly decreased the antibacterial activity.

3. Conclusions

Twenty-six enamines (**1a**–**12a**, **1b**–**12b**, **13** and **14**) were synthesized and the molecular structures of three (**2b**, **6a** and **14**) were determined by X-ray diffraction analysis. In general, these synthesized compounds were shown to be more effective to inhibit growth of bacteria than fungi. As a result of optimization for chemical structure of enamine **11b**, compounds **1b**, **2b** and **5b** showed considerable antibacterial activities against *S. aureus* ATCC 6538 (MIC = 0.5, 0.9 and 1.0 μg/mL) and *P. fluorescens* ATCC 13525 (MIC = 1.5, 1.6 and 3.7 μg/mL). As for A-ring, the SAR disclosed that introduction of groups at 3,5-positions gave much more potent compounds than that at 2,4-positions, and halo-substituted analogs at 2-position had essentially same activities against *S. aureus* ATCC 6538 and *P. fluorescens* ATCC 13525 as those at 4-position. Increase of steric hindrance around the nitrogen atom led to an inactive compound.

4. Experiments

4.1. Crystallographic studies

X-ray single-crystal diffraction data for compounds **2b**, **6a** and **14** 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 the integration of the diffraction profiles. All the structures were solved by direct methods using the SHELXS program of the SHELXTL package and refined by full-matrix least-squares methods with SHELXL [9]. All non-hydrogen atoms of compounds **2b**, **6a** and **14** were refined with anisotropic thermal parameters except

Table 3
Selected bond lengths (Å) and torsion angles (°) of compounds **2b**, **6a** and **14**

Compounds	**2b**	**6a**	**14**
Bond lengths (Å)			
C1–N1	1.418(3)	1.400(7)	1.425(3)
C7–C14	1.485(3)	1.499(8)	1.475(3)
C13–N1	1.353(3)	1.347(7)	1.361(3)
C13–C14	1.345(3)	1.343(8)	1.358(3)
C14–C15	1.467(3)	1.476(8)	1.471(3)
C16–O2	1.450(3)	1.4006(11)/ 1.4008(11)[a]	1.447(3)
C16–C17	1.503(4)	1.4995(12)/ 1.4995(11)[a]	1.5143(12)/ 1.5092(11)[a]
Bond angles (°)			
C14–C13–N1	126.6(2)	127.2(6)	130.1(2)
C13–C14–C15	115.3(2)	119.7(6)	113.5(2)
C13–C14–C7	123.5(2)	118.2(6)	126.0(2)
C15–C14–C7	121.2(2)	122.1(5)	119.8(2)
C13–N1–C1	125.1(2)	125.8(5)	119.2(2)
Torsion angles (°)			
N1–C13–C14–C15	−178.1(2)	3.6(10)	175.4(2)
N1–C13–C14–C7	2.4(4)	−179.4(6)	−14.0(4)
C8–C7–C14–C13	−121.1(3)	−117.8(7)	134.6(3)
C12–C7–C14–C13	59.3(3)	57.4(8)	−48.4(3)
C13–C14–C15–O1	−2.3(4)	2.7(10)	−10.1(4)
C13–C14–C15–O2	177.6(2)	−176.4(6)	167.9(2)
C14–C13–N1–C1	−176.2(3)	−171.7(6)	164.3(2)
C6–C1–N1–C13	−159.1(3)	11.8(9)	147.3(2)
C2–C1–N1–C13	21.8(4)	−169.9(6)	−34.5(4)

[a] There are two values for disordered C16(A), C16(B) or C17(A), C17(B).

disordered C16A and C16B in **6a** and C17A and C17B in **14**. All hydrogen atoms were generated theoretically onto the parent atoms and refined isotropically with fixed thermal factors except that the hydrogen atom of N1 in **2b** was located in a difference Fourier map.

4.2. 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 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 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 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. The observed MICs are presented in Table 5.

4.3. 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 were reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

4.3.1. General synthesis method of enamines

The starting materials (aldehyde **n–o**) for the synthesis of enamines have been previously published [11–13]. Equimolar quantities (6 mmol) of the appropriate substituted aromatic amines and the aldehyde (**n–o**) 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. But as for compounds **13** and **14**, chromatography was unnecessary due to the formation of only one isomer. They were alternatively purified by crystallization from EtOAc–petroleum.

4.3.2. (*Z*)-Ethyl 3-(4-hydroxyphenylamino)-2-(4-chlorophenyl)acrylate (*1a*)

Light yellow powder, ^{1}H NMR (500 MHz, DMSO-d_6): 1.21 (t, $J = 7.0$ Hz, 3H); 4.17 (q, $J = 7.0$ Hz, 2H); 6.73 (d, $J = 8.8$ Hz, 2H); 7.11 (d, $J = 8.8$ Hz, 2H); 7.32 (d, $J = 8.6$ Hz, 2H); 7.37 (d, $J = 8.6$ Hz, 2H); 7.53 (d, $J = 13.1$ Hz, 1H); 10.19 (d, $J = 13.2$ Hz, 1H). MS (ESI): 318.1 ($C_{17}H_{17}ClNO_3$, $[M + H]^+$). Anal. Calcd for $C_{17}H_{16}ClNO_3$: C, 64.26; H, 5.08; N, 4.41; found: C, 64.37; H, 5.06; N, 4.38.

4.3.3. (*E*)-Ethyl 3-(4-hydroxphenylamino)-2-(4-chlorophenyl)acrylate (*1b*)

Light yellow crystal, ^{1}H NMR (300 MHz, DMSO-d_6): 1.18 (t, $J = 6.9$ Hz, 3H); 4.08 (q, $J = 6.9$ Hz, 2H); 6.70 (d, $J = 8.8$ Hz, 2H); 6.99 (d, $J = 8.9$ Hz, 2H); 7.26 (d, $J = 8.6$ Hz, 2H); 7.42 (d, $J = 8.6$ Hz, 2H); 7.92 (s, 1H). MS (ESI): 318.1 ($C_{17}H_{17}ClNO_3$, $[M + H]^+$). Anal. Calcd for $C_{17}H_{16}ClNO_3$: C, 64.26; H, 5.08; N, 4.41; found: C, 64.37; H, 5.06; N, 4.38.

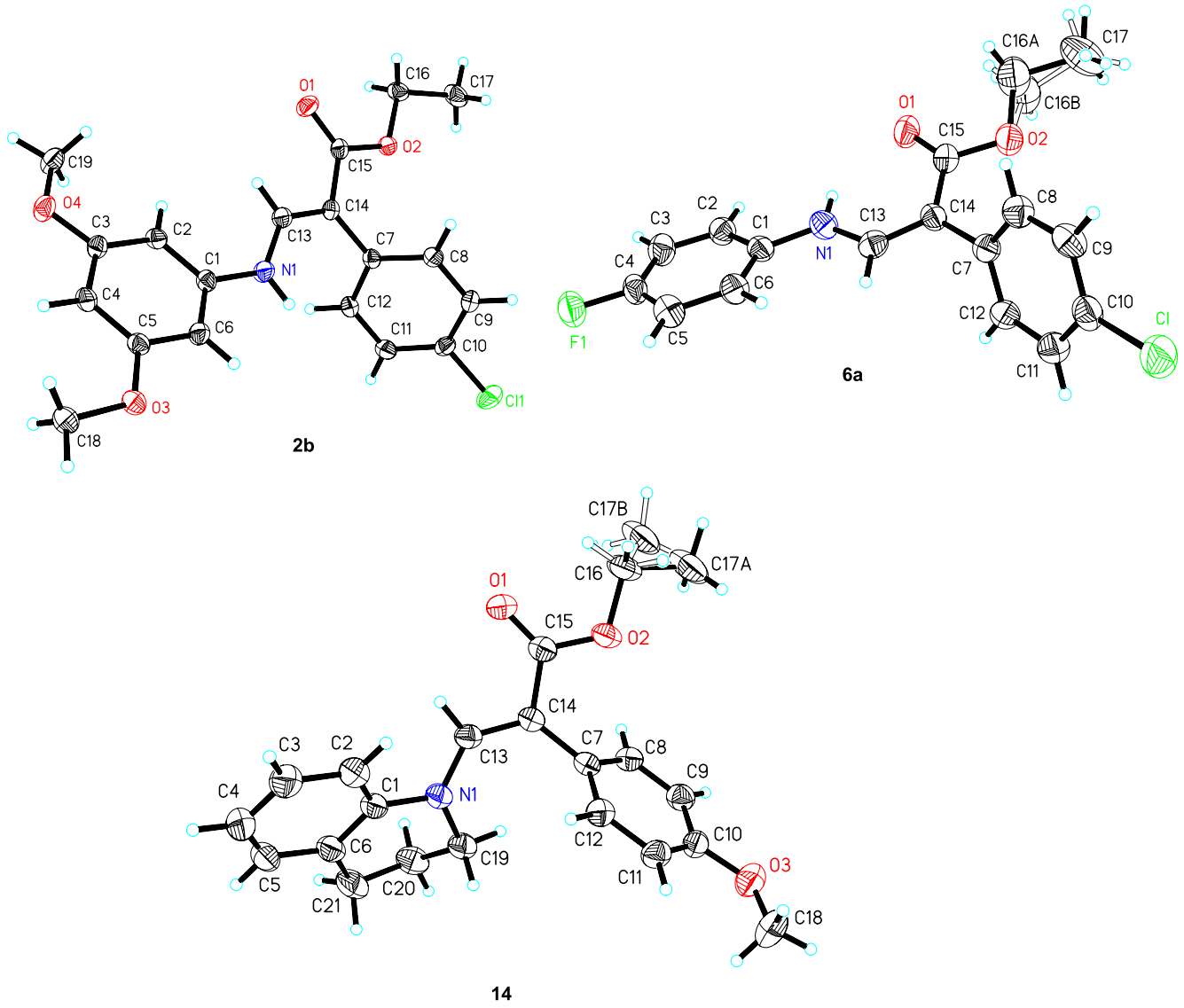


Fig. 1. Molecular structures of **2b**, **6a** and **14**. Displacement ellipsoids are drawn at the 30% probability level.

*4.3.4. (Z)-Ethyl 3-(3,5-dimethoxyphenylamino)-2-(4-chlorophenyl)acrylate (**2a**)*

White powder, ^{1}H NMR (500 MHz, DMSO-d_6): 1.20 (t, $J = 7.1$ Hz, 3H); 3.74 (s, 6H); 4.26 (q, $J = 7.1$ Hz, 2H); 6.16 (d, $J = 1.9$ Hz, 1H); 6.48 (d, $J = 1.8$ Hz, 2H); 7.35 (d, $J = 8.6$ Hz, 2H); 7.38 (d, $J = 8.8$ Hz, 2H); 7.68 (d, $J = 12.7$ Hz, 1H); 10.26 (d, $J = 12.8$ Hz, 1H). MS (ESI): 362.1 ($C_{19}H_{21}ClNO_4$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{20}ClNO_4$: C, 63.07; H, 5.57; N, 3.87; found: C, 63.13; H, 5.55; N, 3.84.

Table 4
Hydrogen-bond geometry (Å, °) in compounds **2b**, **6a** and **14**

D–H···A	D–H	H···A	D···A	D–H···A
Compound **2b**				
C13–H13···O1	0.930	2.390	2.768(3)	104.1
N1–H1···Cl1#1	0.88(3)	3.03(3)	3.848(3)	156(2)
Compound **6a**				
N1–H1···O1	0.86	2.10	2.711(7)	127.9
C16B–H16B···O1	0.97	2.28	2.630(3)	100.3
Compound **14**				
C13––H13···O1	0.93	2.36	2.760(3)	105.9

Symmetry code: #1 $-x + 1$, $-y + 1$, $-z + 2$.

*4.3.5. (E)-Ethyl 3-(3,5-dimethoxyphenylamino)-2-(4-chlorophenyl)acrylate (**2b**)*

Colorless crystal, ^{1}H NMR (300 MHz, DMSO-d_6): 1.19 (t, $J = 7.1$ Hz, 3H); 3.70 (s, 6H); 4.10 (q, $J = 7.1$ Hz, 2H); 6.12 (d, $J = 2.1$ Hz, 1H); 6.34 (d, $J = 2.2$ Hz, 2H); 7.25 (d, $J = 8.6$ Hz, 2H); 7.40 (d, $J = 8.6$ Hz, 2H); 7.99 (s, 1H). MS (ESI): 362.1 ($C_{19}H_{21}ClNO_4$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{20}ClNO_4$: C, 63.07; H, 5.57; N, 3.87; found: C, 63.15; H, 5.56; N, 3.87.

*4.3.6. (Z)-Ethyl 3-(4-benzyloxy-3,5-dibromophenylamino)-2-(4-chlorophenyl)acrylate (**3a**)*

White powder, ^{1}H NMR (300 MHz, DMSO-d_6): 1.22 (t, $J = 7.1$ Hz, 3H); 4.20 (q, $J = 7.0$ Hz, 2H); 4.93 (s, 2H); 7.39 (m, 5H); 7.45 (d, $J = 8.3$ Hz, 2H); 7.60 (d, $J = 8.1$ Hz, 2H); 7.66 (d, $J = 12.6$ Hz, 1H); 7.77 (s, 2H); 10.20 (d, $J = 12.6$ Hz,

Table 5
MICs of the synthetic compounds

Compound	Minimum inhibitory concentrations (μg/mL)						
	A	B	C	D	E	F	G
1a	>50	38.9	13.1	44.9	>50	>50	>50
1b	>50	>50	1.5	0.5	>50	>50	>50
2a	>50	>50	36.3	>50	>50	>50	>50
2b	>50	>50	1.6	0.9	>50	>50	>50
3a	>50	>50	>50	>50	>50	>50	>50
3b	>50	>50	8.5	3.4	>50	>50	>50
4a	43.3	48.6	48.1	47.7	>50	>50	>50
4b	>50	>50	6.1	2.2	>50	>50	>50
5a	>50	>50	>50	33.5	>50	>50	>50
5b	>50	>50	3.7	1.0	>50	>50	>50
6a	49.8	43.4	>50	>50	>50	>50	>50
6b	>50	>50	>50	35.6	>50	>50	>50
7a	46.6	>50	46	>50	>50	>50	>50
7b	>50	>50	>50	41.2	>50	>50	>50
8a	>50	>50	>50	>50	>50	>50	>50
8b	>50	>50	7.1	12.3	>50	>50	>50
9a	>50	47.3	>50	>50	>50	>50	>50
9b	>50	48	>50	40.9	>50	>50	>50
10a	>50	>50	23.8	32.1	>50	>50	>50
10b	>50	>50	4.5	8.2	>50	>50	>50
11a	>50	>50	>50	>50	>50	>50	>50
11b	>50	>50	25.7	1.1	>50	>50	>50
12a	>50	>50	>50	>50	>50	>50	>50
12b	48.2	13.5	31.5	1.9	>50	>50	>50
13	>50	>50	38.5	>50	>50	>50	>50
14	>50	>50	41.5	>50	>50	>50	>50
Ketoconazole	–	–	–	–	7.8	3.9	3.9
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; E, *Aspergillus niger* ATCC 16404; F, *Candida albicans* ATCC 10231; G, *Trichophyton rubrum* ATCC 10218.

1H). MS (ESI): 564.0 ($C_{24}H_{21}Br_2ClNO_3$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{20}Br_2ClNO_3$: C, 50.96; H, 3.56; N, 2.48; found: C, 50.75; H, 3.55; N, 2.47.

*4.3.7. (E)-Ethyl 3-(4-benzyloxy-3,5-dibromophenylamino)-2-(4-chlorophenyl)acrylate (**3b**)*

White powder, ^{1}H NMR (300 MHz, DMSO-d_6): 1.20 (t, $J = 7.1$ Hz, 3H); 4.12 (q, $J = 7.0$ Hz, 2H); 4.92 (s, 2H); 7.26 (d, $J = 8.4$ Hz, 2H); 7.44 (m, 5H); 7.53 (s, 2H); 7.56 (d, $J = 8.0$ Hz, 2H); 7.93 (d, $J = 13.2$ Hz, 1H); 8.68 (d, $J = 13.0$ Hz, 1H). MS (ESI): 564.1 ($C_{24}H_{21}Br_2ClNO_3$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{20}Br_2ClNO_3$: C, 50.96; H, 3.56; N, 2.48; found: C, 50.82; H, 3.56; N, 2.47.

*4.3.8. (Z)-Ethyl 3-(3,5-dibromo-4-methoxyphenylamino)-2-(4-chlorophenyl)acrylate (**4a**)*

White powder, ^{1}H NMR (500 MHz, DMSO-d_6): 1.21 (t, $J = 7.1$ Hz, 3H); 3.75 (s, 3H); 4.19 (q, $J = 7.1$ Hz, 2H); 7.35 (d, $J = 8.6$ Hz, 2H); 7.39 (d, $J = 8.6$ Hz, 2H); 7.64 (d, $J = 12.5$ Hz, 1H); 7.72 (s, 2H); 10.17 (d, $J = 12.5$ Hz, 1H). MS (ESI): 487.9 ($C_{18}H_{17}Br_2ClNO_3$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}Br_2ClNO_3$: C, 44.16; H, 3.29; N, 2.86; found: C, 44.34; H, 3.26; N, 2.88.

*4.3.9. (E)-Ethyl 3-(3,5-dibromo-4-methoxyphenylamino)-2-(4-chlorophenyl)acrylate (**4b**)*

Colorless crystal, ^{1}H NMR (300 MHz, DMSO-d_6): 1.19 (t, $J = 7.1$ Hz, 3H); 3.74 (s, 3H); 4.11 (q, $J = 7.0$ Hz, 2H); 7.25 (d, $J = 8.3$ Hz, 2H); 7.45 (d, $J = 8.2$ Hz, 2H); 7.48 (s, 2H); 7.91 (d, $J = 13.0$ Hz, 1H); 8.65 (d, $J = 13.0$ Hz, 1H). MS (ESI): 487.8 ($C_{18}H_{17}Br_2ClNO_3$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}Br_2ClNO_3$: C, 44.16; H, 3.29; N, 2.86; found: C, 44.35; H, 3.27; N, 2.84.

*4.3.10. (Z)-Ethyl 3-(3,5-dibromo-4-hydroxyphenylamino)-2-(4-chlorophenyl)acrylate (**5a**)*

Light yellow powder, ^{1}H NMR (300 MHz, DMSO-d_6): 1.22 (t, $J = 7.1$ Hz, 3H); 4.19 (q, $J = 7.0$ Hz, 2H); 7.35 (d, $J = 8.8$ Hz, 2H); 7.40 (d, $J = 8.8$ Hz, 2H); 7.59 (d, $J = 12.5$ Hz, 1H); 7.87 (s, 2H); 10.16 (d, $J = 12.4$ Hz, 1H). MS (ESI): 473.9 ($C_{17}H_{15}Br_2ClNO_3$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Br_2ClNO_3$: C, 42.94; H, 2.97; N, 2.95; found: C, 42.83; H, 2.96; N, 2.94.

*4.3.11. (E)-Ethyl 3-(3,5-dibromo-4-hydroxyphenylamino)-2-(4-chlorophenyl)acrylate (**5b**)*

Light yellow powder, ^{1}H NMR (300 MHz, DMSO-d_6): 1.18 (t, $J = 7.1$ Hz, 3H); 4.10 (q, $J = 7.0$ Hz, 2H); 6.78 (s, 1H); 7.24 (d, $J = 8.2$ Hz, 2H); 7.40 (s, 2H); 7.43 (d, $J = 8.4$ Hz, 2H); 7.87 (d, $J = 12.3$ Hz, 1H); 8.65 (d, $J = 12.4$ Hz, 1H). MS (ESI): 473.9 ($C_{17}H_{15}Br_2ClNO_3$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Br_2ClNO_3$: C, 42.94; H, 2.97; N, 2.95; found: C, 42.50; H, 2.99; N, 2.96.

*4.3.12. (Z)-Ethyl 3-(4-fluorophenylamino)-2-(4-chlorophenyl)acrylate (**6a**)*

White powder, ^{1}H NMR (300 MHz, DMSO-d_6): 1.22 (t, $J = 7.1$ Hz, 3H); 4.19 (q, $J = 7.1$ Hz, 2H); 7.16 (t, $J = 8.4$ and 9.1 Hz, 2H); 7.34 (m, 4H); 7.34 (d, $J = 8.9$ Hz, 2H); 7.99 (d, $J = 12.5$ Hz, 1H); 10.31 (d, $J = 12.6$ Hz, 1H). MS (ESI): 320.1 ($C_{17}H_{16}ClFNO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}ClFNO_2$: C, 63.86; H, 4.73; N, 4.38; found: C, 63.94; H, 4.70; N, 4.34.

*4.3.13. (E)-Ethyl 3-(4-fluorophenylamino)-2-(4-chlorophenyl)acrylate (**6b**)*

Colorless crystal, ^{1}H NMR (300 MHz, DMSO-d_6): 1.19 (t, $J = 6.9$ Hz, 3H); 4.10 (q, $J = 7.0$ Hz, 2H); 7.14 (m, 4H); 7.27 (d, $J = 8.2$ Hz, 2H); 7.44 (d, $J = 8.4$ Hz, 2H); 7.97 (d, $J = 12.9$ Hz, 1H); 8.53 (d, $J = 12.2$ Hz, 1H). MS (ESI): 320.1 ($C_{17}H_{16}ClFNO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}ClFNO_2$: C, 63.86; H, 4.73; N, 4.38; found: C, 63.94; H, 4.70; N, 4.34.

*4.3.14. (Z)-Ethyl 3-(2-fluorophenylamino)-2-(4-chlorophenyl)acrylate (**7a**)*

White powder, ^{1}H NMR (500 MHz, DMSO-d_6): 1.22 (t, $J = 7.1$ Hz, 3H); 4.21 (q, $J = 7.0$ Hz, 2H); 7.02 (m, 1H); 7.17 (t, $J = 7.6$ Hz, 1H); 7.29 (m, 1H); 7.37 (d, $J = 8.6$ Hz, 2H); 7.41 (d, $J = 8.8$ Hz, 2H); 7.64 (t, $J = 8.6$ Hz, 1H); 7.77 (d, $J = 12.5$ Hz, 1H); 10.50 (d, $J = 12.6$ Hz, 1H). MS (ESI): 320.1 ($C_{17}H_{16}ClFNO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}ClFNO_2$: C, 63.86; H, 4.73; N, 4.38; found: C, 63.66; H, 4.76; N, 4.40.

*4.3.15. (E)-Ethyl 3-(2-fluorophenylamino)-2-(4-chlorophenyl)acrylate (**7b**)*

Colorless crystal, ^{1}H NMR (500 MHz, DMSO-d_6): 1.18 (t, $J = 7.1$ Hz, 3H); 4.10 (q, $J = 7.0$ Hz, 2H); 7.05 (t, $J = 5.5$ and 7.0 Hz, 1H); 7.15 (t, $J = 7.7$ Hz, 1H); 7.22 (t, $J = 8.4$ Hz, 1H); 7.29 (d, $J = 8.3$ Hz, 2H); 7.33 (d, $J = 8.3$ Hz, 1H); 7.45 (d, $J = 8.3$ Hz, 2H); 7.98 (d, $J = 12.0$ Hz, 1H); 8.83 (d, $J = 12.1$ Hz, 1H). MS (ESI): 320.1 ($C_{17}H_{16}ClFNO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}ClFNO_2$: C, 63.86; H, 4.73; N, 4.38; found: C, 63.66; H, 4.70; N, 4.39.

*4.3.16. (Z)-Ethyl 3-(2,4-dichlorophenylamino)-2-(4-chlorophenyl)acrylate (**8a**)*

White powder, ^{1}H NMR (500 MHz, DMSO-d_6): 1.22 (t, $J = 7.4$ Hz, 3H); 4.22 (q, $J = 7.0$ Hz, 2H); 7.38 (d, $J = 8.9$ Hz, 3H); 7.42 (d, $J = 8.9$ Hz, 2H); 7.66 (d, $J = 2.5$ Hz, 1H); 7.70 (d, $J = 9.2$ Hz, 1H); 7.80 (d, $J = 12.2$ Hz, 1H); 10.78 (d, $J = 12.2$ 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, 55.29; H, 3.78; N, 3.74.

*4.3.17. (E)-Ethyl 3-(2,4-dichlorophenylamino)-2-(4-chlorophenyl)acrylate (**8b**)*

White powder, ^{1}H NMR (500 MHz, DMSO-d_6): 1.16 (t, $J = 7.0$ Hz, 3H); 4.09 (q, $J = 7.0$ Hz, 2H); 7.31 (d, $J = 8.2$ Hz, 2H); 7.34 (d × d, $J = 8.9$, 2.5 Hz, 1H); 7.42 (d, $J = 13.4$ Hz, 1H); 7.43 (d, $J = 8.9$ Hz, 1H); 7.46 (d, $J = 8.6$ Hz, 2H); 7.53 (d, $J = 2.5$ Hz, 1H); 7.96 (d, $J = 13.1$ Hz, 1H). MS (ESI): 369.0 ($C_{17}H_{15}Cl_3NO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Cl_3NO_2$: C, 55.09; H, 3.81; N, 3.78; found: C, 55.24; H, 3.77; N, 3.76.

*4.3.18. (Z)-Ethyl 3-(2,4-difluorophenylamino)-2-(4-chlorophenyl)acrylate (**9a**)*

White powder, ^{1}H NMR (300 MHz, DMSO-d_6): 1.22 (t, $J = 7.0$ Hz, 3H); 4.20 (q, $J = 7.3$ Hz, 2H); 7.08 (t, $J = 7.8$ Hz, 1H); 7.36 (d, $J = 8.6$ Hz, 3H); 7.40 (d, $J = 8.8$ Hz, 2H); 7.68 (m, 1H); 7.73 (d, $J = 12.5$ Hz, 1H); 10.43 (d, $J = 12.2$ 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, 60.26; H, 4.17; N, 4.18.

*4.3.19. (E)-Ethyl 3-(2,4-difluorophenylamino)-2-(4-chlorophenyl)acrylate (**9b**)*

Colorless crystal, ^{1}H NMR (500 MHz, DMSO-d_6): 1.22 (t, $J = 7.3$ Hz, 3H); 4.20 (q, $J = 7.4$ Hz, 2H); 7.07 (t, $J = 8.8$ Hz, 1H); 7.28 (d, $J = 8.3$ Hz, 1H); 7.36 (d, $J = 8.5$ Hz, 2H); 7.42 (d, $J = 8.3$ Hz, 2H); 7.68 (m, 1H); 7.72 (d, $J = 12.0$ Hz, 1H); 7.86 (d, $J = 12.2$ 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, 60.25; H, 4.20; N, 4.19.

*4.3.20. (Z)-Ethyl 3-(2,4-dibromophenylamino)-2-(4-chlorophenyl)acrylate (**10a**)*

White powder, ^{1}H NMR (500 MHz, DMSO-d_6): 1.22 (t, $J = 7.1$ Hz, 3H); 4.22 (q, $J = 7.1$ Hz, 2H); 7.37 (d, $J = 8.6$ Hz, 2H); 7.42 (d, $J = 8.6$ Hz, 2H); 7.53 (d, $J = 8.8$ Hz, 1H); 7.60 (d, $J = 8.8$ Hz, 1H); 7.76 (d, $J = 12.2$ Hz, 1H); 7.88 (s, 1H); 10.75 (d, $J = 12.5$ Hz, 1H). MS (ESI): 457.9 ($C_{17}H_{15}Br_2ClNO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Br_2ClNO_2$: C, 44.43; H, 3.07; N, 3.05; found: C, 44.53; H, 3.04; N, 3.02.

*4.3.21. (E)-Ethyl 3-(2,4-dibromophenylamino)-2-(4-chlorophenyl)acrylate (**10b**)*

Colorless crystal, ^{1}H NMR (500 MHz, DMSO-d_6): 1.20 (t, $J = 7.3$ Hz, 3H); 4.13 (q, $J = 7.4$ Hz, 2H); 7.36 (d, $J = 8.2$ Hz, 2H); 7.40 (d, $J = 8.8$ Hz, 2H); 7.52 (m, 2H); 7.55 (d, $J = 2.2$ Hz, 1H); 7.81 (d, $J = 12.8$ Hz, 1H); 8.01 (d, $J = 13.1$ Hz, 1H). MS (ESI): 457.9 ($C_{17}H_{15}Br_2ClNO_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Br_2ClNO_2$: C, 44.43; H, 3.07; N, 3.05; found: C, 44.54; H, 3.06; N, 3.01.

*4.3.22. (Z)-Ethyl 3-(3,5-dichlorophenylamino)-2-(4-chlorophenyl)acrylate (**11a**)*

White powder, ^{1}H NMR (500 MHz, DMSO-d_6): 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.

*4.3.23. (E)-Ethyl 3-(3,5-dichlorophenylamino)-2-(4-chlorophenyl)acrylate (**11b**)*

Colorless crystal, ^{1}H NMR (500 MHz, DMSO-d_6): 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.

*4.3.24. (Z)-Ethyl 3-(3,5-difluorophenylamino)-2-(4-chlorophenyl)acrylate (**12a**)*

Colorless crystal, ^{1}H NMR (300 MHz, DMSO-d_6): 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.

*4.3.25. (E)-Ethyl 3-(3,5-difluorophenylamino)-2-(4-chlorophenyl)acrylate (**12b**)*

Colorless crystal, ^{1}H NMR (500 MHz, DMSO-d_6): 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.

*4.3.26. (E)-Ethyl 3-(1,2,3,4-tetrahydroquinolin-1-yl)-2-(4-chlorophenyl)acrylate (**13**)*

Light yellow crystal, ^{1}H NMR (500 MHz, $CDCl_3$): 1.26 (t, $J = 7.0$ Hz, 3H); 1.76 (m, 2H); 2.73 (t, $J = 6.4$ Hz, 2H); 3.01

(t, $J = 5.8$ Hz, 2H); 4.21 (q, $J = 7.0$ Hz, 2H); 6.98 (t, $J = 7.4$ Hz, 1H); 7.01 (d, $J = 8.0$ Hz, 1H); 7.06 (d, $J = 7.7$ Hz, 1H); 7.19 (d, $J = 8.6$ Hz, 2H); 7.20 (t, $J = 7.2$ Hz, 1H); 7.28 (d, $J = 8.6$ Hz, 2H); 8.00 (s, 1H). MS (ESI): 342.1 ($C_{20}H_{21}ClNO_2$, $[M + H]^+$). Anal. Calcd for $C_{20}H_{20}ClNO_2$: C, 70.27; H, 5.90; N, 4.10; found: C, 70.34; H, 5.89; N, 4.12.

*4.3.27. (E)-Ethyl 3-(1,2,3,4-tetrahydroquinolin-1-yl)-2-(4-methoxyphenyl)acrylate (**14**)*

Light yellow crystal, ^{1}H NMR (300 MHz, DMSO-d_6): 1.17 (t, $J = 7.0$ Hz, 3H); 1.65 (m, 2H); 2.66 (t, $J = 6.2$ Hz, 2H); 2.95 (t, $J = 5.6$ Hz, 2H); 3.74 (s, 3H); 4.09 (q, $J = 7.0$ Hz, 2H); 6.88 (d, $J = 8.5$ Hz, 2H); 6.95 (t, $J = 7.7$ Hz, 1H); 6.96 (d, $J = 7.8$ Hz, 1H); 7.07 (d, $J = 7.7$ Hz, 1H); 7.10 (d, $J = 8.6$ Hz, 2H); 7.20 (t, $J = 8.1$ Hz, 1H); 7.81 (s, 1H). MS (ESI): 338.2 ($C_{21}H_{24}NO_3$, $[M + H]^+$). Anal. Calcd for $C_{21}H_{23}NO_3$: C, 74.75; H, 6.87; N, 4.15; found: C, 74.67; H, 6.89; N, 4.13.

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References

[1] Paola Vicini, Athina Geronikaki, Matteo Incerti, Bernadetta Busonera, Graziella Poni, Carla Alba Cabras, Paolo La Colla, Bioorg. Med. Chem. 11 (2003) 4785–4789.
[2] Perumal Panneerselvam, Rajasree R Nair, Gudaparthi Vijayalakshmi, Ekambaram Harihara Subramanian, Seshaiah Krishnan Sridhar, Eur. J. Med. Chem. 40 (2005) 225–229.
[3] M. Karatepe, F. Karatas, Cell Biochem. Funct. 24 (2006) 547–554.
[4] S.D. Paget, C.M. Boggs, B.D. Foleno, R.M. Goldschmidt, D.J. Hlasta, M.A. Weidner-Wells, H.M. Werblood, K. Bush, M.J. Macielag, Bioorg. Med. Chem. Lett. 16 (2006) 4537–4542.
[5] Z.L. You, D.H. Shi, H.L. Zhu, Inorg. Chem. Commun. 9 (2006) 642–644.
[6] Z.L. You, H.L. Zhu, Z. Anorg. Allg. Chem. 632 (2006) 140–146.
[7] Z.L. You, H.L. Zhu, Z. Anorg. Allg. Chem. 630 (2004) 2754–2760.
[8] Z.P. Xiao, J.Y. Xue, S.H. Tan, H.Q. Li, H.L. Zhu, Bioorg. Med. Chem. 15 (2007) 4212–4219.
[9] G.M. Sheldrick, SHELXTL-97, Program for Crystal Structure Solution and Refinement, University of Göttingen, Göttingen, Germany, 1997.
[10] J. Meletiadis, J.F. Meis, J.W. Mouton, J.P. Donnelly, P.E. Verweij, J. Clin. Microbiol. 38 (2000) 2949–2954.
[11] E.M. Beccalli, J. Org. Chem. 49 (1984) 4287–4290.
[12] S.J. Davies, A.P. Ayscough, R.P. Beckett, R.A. Bragg, J.M. Clements, S. Doel, C. Grew, S.B. Launchbury, G.M. Perkins, L.M. Pratt, H.K. Smith, Z.M. Spavold, S.W. Thomas, R.S. Todd, M. Whittaker, Bioorg. Med. Chem. Lett. 13 (2003) 2709–2713.
[13] D.F. Taber, J.F. Mack, A.L. Rheingold, S.J. Geib, J. Org. Chem. 54 (1989) 3831–3836.