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Discovery of vinylogous carbamates as a novel class of β-ketoacyl-acyl carrier protein synthase III (FabH) inhibitors

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ABSTRACT

β-Ketoacyl-acyl carrier protein synthase III (FabH) catalyzes the initial step of fatty acid biosynthesis via a type II fatty acid synthase in most bacteria. The important role of this essential enzyme combined with its unique structural features and ubiquitous occurrence in bacteria has made it an attractive new target for the development of new FabH inhibitors. We first used a structure-based approach to develop 24 new vinylogous carbamates (**4a**–**15a**, **4b**–**15b**) that target FabH for the development of new antibiotics in this paper. Potent FabH inhibitory and selective anti- Gram-negative bacteria activities were observed in most of these vinylogous carbamates. Especially, compound **6a** and **7a** showed the most potent FabH inhibitory activity with IC_{50} of 2.6 and 3.3 μM, respectively. Docking simulation was performed to position compound **6a** into the *Escherichia coli* FabH active site and the possible binding conformation of compounds has been proposed. The biological data and molecular docking indicated that compounds **6a** and **7a** were potent inhibitors of *E. coli* FabH as antibiotics deserving further research.

1. Introduction

Bacteria resistant to known therapies are a growing threat across the globe. An increasing fraction of bacterial isolates shows reduced susceptibility to our most trusted antibiotics. In order to prevent this serious medical problem, the discovery of new types of antibacterial agents or the expansion of bioactivity of the previous drugs is a very important task.[1] Therefore, in recent years, the research has been focused on the development of new antibacterial agents, which may act through structure design and novel targets, overcoming the problem of acquired resistance. One of the most attractive biochemical pathways that could be targeted for new antibacterial agents is the fatty acid biosynthesis (FAS). This pathway has been demonstrated to be essential for the bacteria cell survival,[2] and differs considerably from human FAS pathway. While in humans fatty acid synthesis occurs in a homodimeric multifunctional enzyme,[3,4] in bacteria the pathway is composed of various discrete enzymes and each one can be considered a putative molecular target. Those features make the type II FAS pathway a potential target for new antimicrobial agents.

A key enzyme in this pathway is the β-ketoacyl-acyl carrier protein synthase III (FabH), which is the responsible enzyme of the first pathway reaction and play an important regulatory role. FabH has also been demonstrated to be essential for organism survival and it is presented in a wide number of important human pathogens. Furthermore, some chemical compounds have shown to inhibit FabH from diverse microorganisms, including multi-drug resistant strains.[5,6] These facts support the idea that FabH can be used as an effective molecular target for the development of new antimicrobial agents. *Schiff* bases and their metal complexes display several potentially useful biological activities, of which the antibacterial, antifungal, and FabH inhibitory activities has been widely studied.[7–9] However, vinylogous carbamates which are tautomers of *Schiff* bases were rarely reported for biological activities and they mostly appeared as chemical intermediates for cyclization.[10,11] Due to the evident chemical structure similarity as *Schiff* bases, an enamine should bear relevant biological properties as the corresponding *Schiff* base. As a proof for this hypothesis, our recent report showed that vinylogous carbamates have considerable antibacterial activities.[12] To the continuation of this work and together with aforementioned hypothesis, we directed our efforts to the investigation of the FabH inhibitory activity of new vinylogous carbamates. In this paper, we described the synthesis of vinylogous carbamates and the evaluation of these compounds as FabH inhibitors for the first time. The antimicrobial activity of these vinylogous carbamates against two Gram-negative bacterial strains, *Escherichia coli* (*E. coli*) and *Pseudomonas fluorescence* (*P. fluorescence*), and two Gram-positive bacterial strains, *Bacillus Subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) was also determined. Docking simulations were performed using the X-ray crystallographic structure of the FabH

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of *E. coli* in complex with an inhibitor to explore the binding modes of these compounds at the active site.

2. Chemistry

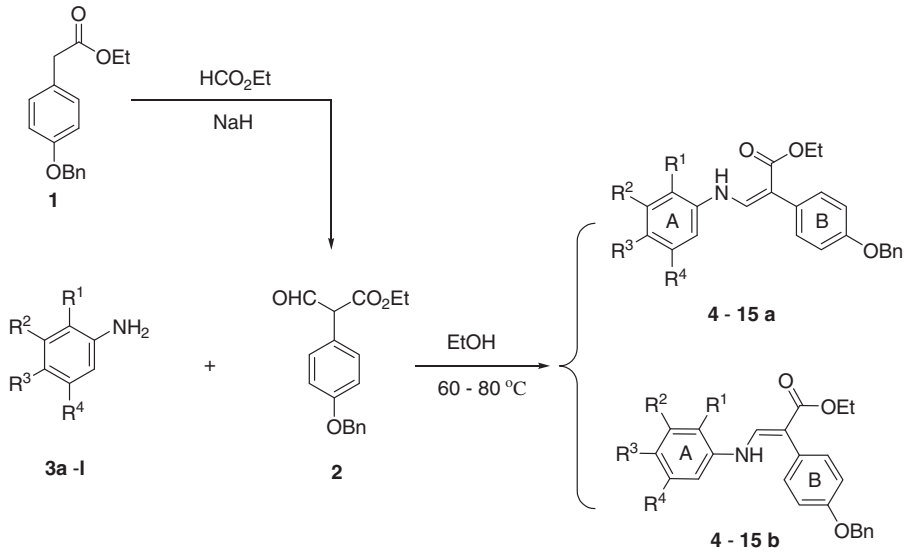
The general method for preparing the final compounds **4a–15a** and **4b–15b** was outlined in Scheme 1.[12] In order to find more effective vinylogous carbamates and convenient to discuss the different trend in SAR from the former report,[12] the substituent on the para position of compound **1** was changed from previously used methoxy group to benzyloxy group. The designed vinylogous carbamates were prepared by a dehydration reaction of the aldehyde (**2**) with different arylamines (**3a**–**3l**). Aldehyde **2**, which was not commercially available, was synthesized using modified procedures of Beccalh.[13] 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 single isomer with high purity. The structures of the vinylogous carbamates (**4a**–**15a** and **4b**–**15b**) were fully characterized by spectroscopic methods and elemental analysis.

3. Results and discussion

3.1. Antibacterial activity

All the synthesized new vinylogous carbamates (**4a–15a** and **4b–15b**) were screened for antibacterial activity against two Gram-negative bacterial strains: *E. coli* and *P. fluorescence* and two Gram-positive bacterial strains: *B. subtilis* and *S. aureus* by MTT method. The MICs (minimum inhibitory concentrations) of the compounds against these bacteria were presented in Table 1. Standard antibacterial agent kanamycin B was also screened under identical conditions for comparison. The results revealed that most of the synthesized vinylogous carbamates exhibited potent antibacterial activity. Of all the compounds tested, **6a, 7a, 10a, 12a, 12b, 13a** demonstrated excellent inhibitory activity against Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. Especially, **6a** (MIC = 1.562 and 1.562 μg/mL) and **7a** (MIC = 0.78 and 1.562 μg/mL) showed comparable activities to positive control kanamycin B.

In general, this series of vinylogous carbamates displayed more effective antibacterial activity than the former report, this indicated that the different substituent (benzyloxy group instead of methoxy group) on the para position of benzene ring B was benefit for the antibacterial activity. As shown in Table 1, all Z-isomers had better antibacterial activity than E-isomers, which implied that configuration did affect isomers' activity, and this trend is opposite to the former report.[12] Compounds with a single substitution at the 4-position on A-ring (Scheme 1) demonstrated higher inhibitory activity, as shown by the most potent six compounds. Multiple substitutions or a single substitution at other positions of the A ring decreased their inhibitory activity. For compounds with single substitution at 4-position on A-ring, **7a** showed the best antibacterial activity followed by **6a**. As to compounds **10a**, **12a** and **13a,**



3a $R^1 = R^2 = R^4 = H$, $R^3 = Cl$;
3b $R^1 = R^3 = Cl$, $R^2 = R^4 = H$;
3c $R^1 = R^2 = R^4 = H$, $R^3 = Br$;
3d $R^1 = R^2 = R^4 = H$, $R^3 = CH_3$;
3e $R^1 = R^3 = Br$, $R^2 = R^4 = H$;
3f $R^1 = Cl$, $R^2 = R^3 = R^4 = H$;
3g $R^1 = R^2 = R^4 = H$, $R^3 = F$;
3h $R^1 = R^3 = F$, $R^2 = R^4 = H$;
3i $R^1 = R^2 = R^4 = H$, $R^3 = CH(CH_3)_2$;
3j $R^1 = R^2 = R^4 = H$, $R^3 = NO_2$;
3k $R^1 = F$, $R^2 = R^3 = R^4 = H$;
3l $R^1 = R^2 = R^3 = R^4 = H$;

4 $R^1 = R^2 = R^4 = H$, $R^3 = Cl$;
5 $R^1 = R^3 = Cl$, $R^2 = R^4 = H$;
6 $R^1 = R^2 = R^4 = H$, $R^3 = Br$;
7 $R^1 = R^2 = R^4 = H$, $R^3 = CH_3$;
8 $R^1 = R^3 = Br$, $R^2 = R^4 = H$;
9 $R^1 = Cl$, $R^2 = R^3 = R^4 = H$;
10 $R^1 = R^2 = R^4 = H$, $R^3 = F$;
11 $R^1 = R^3 = F$, $R^2 = R^4 = H$;
12 $R^1 = R^2 = R^4 = H$, $R^3 = CH(CH_3)_2$;
13 $R^1 = R^2 = R^4 = H$, $R^3 = NO_2$;
14 $R^1 = F$, $R^2 = R^3 = R^4 = H$;
15 $R^1 = R^2 = R^3 = R^4 = H$;

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

Scheme 1. Synthetic routes of vinylogous carbamates.

Table 1
Antibacterial activity of synthetic vinylogous carbamates

Compounds	Minimum inhibitory concentrations (μg /mL)			
	Gram-negative		Gram-positive	
	E. coli	*P. fluorescence*	*B. subtilis*	*S. aureus*
4a	12.5	6.25	25	25
4b	25	25	25	>50
5a	25	25	25	25
5b	25	25	>50	>50
6a	1.56	1.56	50	25
6b	>50	>50	>50	>50
7a	0.78	1.56	25	25
7b	>50	>50	>50	>50
8a	12.5	12.5	25	25
8b	3.13	12.5	12.5	12.5
9a	12.5	25	>50	>50
9b	25	>50	25	12.5
10a	3.125	3.125	25	>50
10b	6.25	12.5	25	12.5
11a	6.25	12.5	>50	>50
11b	25	25	12.5	12.5
12a	3.125	3.125	25	12.5
12b	6.25	6.25	12.5	25
13a	3.125	6.25	>50	>50
13b	>50	>50	>50	>50
14a	12.5	25	>50	25
14b	25	>50	>50	>50
15a	12.5	25	25	50
15b	6.25	6.25	25	25
Kanamycin B	3.125	3.125	0.39	1.56

their inhibitory activity was weaker than **6a** and **7a**. From antibacterial evaluation, it was found that *Z*-isomers had better activity than that of *E*-isomers, and compounds with a single substitution at 4-position on A-ring showed most potent activity.

3.2. *E.* coli FabH inhibitory activity

The *E. coli* FabH inhibitory potency of the representative vinylogous carbamates (**6a**–**10a, 6b, 12b**) was examined and the results are summarized in Table 2. As shown in Table 2, most of the tested compounds displayed potent *E. coli* FabH inhibitory activity. Among them, compounds **8a**, **9a** and **6b** with lower antibacterial activities also had less inhibitory activity toward *E. coli* FabH. In addition to antibacterial activity against Gram-negative *E. coli* and *P. aeruginosa*, *Z*-isomers compounds **6a** and **7a** with a single substitution at the 4-position on A-ring were also good inhibitors of *E. coli* FabH, indicating that **6a** and **7a** may form a hydrophobic interaction of *E. coli* FabH. These biological assays indicated that compounds **6a** and **7a** were potent inhibitors of *E. coli* FabH as antibiotics.

Molecular docking of compound **7a** and *E. coli* FabH was performed on the binding model based on the *E. coli* FabH-CoA complex structure (1HNJ.pdb).[14] The FabH active site generally contains a catalytic triad tunnel consisting of Cys-His-Asn, which is conserved in various bacteria. This catalytic triad plays an important role in the regulation of chain elongation and substrate binding. Since the alkyl chain of CoA is broken by Cys of the catalytic triad of FabH, interactions between Cys and substrate appear to play an important role in substrate binding (Fig. 1). Qiu, et al. refined three-dimensional structure of *E. coli* FabH in the presence or absence of malonyl-CoA by X-ray spectroscopy. Since the malonyl moiety is degraded by *E. coli* FabH, molecular docking studies for FabH and malonyl-CoA were carried out to identify a plausible malonyl-binding mode.[14] They found that in one of the binding modes appeared in the lower scored conformations, the malonyl carboxylate formed hydrogen bonds to the backbone nitrogen of Phe304. Enlightened by these facts, compound **7a** with the most potent inhibitory activity was hit by pharmacophore map I mentioned above. The binding model of compound **7a** and *E. coli* FabH is depicted in Figure 2. In the binding model, the side chain carbonyl group of Gly209 forms a hydrogen bond with N–H group of compound **7a**. The A-ring in compound **7a** projects into a hydrophobic interaction region, which is comprised of the side chains of Met207, Asn247, Asn274 and Ile156. The hydrophobic interaction between **6a** and FabH was important for the potent inhibitory activity of **7a**.

Table 2
E. coli FabH inhibitory activity of the selected compounds

Compound	*E. coli* FabH IC_{50}(μM)	Hemolysis LC30 [a] (mg/ml)
6a	3.3	>10
7a	2.6	>10
8a	24	>10
9a	28	>10
10a	8.2	>10
6b	43	>10
12b	8.5	>10

[a] Lytic concentration 30%.

4. Conclusion

In this paper, 24 vinylogous carbamates (**4a**–**15a** and **4b**–**15b**) were synthesized and evaluated for *E. coli* FabH inhibitory activity as antibacterial agents for the first time. Among the compounds being studied, potent and selective anti-Gram-negative bacteria activities were observed in these vinylogous carbamates. Compounds **6a, 7a, 10a, 12a, 12b** and **13a** exhibited excellent activities against Gram-negative *E. coli* and *Pseudomonas aeruginosa*. Especially, compounds **6a** and **7a** showed the most potent *E. coli* FabH inhibitory activity. Molecular docking simulation was performed to position compound **6a** into the *E. coli* FabH active site to determine the probable binding conformation. Based on the data obtained from this study, we concluded that compounds **6a** and **7a** were potent inhibitors of *E. coli* FabH as antibiotics deserving further research.

5 Experimental section

5.1. Antibacterial activity

The antibacterial activity of the synthesized compounds was tested against *E. coli*, *P. fluorescence*, *B. subtilis* and *S. aureus* using MH medium (Mueller-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL), The MICs (minimum inhibitory concentrations) of the test compounds were determined by a colorimetric method using the dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). A stock solution of the synthesized compound (100 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (Phosphate Buffered Saline 0.01 mol/L, pH 7.4: $Na_2HPO_4{\cdot}12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of

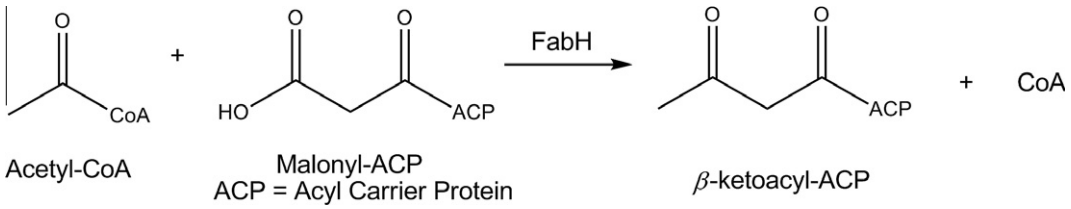


Figure 1. FabH-catalyzed initiation reaction of fatty acid biosynthesis.

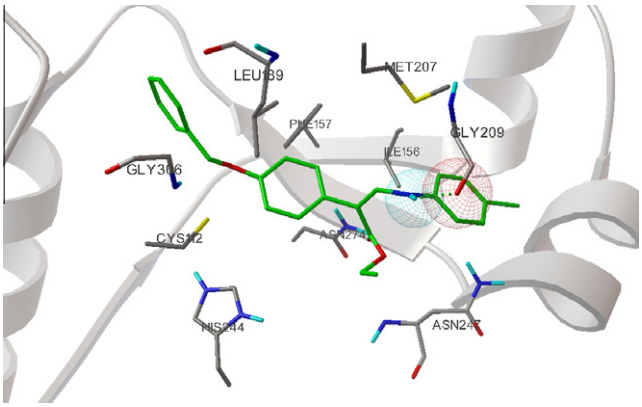


Figure 2. Binding model of compound **7a** and *E. coli* FabH.

incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm.

5.2. *E. coli* FabH purification and activity assay

Native *E. coli* FabH protein was overexpressed in *E. coli* DH10B cells using the pET30 vector and purified to homogeneity in three chromatographic steps (Q-Sepharose, MonoQ, and hydroxyapatite) at 4 °C. The selenomethionine- substituted protein was expressed in *E. coli* BL21(DE3) cells and purified in a similar way. Harvested cells containing FabH were lysed by sonication in 20 mM Tris, pH 7.6, 5 mM imidazole, 0.5 M NaCl and centrifuged at 20000 rpm for 30 min. The supernatant was applied to a Ni-NTA agarose column, washed, and eluted using a 5–500 mM imidazole gradient over 20 column volumes. Eluted protein was dialyzed against 20 mM Tris, pH 7.6, 1 mM DTT, and100 mM NaCl. Purified FabD and FabHs were concentrated up to 2 mg/mL and stored at −80 °C in 20 mM Tris, pH 7.6, 100 mM NaCl, 1 mM DTT, and 20% glycerol for enzymatic assay.

In a final 20 μL reaction, 20 mM Na_2HPO_4, pH 7.0, 0.5 m MDTT, 0.25 mMMgCl_2, and 2.5 μM holo-ACP were mixed with 1 nM FabH, and H_2O was added to 15 μL. After 1 min incubation, a 2 μL mixture of 25 μM acetyl-CoA, 0.5 mMNADH, and 0.5 mM NADPH was added for FabH reaction for 25 min. The reaction was stopped by adding 20 μL of ice-cold 50% TCA, incubating for 5 min on ice, and centrifuging to pellet the protein. The pellet was washed with 10% ice-cold TCA and resuspended with 5 μL of 0.5 M NaOH. The incorporation of the 3*H* signal in the final product was read by liquid scintillation. When determining the inhibition constant (IC_{50}), inhibitors were added from a concentrated DMSO stock such that the final concentration of DMSO did not exceed 2%.

5.3. Docking simulations

Molecular docking of compound **7a** into the three-dimensional X-ray structure of *E. coli* FabH (PDB code: 1HNJ) was carried out using the AutoDock software package (version 4.0) as implemented through the graphical user interface AutoDock Tools (ADT 1.4.6) .[15]

5.4. Chemistry

All chemicals (reagent grade) used were purchased from Aldrich (U.S.A). 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 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 at DPX500 on a Bruker spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

5.5. General method of synthesis for vinylogous carbamates

The starting materials (aldehyde **2**) for the synthesis of vinylogous carbamates have been previously published. Equimolar of the appropriate substituted aromatic amines and the aldehyde (**2**) in absolute alcohol (30 mL) were heated at 80° for 2–4 h. The excess solvent was removed under reduced pressure. The residue was purified by a flash chromatography with chloroform/EtOAc/petroleum ether to afford two fractions. The first fraction gave a *Z*-isomer, and the second fraction gave an *E*-isomer.[12]

5.6. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-chlorophenylamino)acrylate (4a)

Yield: 0.78 g (38%). Shallow yellow powder. Mp 92–93°. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 7.2 Hz, 3H); 4.17(*q*, *J* = 7.2 Hz, 2H); 5.11 (*s*, 2H); 6.95 (*d*, *J* = 8.8 Hz, 2H); 7.26 (*d*, *J* = 8.8 Hz, 2H); 7.31–7.47 (*m*, 9H); 7.53 (*d*, *J* = 12.7 Hz, 1H); 10.2 (*d*, *J* = 12.7 Hz, 1H). MS (ESI): 408.9 ($C_{24}H_{22}ClNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}ClNO_3$: C, 70.67; H, 5.44; N, 3.43. Found: C, 70.79; H, 5.63; N, 3.67.

5.7. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-chlorophenylamino)acrylate (4b)

Yield: 0.88 g (43%). White powder. Mp 114–115°. ^{1}H NMR (DMSO-d_6): 1.18 (*t*, *J* = 5.0 Hz, 3H); 4.17(*q*, *J* = 7.1 Hz, 2H); 5.12 (*s*, 2H); 7.03 (*d*, *J* = 8.7 Hz, 2H); 7.15–7.49 (*m*, 11H); 7.95 (*d*, *J* = 12.9 Hz, 1H); 8.57 (*d*, *J* = 12.9 Hz, 1H). MS (ESI): 408.9 ($C_{24}H_{22}ClNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}ClNO_3$: C, 59.03; H, 4.68; N, 3.43. Found: C, 70.74; H, 5.71; N, 3.64.

5.8. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2,4-dichlorophenylamino)acrylate (5a)

Yield: 1.12 g (51%). White powder. Mp 98–99°. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 7.2 Hz, 3H); 4.20(*q*, *J* = 7.1 Hz, 2H); 5.12 (*s*, 2H); 6.97 (*d*, *J* = 8.7 Hz, 2H); 7.08–7.68(*m*, 11H); 7.71 (*d*, *J* = 12.3 Hz, 1H); 10.73 (*d*, *J* = 12.3 Hz, 1H). MS (ESI): 442.1 ($C_{24}H_{21}Cl_2NO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{21}Cl_2NO_3$: C, 65.17; H, 4.79; N, 3.17. Found: C, 65.41; H, 4.90; N, 3.25.

5.9. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2,4-dichlorophenylamino)acrylate (5b)

Yield: 0.75 g (34%). White powder. Mp 64–65 °C. ^{1}H NMR (DMSO-d_6): 1.19 (*t*, *J* = 7.2 Hz, 3H); 4.12(*q*, *J* = 7.0 Hz, 2H); 5.14 (*s*, 2H); 7.10 (*d*, *J* = 8.4 Hz, 2H); 7.23 (*d*, *J* = 8.4 Hz, 2H); 7.36–7.42 (*m*, 9H); 7.47 (*d*, *J* = 12.5 Hz, 1H); 8.02 (*d*, *J* = 12.5 Hz, 1H). MS (ESI): 442.1 ($C_{24}H_{21}Cl_2NO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{21}Cl_2NO_3$: C, 65.17; H, 4.79; N, 3.17. Found: C, 65.41; H, 4.90; N, 3.25.

5.10. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-bromophenylamino)acrylate (6a)

Yield: 1.02 g (45%). Shallow yellow powder. Mp 91–92 °C. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 7.2 Hz, 3H); 4.07(*q*, *J* = 7.2 Hz, 2H); 5.11 (*s*, 2H); 6.95 (*d*, *J* = 8.8 Hz, 2H); 7.22–7.49 (*m*, 11H); 7.53 (*d*, *J* = 12.7 Hz, 1H); 10.19 (*d*, *J* = 12.9 Hz, 1H). MS (ESI): 453.3 ($C_{24}H_{22}BrNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}BrNO_3$: C, 63.73; H, 4.90; N, 3.10. Found: C, 63.82; H, 5.05; N, 3.21.

5.11. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-bromophenylamino)acrylate (6b)

Yield: 0.84 g (37%). White powder. Mp 108–109 °C. ^{1}H NMR (DMSO-d_6): 1.18 (*t*, *J* = 7.0 Hz, 3H); 4.10(*q*, *J* = 7.1 Hz, 2H); 5.12 (*s*, 2H); 7.03 (*d*, *J* = 8.8 Hz, 2H); 7.11–7.49 (*m*, 11H); 7.94 (*d*, *J* = 13.2 Hz, 1H); 8.57 (*d*, *J* = 13.2 Hz, 1H). MS (ESI): 453.3 ($C_{24}H_{22}BrNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}BrNO_3$: C, 63.73; H, 4.90; N, 3.10. Found: C, 63.92; H, 5.12; N, 3.25.

5.12. (*Z*)-*Ethyl* 3-(*p*-toluidino)-2-(4-(benzyloxy)phenyl)acrylate (7a)

Yield: 1.06 g (55%). Shallow yellow powder. Mp 75–76 °C. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 7.0 Hz, 3H); 4.17(*q*, *J* = 6.9 Hz, 2H); 5.11 (*s*, 2H); 6.95 (*d*, *J* = 8.6 Hz, 2H); 7.10 (*d*, *J* = 8.6 Hz, 2H); 7.15 (*d*, *J* = 8.6 Hz, 2H); 7.25 (*d*, *J* = 8.6 Hz, 2H); 7.30–7.47 (*m*, 5H); 7.53 (*d*, *J* = 12.8 Hz, 1H); 10.2 (*d*, *J* = 12.8 Hz, 1H). MS (ESI): 388.18 ($C_{25}H_{25}NO_3$, $[M+H]^+$). Anal. calcd for $C_{25}H_{25}NO_3$: C, 77.49; H, 6.50; N, 3.61. Found: C, 77.38; H, 6.67; N, 3.80.

5.13. (*Z*)-Ethyl 3-(*p*-toluidino)-2-(4-(benzyloxy)phenyl)acrylate (7b)

Yield: 0.54 g (28%). White powder. Mp 95–96 °C. ^{1}H NMR (DMSO-d_6): 1.18 (*t*, *J* = 7.0 Hz, 3H); 4.08(*q*, *J* = 7.0 Hz, 2H); 5.12 (*s*, 2H); 7.03 (*d*, *J* = 8.8 Hz, 2H); 7.01–7.49 (*m*, 11H); 7.98 (*d*, *J* = 10.1 Hz, 1H); 8.35 (*d*, *J* = 10.5 Hz, 1H). MS (ESI): 388.18 ($C_{25}H_{25}NO_3$, $[M+H]^+$). Anal. calcd for $C_{25}H_{25}NO_3$: C, 77.49; H, 6.50; N, 3.61. Found: C, 77.32; H, 6.64; N, 3.78.

5.14. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2,4-dibromophenylamino)acrylate (8a)

Yield: 1.35 g (51%). Shallow yellow powder. Mp 88–90 °C. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 7.0 Hz, 3H); 4.21(*q*, *J* = 6.9 Hz, 2H); 5.13 (*s*, 2H); 6.97 (*d*, *J* = 8.6 Hz, 2H); 7.28–7.67 (*m*, 5H); 7.83 (*d*, *J* = 12.1 Hz, 1H); 10.69 (*d*, *J* = 12.1 Hz, 1H). MS (ESI): 532.2 ($C_{24}H_{21}Br_2NO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{21}Br_2NO_3$: C, 54.26; H, 3.98; N, 2.64. Found: C, 54.32; H, 3.97; N, 2.80.

5.15. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2,4-dibromophenylamino)acrylate (8b)

Yield: 0.74 g (28%). Shallow yellow powder. Mp 92–94 °C. ^{1}H NMR (DMSO-d_6): 1.18 (*t*, *J* = 7.0 Hz, 3H); 4.16(*q*, *J* = 7.0 Hz, 2H); 5.13 (*s*, 2H); 7.03 (*d*, *J* = 8.8 Hz, 2H); 7.09–7.59 (*m*, 11H); 7.79 (*d*, *J* = 12.9 Hz, 1H); 8.35 (*d*, *J* = 12.9 Hz, 1H). MS (ESI): 532.2 ($C_{24}H_{21}Br_2NO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{21}Br_2NO_3$: C, 54.26; H, 3.98; N, 2.64. Found: C, 54.37; H, 4.07; N, 2.83.

5.16. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2-chlorophenylamino)acrylate (9a)

Yield: 1.12 g (55%). Shallow yellow powder. Mp 70–72 °C. ^{1}H NMR (DMSO-d_6): 1.21 (*t*, *J* = 7.0 Hz, 3H); 4.20(*q*, *J* = 7.0 Hz, 2H); 5.12 (*s*, 2H); 6.97 (*d*, *J* = 8.79 Hz, 2H); 7.22–7.65 (*m*, 11H); 7.63(*d*, *J* = 12.2 Hz, 1H); 10.71 (*d*, *J* = 12.2 Hz, 1H). MS (ESI): 408.9 ($C_{24}H_{22}ClNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}ClNO_3$: C, 70.67; H, 5.44; N, 3.43. Found: C, 70.72; H, 5.55; N, 3.62.

5.17. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2-chlorophenylamino)acrylate (9b)

Yield: 0.61 g (30%). White powder. Mp 78–80 °C. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 7.2 Hz, 3H); 4.19(*q*, *J* = 7.1 Hz, 2H); 5.12 (*s*, 2H); 7.00 (*d*, *J* = 8.7 Hz, 2H); 7.23–7.59 (*m*, 11H); 7.90 (*d*, *J* = 12.9 Hz, 1H); 8.17 (*d*, *J* = 12.9 Hz, 1H). MS (ESI): 408.9 ($C_{24}H_{22}ClNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}ClNO_3$: C, 59.03; H, 4.68; N, 3.43. Found: C, 70.79; H, 5.78; N, 3.65.

5.18. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-fluorophenylamino)acrylate (10a)

Yield: 1.13 g (58%). Shallow yellow powder. Mp 112–113 °C. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 6.9 Hz, 3H); 4.11(*q*, *J* = 6.9 Hz, 2H); 5.13 (*s*, 2H); 6.99 (*d*, *J* = 6.6 Hz, 2H); 7.15–7.48 (*m*, 11H); 7.52 (*d*, *J* = 12.7 Hz, 1H); 10.2 (*d*, *J* = 12.7 Hz, 1H). MS (ESI): 392.4 ($C_{24}H_{22}FNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}FNO_3$: C, 73.64; H, 5.66; N, 3.58. Found: C, 73.79; H, 5.88; N, 3.75.

5.19. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-fluorophenylamino)acrylate (10b)

Yield: 0.63 g (32%). White powder. Mp 114–115 °C. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 6.9 Hz, 3H); 4.10(*q*, *J* = 6.9 Hz, 2H); 5.13 (*s*, 2H); 7.03 (*d*, *J* = 6.6 Hz, 2H); 7.19–7.49 (*m*, 11H); 7.57 (*d*, *J* = 13.8 Hz, 1H); 8.00 (*d*, *J* = 13.8 Hz, 1H). MS (ESI): 392.4 ($C_{24}H_{22}FNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}FNO_3$: C, 73.64; H, 5.66; N, 3.58. Found: C, 73.78; H, 5.70; N, 3.69.

5.20. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2,4-difluorophenylamino)acrylate (11a)

Yield: 0.65 g (32%). Shallow yellow powder. Mp 149–150 °C. ^{1}H NMR (DMSO-d_6): 1.18 (*t*, *J* = 6.9 Hz, 3H); 4.22(*q*, *J* = 6.9 Hz, 2H); 5.13 (*s*, 2H); 6.97 (*d*, *J* = 8.6 Hz, 2H); 7.21–7.63 (*m*, 11H); 7.53 (*d*, *J* = 12.7 Hz, 1H); 10.37 (*d*, *J* = 11.3 Hz, 1H). MS (ESI): 410.2 ($C_{24}H_{21}F_2NO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{21}F_2NO_3$: C, 70.41; H, 5.17; N, 3.42. Found: C, 70.59; H, 5.28; N, 9.60.

5.21. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2,4-difluorophenylamino)acrylate (11b)

Yield: 0.86 g (42%). White powder. Mp 97–98 °C. ^{1}H NMR (DMSO-d_6): 1.22 (*t*, *J* = 7.2 Hz, 3H); 4.10(*q*, *J* = 7.2 Hz, 2H); 5.14 (*s*, 2H); 7.00 (*d*, *J* = 8.6 Hz, 2H); 7.05 (*d*, *J* = 8.6 Hz, 2H); 7.18–7.60 (*m*, 9H); 7.72 (*d*, *J* = 11.3 Hz, 1H); 10.37 (*d*, *J* = 11.3 Hz, 1H). MS (ESI): 410.2 ($C_{24}H_{21}F_2NO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{21}F_2NO_3$: C, 70.41; H, 5.17; N, 3.42. Found: C, 70.59; H, 5.28; N, 9.60.

5.22. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-isopropylphenylamino)acrylate (12a)

Yield: 1.21 g (58%). Shallow yellow powder. Mp 88–89 °C. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 6.9 Hz, 3H); 4.11(*q*, *J* = 6.9 Hz, 2H); 5.13 (*s*,

2H); 7.00 (*d*, *J* = 6.6 Hz, 2H); 7.17 (*d*, *J* = 6.6 Hz, 2H); 7.28–7.47 (*m*, 9H); 7.57 (*d*, *J* = 13.5 Hz, 1H); 10.22 (*d*, *J* = 13.5 Hz, 1H). MS (ESI): 416.5 ($C_{27}H_{29}NO_3$, $[M+H]^+$). Anal. calcd for $C_{27}H_{29}NO_3$: C, 78.04; H, 7.03; N, 3.37. Found: C, 78.19; H, 5.15; N, 3.47.

5.23. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-isopropylphenylamino)acrylate (12b)

Yield: 0.52 g (25%). Shallow yellow powder. Mp 179–180 °C. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 6.9 Hz, 3H); 4.17(*q*, *J* = 6.9 Hz, 2H); 5.13 (*s*, 2H); 7.03 (*d*, *J* = 6.9 Hz, 2H); 7.15–7.49 (*m*, 11H); 7.52 (*d*, *J* = 13.5 Hz, 1H); 8.00 (*d*, *J* = 13.5 Hz, 1H). MS (ESI): 416.5 ($C_{27}H_{29}NO_3$, $[M+H]^+$). Anal. calcd for $C_{27}H_{29}NO_3$: C, 78.04; H, 7.03; N, 3.37. Found: C, 78.19; H, 5.15; N, 3.47.

5.24. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-nitrophenylamino)acrylate (13a)

Yield: 0.75 g (57%). Yellow powder. Mp 179–180 °C. ^{1}H NMR (DMSO-d_6): 1.18 (*t*, *J* = 7.2 Hz, 3H); 4.15(*q*, *J* = 7.2 Hz, 2H); 5.10 (*s*, 2H); 6.92 (*d*, *J* = 8.4 Hz, 2H); 7.14–7.53 (*m*, 11H); 8.16 (*d*, *J* = 12.3 Hz, 1H); 10.18 (*d*, *J* = 12.3 Hz, 1H). MS (ESI): 419.4 ($C_{24}H_{22}N_2O_5$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}N_2O_5$: C, 68.89; H, 5.30; N, 6.69. Found: C, 68.96; H, 5.43; N, 6.85.

5.25. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(4-nitrophenylamino)acrylate (13b)

Yield: 0.26 g (20%). Yellow powder. Mp 130–131 °C. ^{1}H NMR (DMSO-d_6): 1.20 (*t*, *J* = 7.2 Hz, 3H); 4.12(*q*, *J* = 7.2 Hz, 2H); 5.13 (*s*, 2H); 7.05 (*d*, *J* = 8.1 Hz, 2H); 7.18 (*d*, *J* = 8.1 Hz, 2H); 7.33–7.49 (*m*, 8H); 8.02 (*d*, *J* = 12.9 Hz, 1H); 8.13 (*d*, *J* = 8.7 Hz, 1H); 9.14 (*d*, *J* = 12.9 Hz, 1H). MS (ESI): 419.4 ($C_{24}H_{22}N_2O_5$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}N_2O_5$: C, 68.89; H, 5.30; N, 6.69. Found: C, 68.96; H, 5.43; N, 6.85.

5.26. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2-fluorophenylamino)acrylate (14a)

Yield: 0.44 g (36%). White powder. Mp 124–125 °C. ^{1}H NMR (DMSO-d_6): 1.18 (*t*, *J* = 7.2 Hz, 3H); 4.15(*q*, *J* = 7.2 Hz, 2H); 5.11 (*s*, 2H); 6.93 (*d*, *J* = 8.4 Hz, 2H); 7.14–7.45 (*m*, 11H); 7.52 (*d*, *J* = 12.9 Hz, 1H); 10.49 (*d*, *J* = 12.9 Hz, 1H). MS (ESI): 392.4 ($C_{24}H_{22}FNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}FNO_3$: C, 73.64; H, 5.66; N, 3.58. Found: C, 73.74; H, 5.78; N, 3.65.

5.27. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(2-fluorophenylamino)acrylate (14b)

Yield: 0.55 g (45%). Shallow yellow powder. Mp 75–77 °C. ^{1}H NMR (DMSO-d_6): 1.19 (*t*, *J* = 6.9 Hz, 3H); 4.11(*q*, *J* = 6.9 Hz, 2H); 5.13 (*s*, 2H); 7.00 (*d*, *J* = 6.6 Hz, 2H); 7.05 (*d*, *J* = 6.6 Hz, 2H); 7.14–7.54 (*m*, 9H); 7.57 (*d*, *J* = 13.5 Hz, 1H); 8.00 (*d*, *J* = 13.5 Hz, 1H). MS (ESI): 392.4 ($C_{24}H_{22}FNO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{22}FNO_3$: C, 73.64; H, 5.66; N, 3.58. Found: C, 73.74; H, 5.78; N, 3.65.

5.28. (*Z*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(phenylamino) acrylate (15a)

Yield: 0.99 g (63%). White powder. Mp 95–96 °C. ^{1}H NMR (DMSO-d_6): 1.18 (*t*, *J* = 7.2 Hz, 3H); 4.09(*q*, *J* = 7.2 Hz, 2H); 5.12 (*s*, 2H); 6.95 (*d*, *J* = 6.9 Hz, 2H); 7.26 (*d*, *J* = 8.8 Hz, 2H); 7.02–7.38 (*m*, 11H); 7.50 (*d*, *J* = 13.5 Hz, 1H); 10.35 (*d*, *J* = 13.5 Hz, 1H). MS (ESI): 374.4 ($C_{24}H_{23}NO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{23}NO_3$: C, 77.19; H, 6.21; N, 3.75. Found: C, 77.32; H, 6.33; N, 3.87.

5.29. (*E*)-*Ethyl* 2-(4-(benzyloxy)phenyl)-3-(phenylamino) acrylate (15b)

Yield: 0.58 g (43%). White powder. Mp 89–90 °C. ^{1}H NMR (DMSO-d_6): 1.18 (*t*, *J* = 6.6 Hz, 3H); 4.10 (*q*, *J* = 6.6 Hz, 2H); 5.12 (*s*, 2H); 6.94 (*d*, *J* = 6.9 Hz, 2H); 7.03–7.49 (*m*, 11H); 7.99 (*d*, *J* = 13.5 Hz, 1H); 8.57 (*d*, *J* = 13.5 Hz, 1H). MS (ESI): 374.4 ($C_{24}H_{23}NO_3$, $[M+H]^+$). Anal. calcd for $C_{24}H_{23}NO_3$: C, 77.19; H, 6.21; N, 3.75. Found: C, 77.32; H, 6.33; N, 3.87.

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References and notes

1. Leeb, M. *Nature* **2004**, *431*, 892.
2. Lee, J.-Y.; Jeong, K.-W.; Lee, J.-U.; Kang, D.-I.; Kim, Y. *Bioorg. Med. Chem.* **2009**, *17*, 5408.
3. Lai, C. Y.; Cronan, J. E. *J. Biol. Chem.* **2003**, *19*, 51494.
4. Lu, Y. J.; Zhang, Y. M.; Rock, C. O. *Biochem. Cell. Biol.* **2004**, *82*, 145.
5. White, S. W.; Zheng, J.; Zhang, Y. M.; Rock, C. O. *Annu. Rev. Biochem.* **2005**, *74*, 791.
6. Asheka, A.; Cho, S. J. *Bioorg. Med. Chem.* **2006**, *14*, 1474.
7. Adsule, S.; Barve, V.; Chen, D.; Ahmed, F.; Dou, Q. P.; Padhye, S.; Sarkar, F. H. *J. Med. Chem.* **2006**, *49*, 7242.
8. Li, H. Q.; Shi, L.; Li, Q. S.; Liu, P. G.; Luo, Y.; Zhao, J.; Zhu, H. L. *Bioorg. Med. Chem.* **2009**, *17*, 6264.
9. Wang, B. D.; Yang, Z. Y.; Wang, Q.; Caib, T. K.; Crewdson, P. *Bioorg. Med. Chem.* **2006**, *14*, 1880.
10. Zewge, D.; Chen, C. Y.; Deer, C.; Dormer, P. G.; Hughes, D. L. *J. Org. Chem.* **2007**, *72*, 4276.
11. Prabhakaran, E. N.; Nugent, B. M.; Williams, A. L.; Nailor, K. E.; Johnston, J. N. *Org. Lett.* **2002**, *4*, 4197.
12. Xiao, Z.-P.; Xue, J.-Y.; Tan, S.-H.; Li, H.-Q.; Zhu, H.-L. *Bioorg. Med. Chem.* **2007**, *15*, 4212.
13. Beccalh, E. M. *J. Org. Chem.* **1984**, *49*, 4286.
14. Qiu, X.; Janson, C. A.; Smith, W. W.; Head, M.; Lonsdale, J.; Konstantinidis, A. K. *J. Mol. Biol.* **2001**, *307*, 341.
15. Huey, R.; Morris, G. M.; Olson, A. J.; Goodsell, D. S. *J. Comput. Chem.* **2007**, *28*, 1145.