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Synthesis and structure activity relationship studies of novel *Staphylococcus aureus* Sortase A inhibitors

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Abstract

Synthetic methods have been developed for the lead Sortase A inhibitors identified from previous studies. Several derivatives of the lead inhibitor were synthesized to derive preliminary structure activity relationships (SAR). Different regions of the lead inhibitor that are critical for the enzyme activity have been determined by systematic SAR studies. The *E* stereochemistry of the lead compound was found to be critical for its activity. Replacement of the *E* double bond with *Z* double bond or a rigid triple bond reduced the enzyme inhibitory activity in most cases. Reduction of the double bond to a C-C single bond resulted in complete loss of activity. Amide carbonyl and NH groups were also found to be crucial for the activity of this class of inhibitors, as well. Morpholine ring oxygen atom was also found to be important factor for the activity of the lead inhibitor. Preliminary SAR studies led to the identification of compounds with improved enzyme inhibition. The most active compound was found to have an IC_{50} value of 58εM against the enzyme.

Keywords

Staphylococcus aureus; Sortase; Inhibitor; Antibacterial; Structure Activity Relationship; Synthesis

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1. Introduction

S. aureus causes a variety of human infections, ranging from superficial abscesses to life threatening bacteremias. Staphylococcal infections within the hospital and in the community are increasing, and an ever-growing number of antibiotic-resistant strains make treatment options more difficult. MRSA strains were isolated from 2% of staphylococcal infections in 1974 and from 63% of staphylococcal infections in 2004. Many of the nosocomial MRSA strains are multi-drug resistant, and even methicillin-sensitive strains can be deadly. A recent report using population-based, active case finding revealed that 94,360 invasive MRSA infections occurred in the U.S. in 2005, and that the majority of these (58%) occurred outside of the hospital [1].

Notorious as a major source of nosocomial infections, *S. aureus* has recently taken on a new role in causing an escalating number of community-acquired infections in non-hospitalized persons without predisposing risk factors. A single *S. aureus* clone, designated as USA300, causes the majority of community acquired-MRSA infections in the U.S., and its dissemination has been observed globally [2–6]. Vancomycin is most commonly used for treatment of systemic infections caused by MRSA. However, *S. aureus* isolates with reduced susceptibility to vancomycin have been reported since 1997 [7]. These isolates are also methicillin resistant [8–10]. Because *S. aureus* cannot always be controlled by antibiotics and MRSA isolates are becoming increasingly prevalent in the community, additional control strategies and novel therapeutic approaches are sorely needed.

New approaches for the prevention and treatment of bacterial infections require greater understanding of the molecular structure and mechanisms of the chosen intervention targets and of the pathogenic role played by the target in the infection process. Bacterial infections are complex and involve the action of a large, sophisticated arsenal of virulence factors, many of which are surface-bound or secreted. Gram-positive bacteria such as *S. aureus* are endowed with a multitude of cell-wall anchored proteins that serve as an interface between the microbe and its host. Bacterial sortases are cysteine transpeptidases that participate in secretion and anchoring of many cell wall proteins by a mechanism conserved in almost the entire class of Gram-positive bacteria. Surface proteins can be attached to the bacterial surface in several fashions [11,12]. Proteins that are covalently attached to the cell wall share conserved regions known as the “sorting signal” or cell wall anchors [13,14]. The sorting signal includes a conserved amino acid motif, usually LPXTG. Precursor proteins are directed into a secretory pathway by their N-terminal signal peptides. They are translocated across the membrane and the signal peptide is cleaved [14,15]. Then, the C-terminal sorting signal retains the protein in the secretory pathway. The enzyme sortase acts at this point to cleave the protein between the threonine (T) and the glycine (G) of the LPXTG motif [11,16]. The carboxyl group of the Thr is then amide-linked to the amino group of a “cross-bridge” peptide in the lipid II precursor for cell wall synthesis [11,17]. Sortase-defective strains of various pathogens were shown to be faulty in the display of surface proteins and are less virulent [18,19]. In a number of studies, individual sortase genes have been deleted and the loss-of-sortase function has resulted in less virulence in

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several animal models of the disease [18, 20–24]. Hence, sortases are attractive pharmacotherapeutic targets [12].

Currently, there have only been a few reports of specific sortase inhibitors [25–28]. Recently, Oh et al [29] identified a small molecule reversible inhibitor of SrtA with a low micromolar IC_{50} value by structurally modifying a lead compound identified by random screening of a group of small molecules. We have recently identified an inhibitor (**1**) of *S. aureus* SrtA aided by *in-silico* virtual screening (Figure 1) [30]. We have conducted preliminary structure activity relationship (SAR) studies with the goal of improving the activity of inhibitor **1**. This manuscript describes the synthesis of inhibitor **1** and several of its derivatives and the results of their *in vitro* enzymatic evaluation against $SrtA_{\Delta 59}$, a fully active variant of SrtA with 59 N-terminus residues removed.

2. Results and discussion

Inhibitor **1** was initially obtained from a commercial source in milligram quantities. For SAR studies we needed this compound in larger quantities. So, a general method for the synthesis of inhibitor **1** was developed in our laboratory. The synthetic procedures for all analogues were modelled around this synthesis. Chemistry employed for the synthesis of inhibitor **1** is outlined in Scheme 1.

Commercially available methyl 2-(N-morpholino)-5-nitrobenzoate (**2**) was reduced using hydrogen in the presence of Pd/C catalyst in anhydrous ethyl acetate to afford the corresponding amino compound **3**. The compound **3** was coupled with commercially available *trans*-3-(thiophene-2-yl)acrylic acid (**4a**) in the presence of ethyl(*N*,*N*-dimethylaminopropyl) carbodiimide (EDAC) and *N*,*N*-dimethylaminopyridine (DMAP) in 1,2-dichloroethane to form the amide compound **5a** [31]. Basic hydrolysis of the ester methyl group present in compound **5a** afforded the inhibitor **1** as a white crystalline solid.

We have synthesized several derivatives of inhibitor **1** in order to derive preliminary structure activity relationship data. Their activities against $SrtA_{\Delta 59}$ were determined using a fluorescence resonance energy transfer (FRET) assay which is a modification of a previously reported procedure. In this assay, the IC_{50} values were determined by monitoring the effect of the compounds on the steady state cleavage of a model substrate peptide, Dabcyl-QALPETGEE-EDANS [29,32,33].Structures and IC_{50} values of the newly synthesized derivatives of inhibitor **1** are given in Table 1.

Compound **6** is a furan analogue of inhibitor **1**. Compound **6** was synthesized by a similar procedure as described for the synthesis of inhibitor **1** (Scheme 1). We have used *trans*-3-(furan-2-yl)acrylic acid (**4b**) instead of *trans*-3-(thiophene-2-yl)acrylic acid (**4a**) in this procedure. Compound **3** was coupled with commercially available **4b** in the presence of EDAC and DMAP in 1,2-dichloroethane to form the amide compound **5b**. Basic hydrolysis of the ester present in compound **5b** afforded compound **6**. Compound **6** along with the synthetic intermediate methyl esters **5a** and **5b** were evaluated for their enzyme inhibitory activity. The results of the enzyme assays for these and all other compounds described in this report are summarized in Table 1. Compound **6** did not show an enhancement of

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activity. Instead, it was found to be less active compared to inhibitor **1** in the enzymatic assay (IC_{50} = 181 μM). But, the methyl ester intermediates (**5a** and **5b**) showed improved inhibition as compared to the corresponding acid derivatives **1** and **6**. Methyl ester derivative of the thiophene compound (**5a**) showed an IC_{50} value of 71 μM and methyl ester derivative of the furan compound (**5b**) showed a much improved inhibition at an IC_{50} value of 58 μM. Although this increase in activity of methyl ester analogues is counter intuitive of the FlexX model which showed a possible salt bridge interaction between the carboxylic acid and Arg197 side chain, the increased activity may be explained using the FlexX model of the methyl ester in the active site. This model shows a similar fit of the molecule in the active site with the ester methyl group resting in a small hydrophobic pocket alongside the central phenyl ring without completely destroying the electrostatic interaction between Arg197 side chain and ester group O atoms of the inhibitor. The increased activity could be a result of these additional hydrophobic interactions generated by the ester methyl group with hydrophobic residues in the active site.

Compounds **7 – 10** were synthesized to evaluate the importance of the double bond present in compounds **1** and **6** for their activity. Compounds **7** and **9** are the saturated analogues of the carboxylic acid derivatives **1** and **6**. Compounds **8** and **10** are the saturated analogues of the methyl ester derivatives **5a** and **5b**. Our assay showed that all four saturated compounds **7 – 10** were inactive up to a concentration of 600 μM showing that the double bond is crucial for the activity of these inhibitors (Table 1). Synthesis of compounds **7 – 10** is outlined in Scheme 2. Hydrogenation of the thiophene derivatives **1** and **5a** using Pd/C as catalyst did not work, possibly due to the catalyst poisoning effect of the thiophene ring present in these molecules. Use of a stronger catalyst like Pd black in the presence of ammonium formate resulted in the hydrogenation of **1** and **5a** to corresponding hydrogenated compounds **7** and **8**. Hydrogenation of **6** and **5b** were carried out using H2 in the presence of Pd/C catalyst to afford compounds **9** and **10** in good yields.

Our lead inhibitor **1** has a double bond with *E* stereochemistry. We already know that the double bond is critical for the activity of the inhibitor **1**. Our next goal was to examine the effect of stereochemistry of the double bond on the activity. With this goal, we prepared the *Z* isomer **15**. In order to examine the influence of a carbon-carbon triple bond on the activity, we prepared the compound **13**. Synthesis of compounds **13** and **15** is outlined in Scheme 3.

The synthesis started from the known 3-(thiophen-2-yl)propiolic acid **11**, which is prepared according to the literature procedure [34]. The acid **11** was coupled with the amino compound **3** in the presence of EDAC and DMAP in 1,2-dichloroethane afforded the alkyne ester **12**. The ester group present in compound **12** was hydrolyzed using NaOH in MeOH to afford the acetylenic acid **13**. The partial hydrogenation of the acetylenic ester **12** using Lindlar Pd catalyst did not work as expected possibly due to the catalytic poisoning effect of the thiophene ring already present in the molecule. However, reduction worked smoothly to a *cis* double bonded compound **14** when it was carried out with H_2 in the presence of Pd/C in ethyl acetate. The ester group present in compound **14** was hydrolyzed using NaOH in MeOH to afford the cis derivative **15**.

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Compounds **13, 15** and the intermediate esters, **12** and **14** were all evaluated for their enzymatic activity (Table 1). The intermediate acetylenic compound **12** (165 μM) and the corresponding acid **13** (183 μM) showed decreased inhibition of the enzyme compared to the corresponding E alkene derivatives. The Z alkene ester derivative **14** showed more or less similar inhibition (61 μM) as compared to the E derivative, **5a**. However, the Z alkene acid **15** showed a decreased inhibition (154 μM) of the enzyme as compared to E alkene acid, **1**.

We have synthesized compounds **17a** and **17b** in order to examine the effect of interruption of possible H-bonding of NH group present in the inhibitors **1** and **6**. Target compounds in this case were compounds **17a** and **17b** where the amide N was methylated. Synthesis of the target compounds are outlined in Scheme 4.

Compound **5a** and **5b** were methylated using methyl iodide in the presence of NaH in THF to afford the N-methylated esters **16a** and **16b**. Basic hydrolysis of **16a** and **16b** using 1N. NaOH in MeOH afforded the final acid products **17a** and **17b**, respectively.

The compounds **17a–b** and intermediates **16a–b** were evaluated for their enzymatic activity in our assay (Table 1). As expected, all four N-methyl derivatives showed decrease in inhibition as compared to corresponding NH analogues. Compounds **16a** and **16b** showed IC_{50} values 571 μM and 514 μM respectively and the final acids **17a–b** were inactive up to a concentration of 600 μM. This demonstrates that the NH group of the inhibitors **1** and **6** is very important for their activity.

In order to examine the effect of amide carbonyl oxygen on the activity of the lead compound, we have prepared compound **20** in which carbonyl group is replaced with a CH_2 group. Synthesis of compound **20** is outlined in Scheme 5.

Reductive amination of the known aldehyde **18** with the amine **3** in the presence of $NaCNBH_3$ and $ZnCl_2$ in MeOH afforded the ester compound **19**. Hydrolysis of the ester group present in compound **19** using NaOH in MeOH afforded the final acid product **20**. The compound **20** and intermediate **19** were evaluated for their enzymatic activity (Table 1). Both the compounds showed decreased inhibition compared to corresponding amide analogues showing that amide carbonyl group is important for the activity. The acid **20** was inactive up to a concentration of 600 μM while the intermediate ester **19** had a reduced activity at 249 μM.

We wanted to inspect the importance of the oxygen atom in the morpholine ring of the inhibitors **1** and **6**. With this objective, we prepared the compounds **25a** and **25b** where oxygen atom in the morpholine ring is replaced with carbon atom. Synthesis of the compounds **25a** and **25b** is outlined in Scheme 6.

Commercially available methyl 2-fluoro-5-nitro benzoate (**21**) was treated with piperidine in THF to afford the compound **22**, which upon hydrogenation using H_2 in the presence of Pd/C gave the amine **23**. Compound **23** was coupled with *trans*-3-(thiophene-2-yl)acrylic acid (**4a**) or *trans*-3-(furan-2-yl)acrylic acid (**4b**) in the presence of EDAC and DMAP in

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1,2-dichloroethane to form the amides **24a** or **24b**. Basic hydrolysis of the ester methyl group present in compounds **24a** or **24b** afforded the acids **25a** or **25b**.

Compounds **25a–b** along with the intermediate esters, **24a–b** were all evaluated for their enzymatic activity (Table 1). Carboxylic acid derivatives **25a** (181 μM) and **25b** (463 μM) exhibited a decreased inhibition compared to corresponding morpholine analogues. The ester intermediates **24a** (92 μM) and **24b** (131 μM) also showed a similar decrease in activity compared to corresponding morpholine analogues.

We have also made a few derivatives of inhibitor **1** by incorporating different substituents such as a -CH_2OH (**26a**), -CHO (**27a**) or -$CONH_2$ (**28**) in the place of the carboxylic acid group of inhibitors **1**. Substitution with -CH_2OH and -CHO groups did not result in a major change in the activity (**26a**, IC_{50} = 73 μM and **27a**, IC_{50} = 77 μM), while substitution with -$CONH_2$ group resulted in a decrease in activity as compared to inhibitor **1** (**28**, IC_{50} = 105 μM) (Table 1). Derivatives of furan compound **6** incorporating substituents such as a -CH_2OH (**26b**) and -CHO (**27b**) in the place of the carboxylic acid group were also made. These compounds showed improved inhibition as compared to the parent furan compound, **6**. Compound **26b** showed an IC_{50} value of 111 mM, and compound **27b** showed an IC_{50} value of 107 μM. Synthesis of compounds **26a–b** and **27a–b** is outlined in Scheme 7.

Compounds **5a–b** were reduced using DIBAL in a mixture of anhydrous CH_2Cl_2 and THF to afford the alcohol derivatives **26a–b**. Oxidation of alcohols **26a–b** using PCC in anhydrous THF afforded the aldehydes, **27a–b**. Synthesis of amide **28** is outlined in Scheme 8. Compound **28** was prepared from the inhibitor **1** by treatment with $SOCl_2$, followed by the treatment of the acid chloride produced with ammonia.

Strikingly, the IC_{50} values determined for all of the active compounds are well below the previously measured Km value of 5.5 mM for SrtA binding to the LPXTG peptide [32]. This suggests that these inhibitors bind ∼1–2 orders of magnitude tighter than the LPXTG peptide and thus should be effective at blocking the enzymes activity in vivo.

3. Conclusions

In conclusion, we have discovered a novel class of small-molecule inhibitors of *Staphylococcus aureus* Sortase A. Inhibitors were screened for their activity against the enzyme using a FRET assay. Micromolar inhibitors of the enzyme are identified. We have carried out preliminary structure activity relationship studies that have resulted in the identification of inhibitors with improved activity. Regions of the molecular structure of the lead inhibitor that are critical for its activity have been determined by systematic SAR studies. Further SAR studies to refine the activity of the lead using the information gained from these studies as well as attempts to obtain high resolution inhibitor / Sortase A complex co-crystal structures are currently in progress.

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5. Experimental section

5.1. Fluorescence Resonance Energy Transfer (FRET) assay

To determine IC_{50} values for each potential inhibitor, we have implemented the previously published fluorescence resonance energy transfer (FRET) assay [32,33]. This FRET assay employs the use of the donor and quencher pair, EDANS and Dabcyl, respectively. We have purchased the peptide Dabcyl-QALPETGEE-EDANS from a commercial source. The EDANS fluorophore has an excitation wavelength, $\lambda_{ex,d}$ = 336 nm and an emission maximum, $\lambda_{em,d}$ = 490 nm. The fluorescence emission spectra for EDANS overlaps very well with the absorption spectra of Dabcyl, where Dabcyl has an absorption maximum, $\lambda_{max,a}$ = 472 nm. As such, when the two molecules are spatially close, as they are in the intact peptide, the efficiency of transfer from EDANS to Dabcyl is high. Upon excitation of EDANS little emission from the donor is observed because most of the energy from fluorescence is transferred to the dabcyl acceptor and lost through non radiative decay pathways. In contrast, upon cleavage the newly formed fragments will diffuse apart and the two peptide fragments will become spatially separated and the FRET efficiency will diminish. Thus, the emission from the EDANS fluorophore will precipitously increase. Because of these properties, this FRET pair has been successfully employed to monitor SrtA catalyzed peptide cleavage.

Typically, the EDANS fluorophore is excited at 350 nm and fluorescence from EDANS is observed at 495 nm over a period of time. However, to reduce the correction for the inner filter effect we have chosen to monitor emission at 590 nm, where the absorbance for either chromophore is insignificant. At the beginning of the observation, fluorescence is low due to a high FRET efficiency, but, as the peptide is cleaved the fluorescence increases linearly. The slope of this linear increase is taken to be the initial velocity, V_0. Because of the large inner filter correction at high concentrations of substrate, we have chosen to monitor the effect of inhibitors on V_0 at a select enzyme and substrate concentration, where the correction is negligible. These experiments are performed by mixing 5 μM SrtA with 20 μM Dabcyl-EDANS labelled peptide in a 0.5 cm cuvette. This mixture is excited at λ_{ex} = 350 nm and fluorescence is observed at λ_{em} = 590 nm. From this experiment, a time course of fluorescence increase as a function of time is acquired. The slope of this line is considered the maximum velocity in the absence of inhibitor. The identical experiment is then performed in the presence of increasing concentrations of inhibitor and the slopes are compared until a 50 % decrease in the slope is observed. The concentration of inhibitor where 50 % of this maximum velocity is observed is taken to be the IC_{50}. Each experiment was repeated at least three times to ensure the reproducibility and calculate standard deviation of the reported IC_{50} values.

5.2. General Methods for Synthesis

Solvent evaporations were carried out *in vaccuo* with a rotary evaporator. Analytical samples were prepared by drying the samples *in vaccuo* (0.2 mmHg) in an Abderhalden drying apparatus over P2O5 and ethyl acetate at reflux temperature. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator (Whatmann, silica gel, UV254, 25 εm plates). Spots were visualized by UV light (254 and

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365 nm). All analytical samples were single spots on TLC in at least two different solvent systems. Purification by column and flash chromatography was carried out using 'BAKER' silica gel (40 εm) in the solvent systems indicated. The amount (weight) of silica gel for column chromatography was in the range of 50–100 times the amount (weight) of the crude compounds being separated. Melting points were determined on a Mel-Temp II melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Brucker DPX 300 spectrometer using TMS as internal standard. The values of chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz. The chemical shift values are reported as parts per million (ppm) relative to tetramethylsilane as internal standard. Mass spectra were recorded on a MicroMass Platform LCC instrument. Elemental analyses were performed by Atlantic Microlab, Norcross, Georgia and the results indicated by symbols for the elements were within ± 0.4% of theoretical values. Anhydrous solvents used for reactions were purchased in Sure-Seal™ bottles from Aldrich Chemical Company. Other reagents were purchased from Aldrich or Fisher chemical companies and used as received.

Methyl 5-amino-2-morpholinobenzoate (3)—To a solution of Methyl 5-nitro-2-morpholinobenzoate, **2** (1.0 g, 3.76 mmol) in EtOAc (35 mL) 10% Pd/C (100 mg) was added and stirred under an atmosphere of H_2 from a balloon (∼1 atm) for 12 h. TLC analysis (1:1 EtOAc / $CHCl_3$) revealed that the reaction is complete. The catalyst was removed by filtration through a celite bed and the filtrate was concentrated in vacuum to furnish **3** as a solid. (0.88 g, 99 %); Mp 121 – 122 °C; ^{1}H NMR ($CDCl_3$) δ 2.91 – 2.97 (m, 4H), 3.60 (bs, 2H), 3.79 – 3.85 (m, 4H), 3.87 (s, 3H), 6.78 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.7 Hz), 6.95 (d, 1H, *J* = 8.7 Hz) and 7.05 (d, 1H, *J* = 2.7 Hz); ^{13}C NMR ($CDCl_3$) δ 51.7, 53.3, 67.1, 116.7, 118.8, 120.8, 127.0, 141.9, 143.7 and 168.0; MS (ES) *m/z* 237 (M + H); Anal Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.87; H, 6.81; N, 11.75.

Methyl 5-((*E*)-3-(thiophen-2-yl)acrylamido)-2-morpholinobenzoate (5a)—To a solution of **3** (0.430 g, 1.82 mmol) in 1,2-dichloroethane (20 mL) 3-(2-thienyl)acrylic acid **4a** (0.337 g, 2.18 mmol), EDAC (1.12 g, 5.82 mmol), and DMAP (0.022 g, 0.18 mmol) were added and stirred for 16 h at room temperature. TLC analysis (1:1 EtOAc / $CHCl_3$) revealed that the reaction is complete. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and the organic layer was washed with saturated $NaHCO_3$ solution (2 × 25 mL), water (1 × 25 mL), brine (1 × 25 mL) and dried over anhydrous Na_2SO_4. The drying agent was filtered off and the solvent was concentrated *in vacuo* to afford the crude product which was crystallized form a mixture of $CHCl_3$ and hexanes to furnish **5a** as a yellow solid. (0.62 g, 91%); Mp 196 – 197 °C; ^{1}H NMR ($CDCl_3$) δ 3.00 – 3.05 (m, 4H), 3.83 – 3.88 (m, 4H), 3.89 (s, 3H), 6.31 (d, 1H, *J* = 15.3 Hz), 7.03 – 7.08 (m, 2H), 7.24 – 7.28 (m, 2H), 7.33 – 7.38 (m, 1H), 7.80 (d, 1H, *J* = 8.7 Hz), 7.83 – 7.90 (m, 1H) and 7.92 (d, 1H, *J* = 2.3 Hz); ^{13}C NMR ($CDCl_3$) δ 52.2, 53.1, 67.2, 119.3, 119.8, 123.2, 124.6, 124.9, 127.9, 128.2, 130.9, 132.5, 135.1, 139.8, 149.0, 163.7 and 167.5; MS (ES) *m/z* 373 (M + H); Anal Calcd for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52. Found: C, 60.98; H, 5.39; N, 7.45.

Methyl 5-((*E*)-3-(furan-2-yl)acrylamido)-2-morpholinobenzoate (5b)—Compound **5b** was synthesized, following a similar procedure as the one used for preparation of **5a**

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starting from compound **3** (0.426 g, 1.8 mmol), 3-(2-furyl)acrylic acid **4b** (0.298 g, 2.16 mmol), EDAC (1.10 g, 5.76 mmol), and DMAP (0.022 g, 0.182 mmol) to obtain the pure product. (0.639 g, 99 %); Mp 172 – 173 °C; ^{1}H NMR ($CDCl_3$) δ 2.98–3.07 (m, 4H), 3.82–3.88 (m, 4H), 3.89 (s, 3H), 6.42 (d, 1H, *J* = 15.0 Hz), 6.48 (dd, 1H, J_1 = 1.8 Hz, J_2 = 3.3 Hz), 6.60 (d, 1H, *J* = 3.3 Hz), 7.04 (d, 1H, *J* = 9.0 Hz), 7.40 (bs, 1H), 7.46 (d, 1H, *J* = 1.5 Hz), 7.52 (d, 1H, *J* = 15.0 Hz), 7.82 (d, 1H, *J* = 7.2 Hz) and 7.91 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 52.1, 53.0, 67.1, 112.3, 114.4, 118.3, 119.6, 123.2, 124.6, 128.8, 132.7, 144.2, 148.8, 151.1 (2C), 164.1 and 167.6; MS (ES) *m/z* 357 (M + H); Anal Calcd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.66; N, 7.86. Found: C, 64.33; H, 5.63; N, 7.64.

5-((*E*)-3-(Thiophen-2-yl)acrylamido)-2-morpholinobenzoic acid (1)—To a solution of compound **5a** (0.3g, 0.81 mmol) in a mixture of THF (1.5 mL) and MeOH (1.5 mL), 3N. NaOH solution (2.5 mL) was added at room temperature and the resulting mixture was refluxed gently for 45 min. TLC analysis (1:1 EtOAc / $CHCl_3$) revealed that the reaction is complete. The solvent was completely removed *in vaccuo* and the residue obtained was taken up in water (3 mL). The resulting mixture was cooled to 0 °C and acidified to pH ∼2 by carefully adding 3N HCl (∼2.5 mL). The resulting white solid was filtered and dried under vacuum to furnish pure compound **1**. (0.283 g, 98%); Mp 278–279 °C; ^{1}H NMR (DMSO-d_6) δ 3.00–3.09 (m, 4H), 3.74–3.85 (m, 4H), 6.55 (d, 1H, *J* = 15.3 Hz), 7.14 (dd, 1H, J_1 = 3.6 Hz, J_2 = 5.1 Hz), 7.47 (d, 1H, *J* = 3.3 Hz), 7.67 (s, 1H), 7.68 (d, 1H, *J* = 3.0 Hz), 7.77 (d, 1H, *J* = 15.3 Hz), 7.99 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.7 Hz), 8.29 (d, 1H, *J* = 2.7 Hz) and 10.4 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 52.7, 66.3, 120.3, 120.9, 123.8, 124.0, 125.4, 128.5, 128.7, 131.6, 133.7, 137.8, 139.6, 145.2, 163.4 and 166.4; MS (ES) *m/z* 357 (M– H); Anal Calcd for $C_{18}H_{18}N_2O_4S$: C, 60.32; H, 5.06; N, 7.82. Found: C, 60.37; H, 5.14; N, 7.59.

5-((*E*)-3-(Furan-2-yl)acrylamido)-2-morpholinobenzoic acid (6)—Compound **6** was synthesized following a similar procedure as the one used for preparation of compound **1** starting from **5b** (0.200 g, 0.57 mmol) and 3N. NaOH solution to afford the pure product as a white solid. (0.192 g, 99%); Mp 281–282 °C; ^{1}H NMR (DMSO-d_6) δ 3.01–3.11 (m, 4H), 3.75–3.86 (m, 4H), 6.59 (d, 1H, *J* = 15.3 Hz), 6.60–6.69 (m, 1H), 6.87 (d, 1H, *J* = 3.6 Hz), 7.41 (d, 1H, *J* = 15.6 Hz), 7.68 (d, 1H, *J* = 9.0 Hz), 7.84 (d, 1H, *J* = 1.5 Hz), 8.00 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.7 Hz), 8.29 (d, 1H, *J* = 2.7 Hz) and 10.4 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 52.8, 66.4, 112.5, 115.0, 118.9, 120.9, 123.8, 124.0, 125.4, 127.8, 137.8, 145.2, 145.4, 150.8, 163.6 and 166.4; MS (ES) *m/z* 341 (M – H); Anal. ($C_{18}H_{18}N_2O_5$) C, H, N. Anal Calcd for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30; N, 8.18. Found: C, 63.06; H, 5.53; N, 7.89.

5-(3-(Thiophen-2-yl)propanamido)-2-morpholinobenzoic acid (7)—To a solution of compound **1** (0.071 g, 0.20 mmol) in a mixture of MeOH (4 mL) and EtOAc (3 mL), $HCOONH_4$ (0.188 g, 2.97 mmol) and Pd black (40 mg) were added and refluxed for 6 h. TLC analysis (1:1 EtOAc / $CHCl_3$) and mass spectrum revealed that the reaction was complete. The catalyst was removed by filtration through a celite bed, washed with a mixture of MeOH and $CHCl_3$, and the filtrate was concentrated *in vaccuo* to afford the crude product. The crude product was purified by washing thoroughly with water to remove the inorganic salts, filtered and dried under vacuum to furnish pure product **7**. (0.049 g, 69%);

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Mp 226 – 227 °C; ^{1}H NMR (DMSO-d_6) δ 2.68 (t, 2H, *J* = 7.5 Hz), 2.99 – 3.08 (m, 4H), 3.13 (t, 2H, *J* = 7.5 Hz), 3.74 – 3.86 (m, 4H), 6.86 – 6.97 (m, 2H), 7.30 (dd, 1H, J_1 = 1.0 Hz, J_2 = 4.9 Hz), 7.65 (d, 1H, *J* = 8.4 Hz), 7.90 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.7 Hz), 8.21 (d, 1H, *J* = 2.4 Hz) and 10.2 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 24.9, 38.0, 52.7, 66.3, 120.8, 123.7, 123.8, 123.9, 124.7, 125.2, 126.9, 137.7, 143.4, 145.0, 166.3 and 170.1; MS (ES) *m/z* 361 (M + H); Anal Calcd for $C_{18}H_{20}N_2O_4S$: C, 59.98; H, 5.59; N, 7.77. Found: C, 60.15; H, 5.75; N, 7.64.

Methyl 5-(3-(thiophen-2-yl)propanamido)-2-morpholinobenzoate (8)—To a solution of compound **5a** (0.100 g, 0.27 mmol) in MeOH (5 mL), $HCOONH_4$ (0.255 g, 4.05 mmol) and Pd black (0.05 g) were added and refluxed for 6 h. TLC analysis (1:1 EtOAc / $CHCl_3$) and mass spectrum revealed that the reaction is complete. The catalyst was removed by filtration through a celite bed, washed with $CHCl_3$, and the filtrate was concentrated *in vacuo*. The residue obtained was dissolved in CH_2Cl_2 (20 mL) and water (15 mL) was added. The organic layer was separated and washed with saturated $NaHCO_3$ solution (2 × 15 mL), water (2 × 15 mL), brine (1 × 15 mL) and dried (Na_2SO_4). The drying agent was filtered off and the solvent was removed *in vaccuo* to obtain the crude product. This crude product was purified by column chromatography over Si gel (4 × 2 cm) using 1:5 EtOAc / $CHCl_3$ as eluent to afford the pure compound **8**. (0.082 g, 82 %); Mp 115 – 116 °C; ^{1}H NMR ($CDCl_3$) δ 2.70 (t, 2H, *J* = 7.5 Hz), 2.97 – 3.05 (m, 4H), 3.27 (t, 2H, *J* = 7.2 Hz), 3.81 – 3.87 (m, 4H), 3.88 (s, 3H), 6.86 (dd, 1H, J_1 = 0.9 Hz, J_2 = 3.3 Hz), 6.90 – 6.96 (m, 1H), 7.01 (d, 1H, *J* = 9.0 Hz), 7.11 (bs, 1H), 7.14 (dd, 1H, J_1 = 1.2 Hz, J_2 = 5.1 Hz), 7.64 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.7 Hz) and 7.76 (d, 1H, *J* = 2.7 Hz); ^{13}C NMR ($CDCl_3$) δ 25.5, 38.9, 52.0, 52.9, 67.0, 119.5, 123.2, 123.4, 124.6, 124.7 (2C), 126.8, 132.3, 143.0, 148.6, 167.4 and 170.1. MS (ES) *m/z* 375 (M + H). Anal Calcd for $C_{19}H_{22}N_2O_4S$. 0.25H_2O: C, 60.26; H, 5.99; N, 7.40. Found: C, 60.27; H, 6.13; N, 7.22.

5-(3-(Furan-2-yl)propanamido)-2-morpholinobenzoic acid (9)—To a solution of compound **6** (0.075 g, 0.22 mmol) in EtOAc (15 mL) 10% Pd/C (0.02 g) was added and stirred under a hydrogen atmosphere from balloon (∼1 atm) for 25 min. TLC analysis (1:1 EtOAc: $CHCl_3$) and ^{1}H NMR of aliquot revealed that the reaction is complete. (Note: Prolonged reaction times lead to hydrogenation of furan ring). The catalyst was removed by filtration through a celite bed and the filtrate was concentrated *in vaccuo* to afford the crude product which was purified by column chromatography over Si gel (4 × 2 cm) using EtOAc as eluent to afford pure compound **9** as a white solid. (0.066 g, 88 %); Mp 230 – 231 °C; ^{1}H NMR (DMSO-d_6) δ 2.65 (t, 2H, *J* = 7.5 Hz), 2.93 (t, 2H, *J* = 7.3 Hz), 3.00–3.07 (m, 4H), 3.73–3.83 (m, 4H), 6.11 (dd, 1H, J_1 = 1.2 Hz, J_2 = 2.1 Hz), 6.34 (dd, 1H, J_1 = 1.8 Hz, J_2 = 3.0 Hz), 7.51 – 7.52 (m, 1H), 7.65 (d, 1H, *J* = 8.7 Hz), 7.89 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.7 Hz), 8.21 (d, 1H, *J* = 2.7 Hz) and 10.2 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 23.2, 34.5, 52.8, 66.4, 105.2, 110.4, 120.8, 123.8, 123.9, 125.3, 137.8, 141.5, 145.0, 154.4, 166.4 and 170.2; MS (ES) *m/z* 343 (M – H); Anal Calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13. Found: C, 62.50; H, 5.89; N, 8.01.

Methyl 5-(3-(furan-2-yl)propanamido)-2-morpholinobenzoate (10)—Compound **10** was synthesized, following procedure used for the preparation of compound **9** starting

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from compound **5b** (0.136 g, 0.38 mmol) in EtOAc (15 mL) and 10% Pd/C (0.02 g) under a hydrogen atmosphere. (0.106 g, 77 %); Mp 119 – 120 °C; ^{1}H NMR ($CDCl_3$) δ 2.69 (t, 2H, *J* = 7.5 Hz), 2.97–3.03 (m, 4H), 3.07 (t, 2H, *J* = 7.3 Hz), 3.81–3.87 (m, 4H), 3.88 (s, 3H), 6.07 (d, 1H, *J* = 3.3 Hz), 6.27–6.32 (m, 1H), 7.01 (d, 1H, *J* = 9.0 Hz), 7.16 (bs, 1H), 7.32 (d, 1H, *J* = 1.2 Hz), 7.65 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.7 Hz) and 7.78 (d, 1H, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 23.7, 35.4, 52.0, 52.9, 67.1, 105.5, 110.2, 119.5, 123.2, 124.6, 124.7, 132.3, 141.1, 148.7, 154.0, 167.5 and 170.3; MS (ES) *m/z* 357 (M – H). Anal Calcd for $C_{19}H_{22}N_2O_5.0.25H_2O$: C, 62.92; H, 6.25; N, 7.72. Found: C, 62.60; H, 6.13; N, 7.59.

Methyl 5-(3-(thiophen-2-yl)propiolamido)-2-morpholinobenzoate (12)— Compound **12** was prepared similar to compound **5a** by the treatment of methyl 4-amino-2-(4-morpholino)-benzoate **3** (0.542 g, 2.3 mmol) and 3-(thiophen-2-yl)propiolic acid, **11** (0.428 g, 2.8 mmol) in the presence of EDAC (1.42 g, 7.4 mmol) and a DMAP (0.028 g, 0.23 mmol). The crude product was purified by flash chromatography over Si gel using 10% EtOAc in $CHCl_3$ as eluent to afford compound **12** as oil. (0.407 g, 37%); ^{1}HNMR ($CDCl_3$) δ 3.03 (t, 4H, *J* = 4.5 Hz), 3.84 – 3.89 (m, 7H), 7.03 – 7.07 (m, 2H), 7.44 – 7.46 (m, 2H), 7.65 (bs, 1H), 7.77 (dd, 1H, J_1 = 2.7 Hz, J_2 = 9 Hz), 7.86 (d, 1H, *J* = 2.7 Hz); ^{13}CNMR ($CDCl_3$) δ 52.3, 52.9, 67.1, 79.9, 87.4, 119.7, 123.3, 124.6, 124.8, 127.5 (2C), 130.5, 131.9, 135.7, 149.2, 151.1, 167.5; MS (ES+) *m/z* 371 (M+H); Anal Calcd for $C_{19}H_{18}N_2O_4S$: C, 61.61; H, 4.90; N, 7.56. Found: C, 61.47; H, 4.88; N, 7.30.

5-(3-(Thiophen-2-yl)propiolamido)-2-morpholinobenzoic acid (13)—Compound **13** was prepared similar to compound **1** by hydrolysis of **12** (0.049 g, 0.13 mmol) using 1N. NaOH in a MeOH / THF (4 mL, 1:1) medium to afford (0.03 g; 64%) as a white solid. Mp. 232 °C (decomp.); ^{1}HNMR (DMSO-d_6) δ 3.04 (t, 4H, *J* = 4.5 Hz), 3.79 (t, 4H, *J* = 4.5 Hz), 7.21 (dd, 1H, J_1 = 3.6 Hz, J_2 = 5.1 Hz), 7.64 – 7.67 (m, 2H), 7.85 – 7.89 (m, 2H), 8.28 (d, 1H, *J* = 2.7 Hz) and 11.08 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 52.6, 66.4, 78.9, 87.8, 118.7, 121.4, 123.7, 124.5, 125.5, 128.3, 132.2, 136.3, 136.7, 146.0, 150.3, 166.4; MS (ES^+) *m/z* 357 (M+H); Anal Calcd for $C_{18}H_{16}N_2O_4S.0.25H_2O$: C, 59.90; H, 4.61; N, 7.76. Found: C, 59.89; H, 4.56; N, 7.62.

(*Z*)-Methyl 5-(-3-(thiophen-2-yl)acrylamido)-2-morpholinobenzoate (14)—To a solution of **12** (0.042 g, 0.11 mmol) in EtOAc (6 mL) 10% Pd/C (0.02 g) was added and stirred under H_2 atmosphere for 30 min. TLC examination (1:9 EtOAc / $CHCl_3$) showed that the reaction is complete. Catalyst was filtered off through a bed of celite. The filtrate was concentrated *in vaccuo* to obtain the crude product which was purified by flash chromatography over Si gel using 5% EtOAc in $CHCl_3$) to yield compound **14** as a yellow gum. (0.035 g, 82%); ^{1}HNMR ($CDCl_3$) δ 2.99 – 3.02 (m, 4H), 3.83 – 3.88 (m, 7H), 5.78 (d, 1H, *J* = 12.6 Hz), 6.99 – 7.06 (m, 3H), 7.38 – 7.39 (m, 1H), 7.46 – 7.48 (m, 2H), 7.83 (m, 2H). ^{13}CNMR ($CDCl_3$) δ 52.2, 53.0, 67.1, 117.1, 199.7, 123.3, 124.8, 124.9, 126.6, 131.7, 132.4, 133.9, 134.8, 137.6, 148.9, 164.4, 167.6. MS (ES^+) *m/z* 373 (M+H). Anal Calcd for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52. Found: C, 61.28; H, 5.50; N, 7.50.

(*Z*)-5-(3-(thiophen-2-yl)acrylamido)-2-morpholinobenzoic acid (15)—Compound **15** was prepared similar to compound **1** by hydrolysis of compound **14** (0.029 g, 0.08 mmol)

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using 1N. NaOH in a MeOH / THF (2 mL, 1:1) to afford as a white solid. (0.018 g, 65% yield); Mp 190 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ 3.06 (bs, 4H), 3.80 (bs, 4H), 6.02 (d, 1H, *J* = 12.3 Hz), 7.08 – 7.11 (m, 1H), 7.19 (d, 1H, *J* = 12.6 Hz), 7.52 (d, 1H, *J* = 3.0 Hz), 7.68 – 7.71 (m, 2H), 7.98 (dd, 1H, J_1 = 2.1 Hz, J_2 = 8.7 Hz), 8.38 (d, 1H, *J* = 2.1 Hz), 10.59 (s, 1H); ^{13}CNMR (DMSO-d_6) δ 53.6, 67.2, 117.9, 121.7, 124.6, 124.9, 126.1, 127.5, 133.5, 134.7, 136.6, 138.5, 138.7, 145.9, 165.2, 167.3; MS (ES+) *m/z* 359 (M+H); HRMS Calcd for $C_{18}H_{18}N_2O_4S$: 358.0987. Found: 358.0980.

(*E*)-Methyl 5-(N-methyl-3-(thiophen-2-yl)acrylamido)-2-morpholinobenzoate (16a)—To a solution of compound **5a** (0.04 g, 0.11 mmol) in anhydrous THF (10 mL), NaH (0.013 g, 60 %; 0.33 mmol) was added and stirred at 0 °C under a N_2 atm for 30 min. Iodomethane (0.03 mL, 0.4 mmol) was added to the reaction mixture and the solution was stirred for 20 min. at rt. MS analysis confirmed product formation (*m/z* = 387). The reaction was quenched with aqueous $NaHCO_3$ (1M, 20 mL). It was extracted with EtOAc (3 × 25 mL), washed with water (2 × 25 mL) and brine (25 mL), and dried over Na_2SO_4. The drying agent was filtered off and the filtrate was concentrated *in vaccuo* to afford the crude product which was purified by flash chromatography over Si gel (10 × 2 cm) using EtOAc / hexanes (1:1) as eluent to afford compound **16a** as a colorless oil. (0.043 g, 100%); ^{1}HNMR ($CDCl_3$) δ 3.09 – 3.12 (m, 4H), 3.36 (s, 3H), 3.87 – 3.91 (m, 7H), 6.16 (d, 1H, *J* = 15.3 Hz), 6.98 (dd, 1H, J_1 = 3.6 Hz, J_2 = 5.1 Hz), 7.07 (d, 1H, *J* = 8.4 Hz), 7.15 (d, 1H, *J* = 3.6 Hz), 7.25 – 7.29 (m, 2H), 7.66 (d, 1H, *J* = 2.7 Hz), 7.76 – 7.81 (d, 1H, *J* = 15 Hz); ^{13}CNMR ($CDCl_3$) δ 37.6, 52.3, 52.7, 66.9, 117.2, 119.7, 124.4, 127.4, 127.9, 130.1, 130.5, 131.8, 134.7, 136.9, 140.3, 151.4, 165.9, 167.1; MS (ES+) *m/z* 387 (M+H); Anal Calcd for $C_{20}H_{22}N_2O_4S.0.5C_6H_{14}$: C, 64.31; H, 6.80; N, 6.52 Found: C, 64.19; H, 6.56; N, 6.53.

(*E*)-Methyl 5-(3-(furan-2-yl)-N-methylacrylamido)-2-morpholinobenzoate (16b)—Compound **16b** was prepared in a similar fashion to compound **16a** using compound **5b** (0.048 g, 0.13 mmol), NaH (0.016 g, 60 %; 0.4 mmol) and iodomethane (0.03 mL, 0.48 mmol) to afford (0.044 g, 88% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.10 – 3.12 (m, 4H), 3.36 (s, 3H), 3.88 – 3.91 (m, 7H), 6.23 (d, 1H, *J* = 15.1 Hz), 6.39 – 6.41 (m, 1H), 6.51 (d, 1H, *J* = 3.4 Hz), 7.07 (d, 1H, 8.7 Hz), 7.28 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.7 Hz), 7.35 (d, 1H, *J* = 1 Hz), 7.45 (d, 1H, *J* = 15.2 Hz), 7.66 (d, 1H, *J* = 2.7 Hz); ^{13}C NMR ($CDCl_3$) δ 38.0, 52.7, 53.1, 67.4, 112.5, 114.4, 116.3, 120.1, 124.8, 129.2, 130.99, 132.3, 137.4, 144.4, 151.8, 151.9, 166.6, 167.5; MS (ES+) *m/z* 371 (M+H); Anal Calcd for $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.27; H, 6.04; N, 7.39.

(*E*)-5-(N-methyl-3-(thiophen-2-yl)acrylamido)-2-morpholinobenzoic acid (17a)—Compound **17a** was prepared similar to compound **1** by hydrolysis of **16a** (0.035 g, 0.09 mmol) using 1N. NaOH in a MeOH / THF (2 mL, 1:1) to yield (0.026 g, 78%) as a white solid; Mp 220 °C (decomp.); ^{1}HNMR ($CDCl_3$) δ 3.14 (bs, 4H), 3.42 (s, 3H), 3.99 (bs, 4H), 6.14 (d, 1H, *J* = 15.3 Hz), 6.99 (dd, 1H, J_1 = 3.6 Hz, J_2 = 5 Hz), 7.18 (d, 1H, *J* = 3.1 Hz), 7.26 – 7.29 (m, 1H), 7.48 – 7.54 (m, 2H), 7.82 (d, 1H, *J* = 15.1 Hz), 8.20 (d, 1H, *J* = 2.6 Hz); ^{13}CNMR ($CDCl_3$) δ 37.9, 54.0, 67.2, 117.2, 124.2, 127.1, 128.1, 128.5, 130.8, 130.9, 133.3, 135.9, 140.4, 143.5, 149.1, 166.1, 166.2; MS (ES+) *m/z* 373 (M+H); Anal Calcd for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52. Found: C, 61.34; H, 5.54; N, 7.34.

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(*E*)-5-(3-(furan-2-yl)-N-methylacrylamido)-2-morpholinobenzoic acid (17b)— Compound **17b** was prepared similar to compound **1** by hydrolysis of **16b** (0.035 g, 0.095 mmol) using 1N. NaOH in a MeOH / THF (2 mL, 1:1) to afford (0.033 g; 97%) as oil; ^{1}HNMR ($CDCl_3$) δ 3.05–3.15 (m, 4H), 3.42 (s, 3H), 3.90–4.02 (m, 4H), 6.24 (d, 1H, J = 14.7 Hz), 6.42 (dd, 1H, J_1 = 1.8 Hz, J_2 = 3.4 Hz), 6.55 (d, 1H, J = 3.4 Hz), 7.35 (s, 1H), 7.47 – 7.54 (m, 3H), 8.20 (d, 1H, J = 2.9 Hz); ^{13}CNMR ($CDCl_3$) δ 38.0, 54.0, 67.2, 112.7, 115.1, 115.7, 124.3, 127.1, 130.1, 130.9, 133.4, 143.5, 144.6, 149.1, 151.7, 166.2, 166.4; MS (ES+) *m/z* 357 (M+H); Anal Calcd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.66; N, 7.86. Found: C, 63.84; H, 6.06; N, 7.56.

(*E*)-Methyl 5-(3-(thiophen-2-yl)allylamino)-2-morpholinobenzoate (19)—To a solution of (*E*)-3-(thiophen-2-yl)acrylaldehyde, **18** (0.74 g, 5.32 mmol) in MeOH (30 mL). Methyl 5-amino-2-(4-morpholino)-benzoate **3** (1.25 g, 5.32 mmol) was added and the mixture stirred at rt for 30 min. A solution of $ZnCl_2$ (0.362 g, 2.66 mmol) and $NaCNBH_3$ (0.367 g, 5.85 mmol) in MeOH (20 mL) was added to the reaction mixture and stirred at rt for 24h. The reaction was then quenched with water (20 mL) and solvents were completely evaporated *in vaccuo*. The crude residue obtained was dissolved in CH_2Cl_2 (60 mL), washed with water (3 × 50 mL) and brine (1 × 50 mL), and dried over anhydrous Na_2SO_4. Drying agent was filtered off and the filtrate was concentrated to afford the clean product **19** (1.68 g, 88% yield); Mp 131 °C; ^{1}H NMR ($CDCl_3$) δ 2.94 (t, 4H, J = 4.5 Hz), 3.82 (t, 4H, J = 4.5 Hz), 3.87 – 3.89 (m, 5H), 6.06 – 6.19 (m, 1H), 6.70 – 6.76 (m, 2H), 6.93 – 7.01 (m, 4H), 7.14 (d, 1H, J = 5.1 Hz); ^{13}C NMR ($CDCl_3$) δ 46.2, 52.0, 53.7, 67.5, 114.9, 116.8, 121.3, 124.2, 124.8, 125.6, 126.4, 127.4, 127.6, 141.9, 143.3, 143.7, 168.5; MS (ES+) *m/z* 359 (M +H); Anal Calcd for $C_{19}H_{22}N_2O_3S.0.25H_2O$: C, 62.87; H, 6.25; N, 7.72. Found: C, 62.74; H, 6.13; N, 7.52.

(*E*)-5-(3-(thiophen-2-yl)allylamino)-2-morpholinobenzoic acid (20)—Compound **20** was prepared similar to compound **1** by hydrolysis of **19** (1.0 g, 2.79 mmol) using 1N. NaOH in a MeOH / THF (14 mL, 1:1) to yield (0.745 g, 78% yield) as a white solid; Mp 208 °C; ^{1}H NMR ($CDCl_3$) δ 2.92–3.17 (m, 4H), 3.84–4.0 (m, 6H), 6.12 (dt, 1H, J_1 = 15.9 Hz, J_2 = 5.7 Hz), 6.73 (d, 1H, J = 16.5 Hz), 6.82 (dd, 1H, J_1 = 8.7 Hz, J_2 = 3.0 Hz), 6.92–6.96 (m, 2H), 7.15 (d, 1H, J = 4.8 Hz), 7.23 (d, 1H, J = 8.7 Hz), 7.52 (d, 1H, J = 3.0 Hz); ^{13}C NMR ($CDCl_3$) δ 45.6, 53.7, 67.0, 115.0, 117.5, 123.3, 124.3, 125.1, 125.6, 125.75, 125.8, 127.4, 139.5, 141.7, 147.1, 167.5; MS (ES) *m/z* 345 (M+H); Anal. Calc for $C_{18}H_{20}N_2O_3S$: C, 62.77; H, 5.85; N, 8.13. Found: C, 62.31; H, 5.83; N, 8.02.

Methyl 5-nitro-2-(piperidin-1-yl)benzoate (22)—To a solution of Methyl 2-fluoro-5-nitrobenzoate, **21** (0.082 g, 0.41 mmol) and triethylamine (0.11 mL, 0.82 mmol) in THF, piperidine (0.054 g, 0.64 mmol) was added and the reaction mixture was stirred at rt for 30 min. TLC (25% EtOAc in $CHCl_3$) examination revealed the completion of the reaction. The solvent was completely removed and the residue was dissolved in $CHCl_3$ (20 mL), washed with water (2 × 15 mL) and brine (15 mL), and dried over anhydrous Na_2SO_4. Drying agent was filtered off and the filtrate was concentrated to afford clean compound **22** (0.115 g, 100%) as a yellow gum. ^{1}HNMR ($CDCl_3$) δ 1.69 – 1.72 (m, 6H), 3.23 – 3.26 (m, 4H), 3.93 (s, 3H), 6.95 (d, 1H, J = 9.3 Hz), 8.15 (dd, 1H, J_1 = 2.7 Hz, J_2 = 9.3 Hz), 8.55 (d, 1H, J = 2.7

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Hz); ^{13}CNMR ($CDCl_3$) δ 23.8, 25.6, 52.4, 52.6, 117.2, 119.5, 127.7, 128.8, 138.2, 156.3, 167.0; MS (ES+) m/z 265 (M+H); Anal. Calcd. for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.60. Found: C, 58.90; H, 6.29; N, 10.25.

Methyl 5-amino-2-(piperidin-1-yl)benzoate (23)—To a solution of compound **22** (0.102 g, 0.38 mmol) in EtOAc (5 mL) was added 10% Pd/C (37 mg) and stirred under a hydrogen atmosphere (∼1 atm) for 2 h. TLC analysis (25% EtOAc / $CHCl_3$) revealed that the reaction is complete. The catalyst was filtered off on a celite bed and the filtrate was concentrated on vacuum to furnish compound **23** (0.083 g, 93%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.51 – 1.53 (m, 2H), 1.64 – 1.69 (m, 4H), 2.84 – 2.87 (m, 4H), 3.58 (bs, 2H), 3.87 (s, 3H), 6.74 (dd, 1H, J_1 = 3 Hz, J_2 = 8.7 Hz), 6.92 (d, 1H, J = 8.4 Hz), 7.02 (d, 1H, J = 3 Hz); ^{13}C NMR ($CDCl_3$) δ 24.2, 26.5, 51.9, 54.7, 117.3, 119.2, 120.9, 126.7, 141.0, 145.7, 168.8; MS (ES+) m/z 235 (M+H); Anal. Calcd. for $C_{13}H_{18}N_2O_2$: C, 66.64; H, 7.74; N, 11.96; Found C, 67.01; H, 7.59; N, 11.13.

(*E*)-Methyl 5-(3-(thiophen-2-yl)acrylamido)-2-(piperidin-1-yl)benzoate (24a)— Compound **24a** was prepared similar to compound **5a** by the treatment of **23** (0.066 g, 0.28 mmol) and 3-(thiophen-2-yl)acrylic acid, **4a** (0.052 g, 0.34 mmol) in the presence of EDAC (0.162 g, 0.84 mmol) and a DMAP (0.005 g, 0.041 mmol) to yield (0.064 g, 62%) as a yellow gum. ^{1}H NMR ($CDCl_3$) δ 1.49 – 1.55 (m, 2H), 1.64 – 1.71 (m, 4H), 2.91 (t, 4H, J = 5.1 Hz), 3.85 (s, 3H), 6.43 (d, 1H, J = 15.3 Hz), 6.93 – 7.01 (m, 2H), 7.15 (d, 1H, J = 3.3 Hz), 7.28 (d, 1H, J = 6 Hz), 7.79 – 7.87 (m, 3H), 8.28 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 24.2, 26.2, 52.1, 54.1, 119.6, 119.9, 123.2, 124.2, 124.7, 127.6, 128.0, 130.5, 131.7, 134.5, 139.9, 150.1, 164.0, 168.4; MS (ES^+) *m/z* 371 (M+H); HRMS Calcd for $C_{20}H_{22}N_2O_3S$: 370.1351. Found: 370.1341.

(*E*)-Methyl 5-(3-(furan-2-yl)acrylamido)-2-(piperidin-1-yl)benzoate (24b)— Compound **24b** was prepared similar to compound **5a** by the treatment of **23** (0.068 g, 0.29 mmol) and 3-(2-furyl)acrylic acid, **4b** (0.047 g, 0.34 mmol) in the presence of EDAC (0.14 g, 0.73 mmol) and a DMAP (0.005 g, 0.041 mmol) to yield (0.081 g, 78% yield) as a yellow gum. ^{1}H NMR ($CDCl_3$) δ 1.54 – 1.58 (m, 2H), 1.66 – 1.73 (m, 4H), 2.92 – 2.96 (m, 4H), 3.88 (s, 3H), 6.44 – 6.49 (m, 2H), 6.56 (d, 1H, J = 3.3 Hz), 6.98 (d, 1H, J = 9.3 Hz), 7.43 (d, 1H, J = 1.8 Hz), 7.50 (d, 1H, J = 15.3 Hz), 7.76 – 7.8 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 24.6, 26.7, 52.7, 54.5, 112.6, 114.6, 119.2, 119.9, 123.6, 124.5, 125.1, 128.9, 132.2, 144.6, 150.4, 151.7, 164.6, 168.9; MS (ES+) m/z 355 (M+H); Anal Calcd for $C_{20}H_{22}N_2O_4$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.90; H, 6.50; N, 7.70.

(*E*)-5-(3-(thiophen-2-yl)acrylamido)-2-(piperidin-1-yl)benzoic acid (25a)— Compound **25a** was prepared similar to compound **1** by hydrolysis of **24a** (0.056 g, 0.15 mmol) using 1N. NaOH in a MeOH / THF (2 mL, 1:1) to afford (0.048 g; 89%) as a white solid. Mp 239 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ 1.61–1.63 (m, 2H), 1.75 (bs, 4H), 3.02 – 3.04 (m, 4H), 6.56 (d, 1H, J = 15.6 Hz), 7.15 (t, 1H, J = 3.6 Hz), 7.48 (d, 1H, J = 3.3 Hz), 7.67 – 7.79 (m, 3H), 8.01 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.7 Hz), 8.29 (d, 1H, J = 2.4 Hz), 10.44 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 22.6, 26.0, 54.2, 120.8, 121.1, 124.1, 124.2, 125.9, 128.9,

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129.2, 132.0, 134.1, 138.8, 140.0, 145.5, 163.9, 167.0; MS (ES^+) *m/z* 357 (M+H); Anal Calcd for $C_{19}H_{20}N_2O_3S$: C, 64.02; H, 5.66; N, 7.86. Found: C, 63.69; H, 5.67; N, 7.62.

(*E*)-5-(3-(furan-2-yl)acrylamido)-2-(piperidin-1-yl)benzoic acid (25b)—Compound **25b** was prepared similar to compound **1** by hydrolysis of **24b** (0.08 g, 0.23 mmol) using 1N. NaOH in MeOH / THF (2 mL, 1:1) to afford (0.078 g; 100%) as oil; ^{1}H NMR (CD_3OD) δ 1.82 – 2.07 (m, 6H), 3.66 – 3.73 (m, 4H), 6.55 – 6.75 (m, 3H), 7.47 (d, 1H, *J* = 14.4 Hz), 7.63 (s, 1H), 7.89 – 8.16 (m, 2H), 8.59 (bs, 1H); ^{13}C NMR (CD_3OD) δ 22.9, 26.8, 58.6, 114.0, 116.7, 119.4, 124.2, 124.5, 126.9, 130.9, 140.6, 142.4, 146.9 (2C), 152.9, 167.3 (2C); MS (ES+) m/z 341 (M+H); HRMS Calcd for $C_{19}H_{20}N_2O_4$: 340.1423. Found: 340.1413.

(*E*)-N-(3-(Hydroxymethyl)-4-morpholinophenyl)-3-(thiophen-2-yl)acrylamide (26a)—To a solution of compound **5a** (0.500 g, 1.34 mmol) in a mixture of anhydrous CH_2Cl_2 (35 mL) and THF (5 mL), DIBAL-H (6.7 mL, 1M in CH_2Cl_2, excess) was added and stirred at 0 °C for 1 h. Then it was allowed to attain room temperature at which it was stirred for an additional 2 h. TLC analysis (1:1 EtOAc / $CHCl_3$) revealed that the reduction is complete. The reaction mixture was cooled to 0 °C and was quenched by careful addition of MeOH (3 mL) and 1N. HCl (0.5 mL). The solvent was removed under reduced pressure and the residue obtained was dissolved in $CHCl_3$ (300 mL) and washed with water (2 × 75 mL), brine (1 × 150 mL) and dried (Na_2SO_4). The drying agent was filtered off and the solvent was removed *in vaccuo* to afford the crude product which was purified by flash column chromatography on silica gel (20 × 3 cm) using EtOAc / hexanes (3 : 1) as eluent to furnish compound **26a** as a yellow solid. (0.354 g, 77 %); Mp 184 – 185 °C; ^{1}H NMR (DMSO-d_6) δ 2.79 – 2.83 (m, 4H), 3.65 – 3.75 (m, 4H), 4.54 (d, 2H, *J* = 5.1 Hz), 5.12 (t, 1H, *J* = 5.4 Hz), 6.58 (d, 1H, *J* = 15.0 Hz), 7.02 (d, 1H, *J* = 8.7 Hz), 7.13 (dd, 1H, J_1 = 3.6 Hz, J_2 = 5.1 Hz), 7.42 (d, 1H, *J* = 3.3 Hz), 7.60–7.74 (m, 4H) and 10.1 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 52.7, 58.5, 66.6, 118.1, 119.0, 119.1, 121.1, 128.3, 128.4, 131.1, 132.7, 135.0, 137.1, 139.8, 145.6 and 162.9; MS (ES) *m/z* 343 (M – H); Anal Calcd for $C_{18}H_{20}N_2O_3S$: C, 62.77; H, 5.85; N, 8.13. Found: C, 62.78; H, 5.95; N, 8.08.

(*E*)-3-(Furan-2-yl)-N-(3-(hydroxymethyl)-4-morpholinophenyl)acrylamide (26b)—Compound **26b** was synthesized, following the procedure used for preparation of **26a**, by the reaction of **5b** (0.500 g, 1.40 mmol) and DIBAL-H (7.02 mL, 1M in CH_2Cl_2) in CH_2Cl_2 (35 mL) and THF (5 mL) for 2 h at room temperature to furnish pure product. (0.372 g, 81 %); Mp 182 – 183 °C; ^{1}H NMR (DMSO – d_6) δ 2.74–2.83 (m, 4H), 3.65–3.74 (m, 4H), 4.54 (d, 2H, *J* = 5.1 Hz), 5.12 (t, 1H, *J* = 5.4 Hz), 6.58–6.67 (m, 2H), 6.82 (d, 1H, *J* = 3.3 Hz), 7.02 (d, 1H, *J* = 8.7 Hz), 7.35 (d, 1H, *J* = 15.3 Hz), 7.61–7.71 (m, 2H), 7.81 (d, 1H, *J* = 1.5 Hz) and 10.1 (bs, 1H); ^{13}C NMR (DMSO – d_6) δ 52.6, 58.5, 66.6, 112.5, 114.3, 118.2, 119.0, 119.1, 119.6, 126.8, 134.9, 137.0, 145.0, 145.5, 150.9 and 163.0; MS (ES) *m/z* 327 (M – H); Anal Calcd for $C_{18}H_{20}N_2O_4$: C, 65.84; H, 6.14; N, 8.53. Found: C, 65.55; H, 6.27; N, 8.37.

(*E*)-N-(3-Formyl-4-morpholinophenyl)-3-(thiophen-2-yl)acrylamide (27a)—To a solution of compound **26a** (0.500 g, 0.44 mmol) in anhydrous THF at 0 °C, pyridinium chlorochromate (0.188 g, 0.87 mmol) was added and stirred for 30 min and then at room

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temperature for 1 h. TLC analysis (1:1 EtOAc / $CHCl_3$) revealed that the reaction was complete. The solvent was removed under vacuum and the crude product obtained was purified by column chromatography over Si gel using EtOAc/$CHCl_3$ (1:4) as the eluent to afford pure **27a**. (0.107 g, 72 %); Mp 218 – 219 °C; ^{1}H NMR (DMSO) δ 2.94–3.03 (m, 4H), 3.72–3.82 (m, 4H), 6.53 (d, 1H, *J* = 15.3 Hz), 7.11–7.17 (m, 1H), 7.25 (d, 1H, *J* = 8.7 Hz), 7.45 (d, 1H, *J* = 3.3 Hz), 7.66 (d, 1H, *J* = 5.1 Hz), 7.74 (d, 1H, *J* = 15.6 Hz), 7.88 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.7 Hz), 8.06 (d, 1H, *J* = 2.7 Hz), 10.2 (s, 1H) and 10.3 (bs, 1H); ^{13}C NMR (CD_3COCD_3) δ 55.3, 67.4, 120.1, 121.0, 121.4, 126.8, 128.7, 129.1, 130.1, 131.7, 134.6, 136.1, 141.0, 152.5, 164.2 and 191.0; MS (ES) *m/z* 341 (M – H); Anal Calcd for $C_{18}H_{18}N_2O_3S$: C, 63.14; H, 5.30; N, 8.18. Found: C, 63.39; H, 5.24; N, 8.00.

(*E*)-N-(3-Formyl-4-morpholinophenyl)-3-(furan-2-yl)acrylamide (27b)—27b was synthesized, following the procedure used for preparation of **27a**, by the reaction of **26b** (0.188 g, 0.57 mmol) and pyridinium chlorochromate (0.247 g, 1.15 mmol) in anhydrous THF (10 mL) to furnish the pure product. (0.115 g, 61 %); Mp 231 – 232 °C; ^{1}H NMR (DMSO-d_6) δ 2.94–3.03 (m, 4H), 3.73–3.82 (m, 4H), 6.53–6.65 (m, 2H), 6.86 (d, 1H, *J* = 3.6 Hz), 7.24 (d, 1H, *J* = 9.0 Hz), 7.38 (d, 1H, *J* = 15.6 Hz), 7.82 (d, 1H, *J* = 1.5 Hz), 7.89 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.8 Hz), 8.06 (d, 1H, *J* = 2.7 Hz), 10.2 (s, 1H) and 10.3 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 54.1, 66.2, 112.6, 114.8, 119.0, 119.1, 120.2, 126.0, 127.4, 128.2, 134.6, 145.3, 150.9, 151.2, 163.4 and 190.7; MS (ES) *m/z* 325 (M – H); Anal Calcd for $C_{18}H_{18}N_2O_4$. 0.3H_2O: C, 65.20; H, 5.65; N, 8.45. Found: C, 64.90; H, 5.35; N, 8.33.

5-((*E*)-3-(Thiophen-2-yl)acrylamido)-2-morpholinobenzamide (28)—To a solution of compound **1** (0.050 g, 0.14 mmol) in anhydrous CH_2Cl_2 (4 mL) at room temperature a mixture of thionyl chloride (0.2 mL) and anhydrous DMF (0.2 mL) was added and the resulting mixture was stirred for 4 h at room temperature. The solvent was removed under reduced pressure. The resulting gum was dissolved in anhydrous CH_2Cl_2 (3 mL) and excess 50% aqueous ammonium hydroxide (3 mL) was added to achieve basic pH (∼12) and stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the crude compound was purified by flash column chromatography (20 × 1 cm) on silica gel using EtOAc / $CHCl_3$ (1:1) as eluent to furnish the pure amide **28**. (0.013 g, 26 %); ^{1}H NMR (DMSO-d_6) δ 2.86–2.94 (m, 4H), 3.71–3.78 (m, 4H), 6.55 (d, 1H, *J* = 15.3 Hz), 7.14 (dd, 1H, J_1 = 3.6 Hz, J_2 = 5.1 Hz), 7.20 (d, 1H, *J* = 8.7 Hz), 7.45 (d, 1H, *J* = 3.0 Hz), 7.57 (bs, 1H), 7.66 (d, 1H, *J* = 5.1 Hz), 7.73 (d, 1H, *J* = 15.6 Hz), 7.84 (dd, 1H, J_1 = 2.7 Hz, J_2 = 8.7 Hz), 7.99 (d, 1H, *J* = 2.7 Hz), 8.64 (bs, 1H) and 10.2 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 52.9, 66.4, 120.4, 120.8, 121.1, 122.0, 128.5 (2C), 129.3, 131.3, 133.1, 135.1, 139.7, 146.2, 163.1 and 167.7; MS (ES) *m/z* 356 (M – H).

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Abbreviations

Dabsyl	4-(4-Dimethylaminophenylazo)-benzenesulfonyl
DIBAL	Diisobutylaluminum hydride
DMAP	*N, N*-dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
EDAC	Ethyl (*N, N*-dimethylaminopropyl) carbodiimide
EDANS	5-[(2-aminoethyl)amino]naphthalene-1-sulfonyl
FRET	Fluorescence Resonance Energy Transfer
IC_{50}	Half maximal inhibitory concentration of inhibitor
MRSA	Methicillin resistant *Staphylococcus aureus*
MS	Mass spectrometry
PCC	Pyridinium chlorochromate
Pd black	Palladium black
Pd/C	Palladium on activated carbon
ppm	Parts per million
SAR	Structure activity relationship
S. aureus	*Staphylococcus aureus*
SrtA	Sortase A
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin layer chromatography
TMS	Tetramethylsilane

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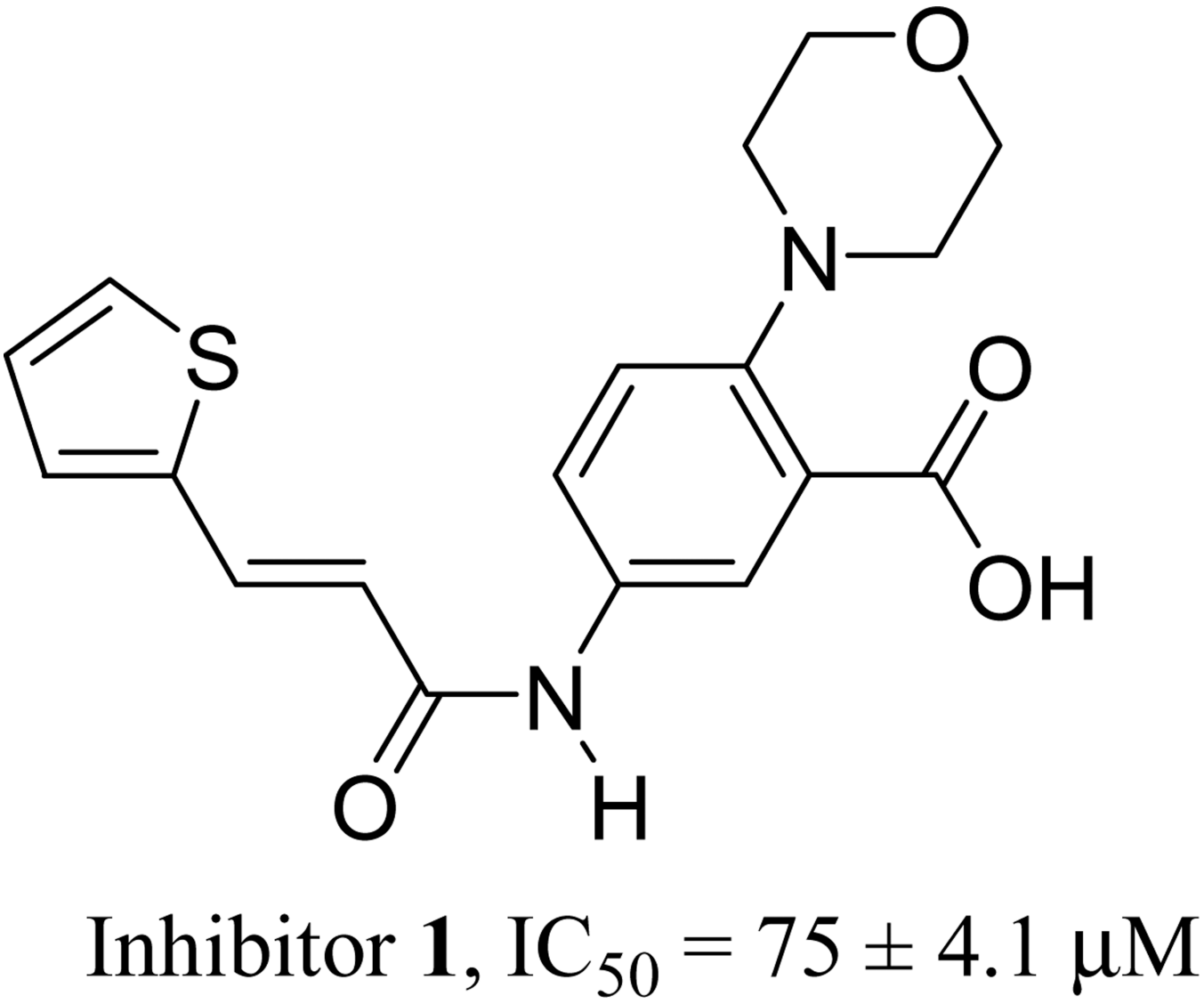

Figure 1.
Structure and IC_{50} value of inhibitor **1**

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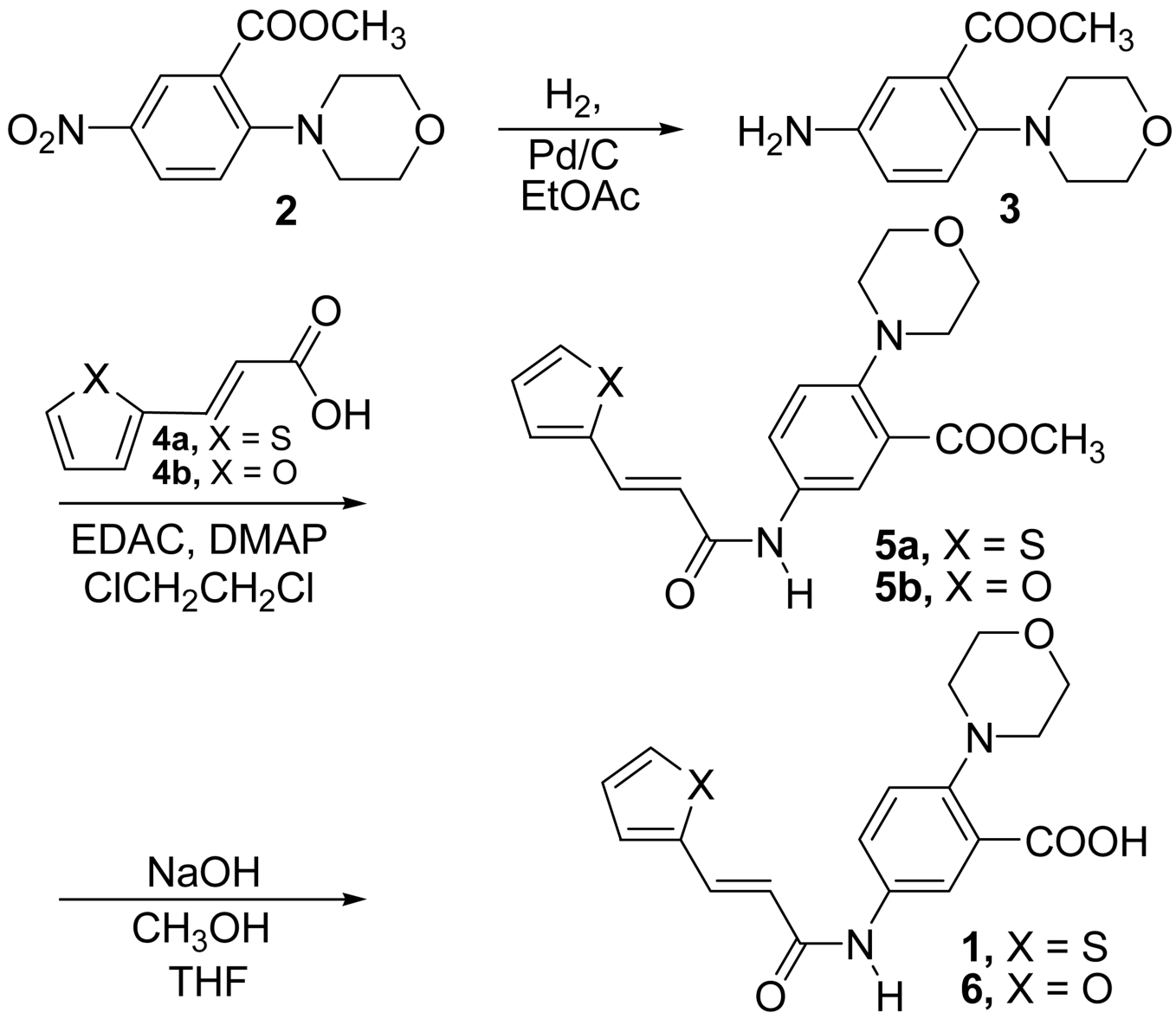

Scheme 1.
Synthesis of inhibitor **1** and its furan analogue **6**

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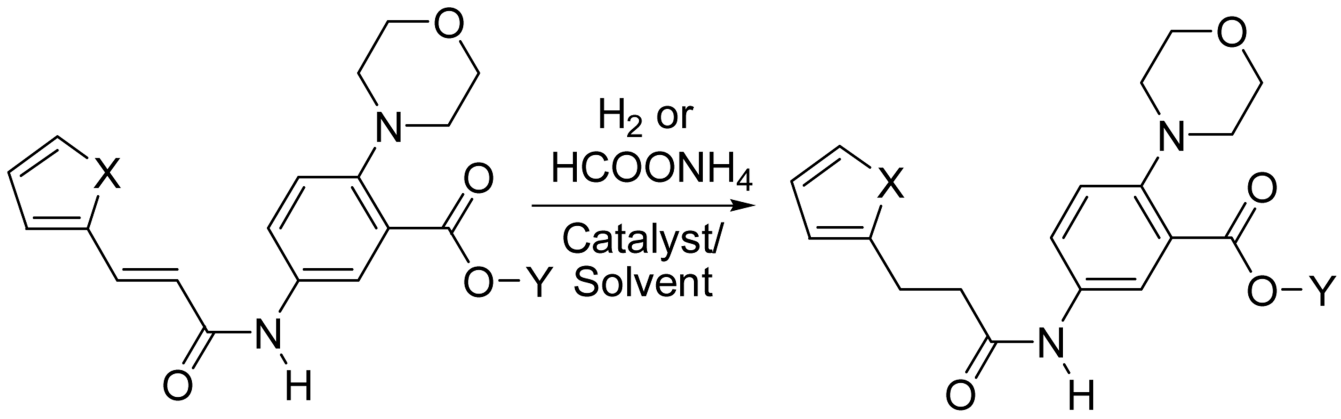

Reactant	X	Y	Catalyst /Solvent	Product	Yield (%)
1	S	H	Pd black/MeOH	**7**	69
5a	S	CH_3	Pd black/MeOH	**8**	82
6	O	H	Pd/C/EtOAc	**9**	88
5b	O	CH_3	Pd/C/EtOAc	**10**	77

Scheme 2.
Synthesis of compounds **7–10**

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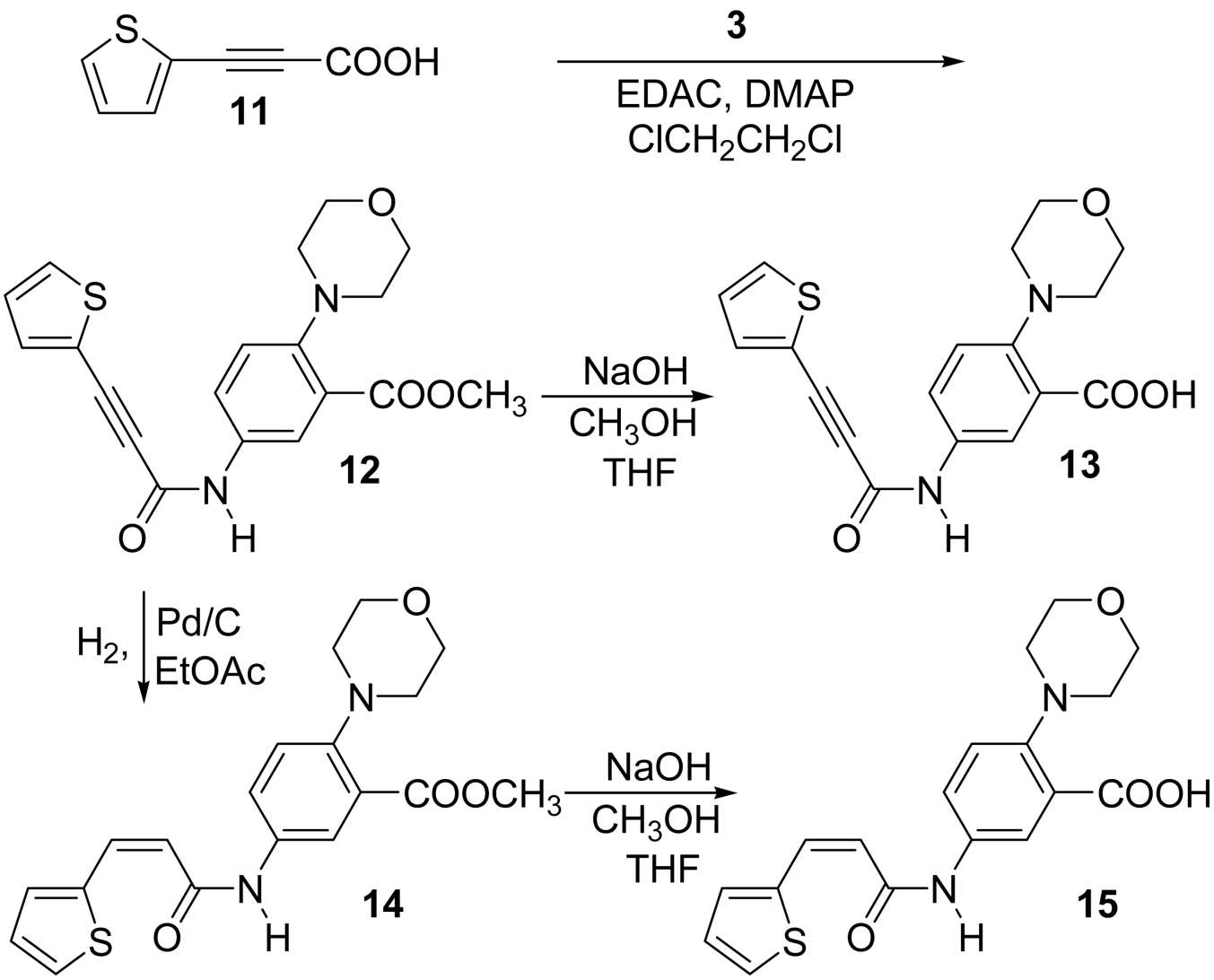

Scheme 3.
Synthesis of acetylenic acid, **13** and the *cis* derivative, **15**

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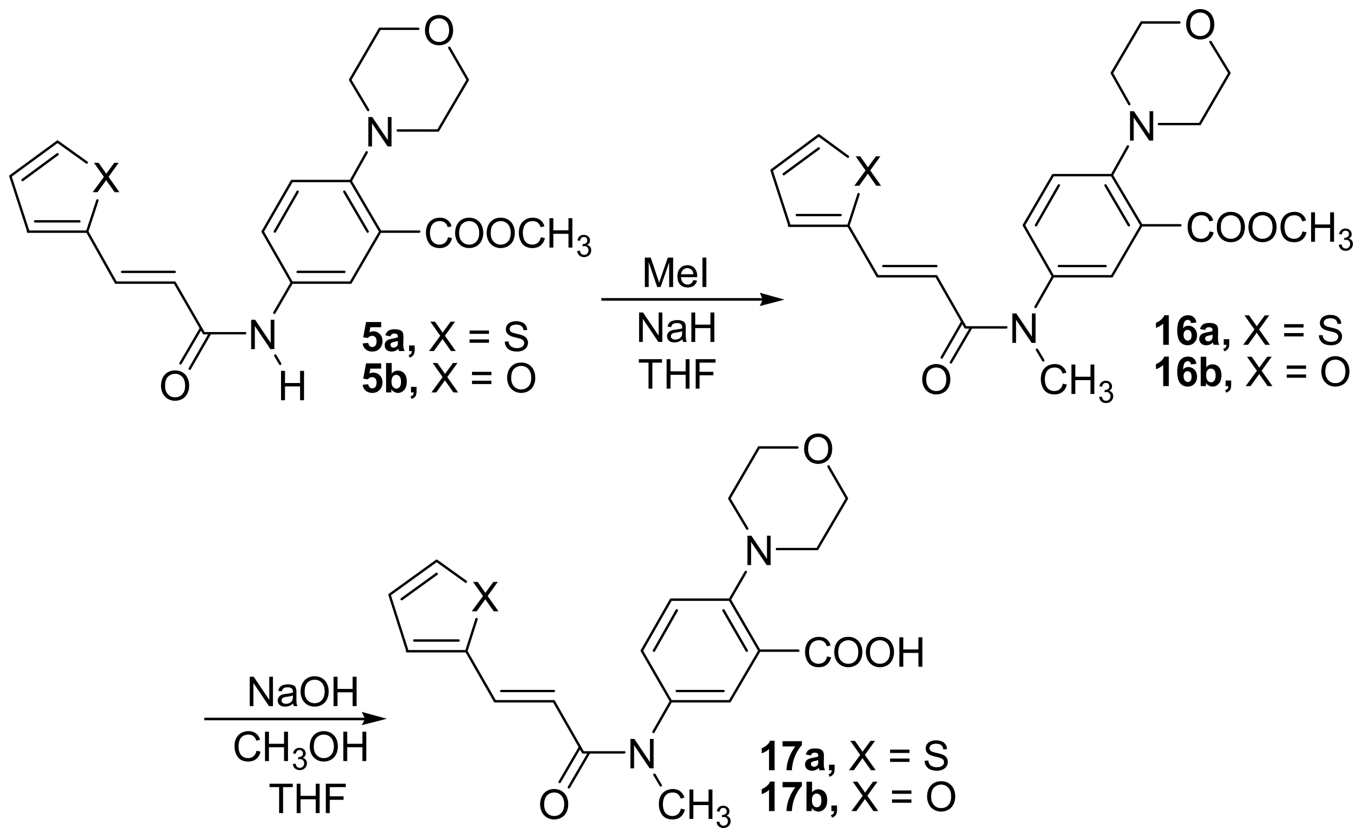

Scheme 4.
Synthesis of N-methyl compounds **17a** and **17b**

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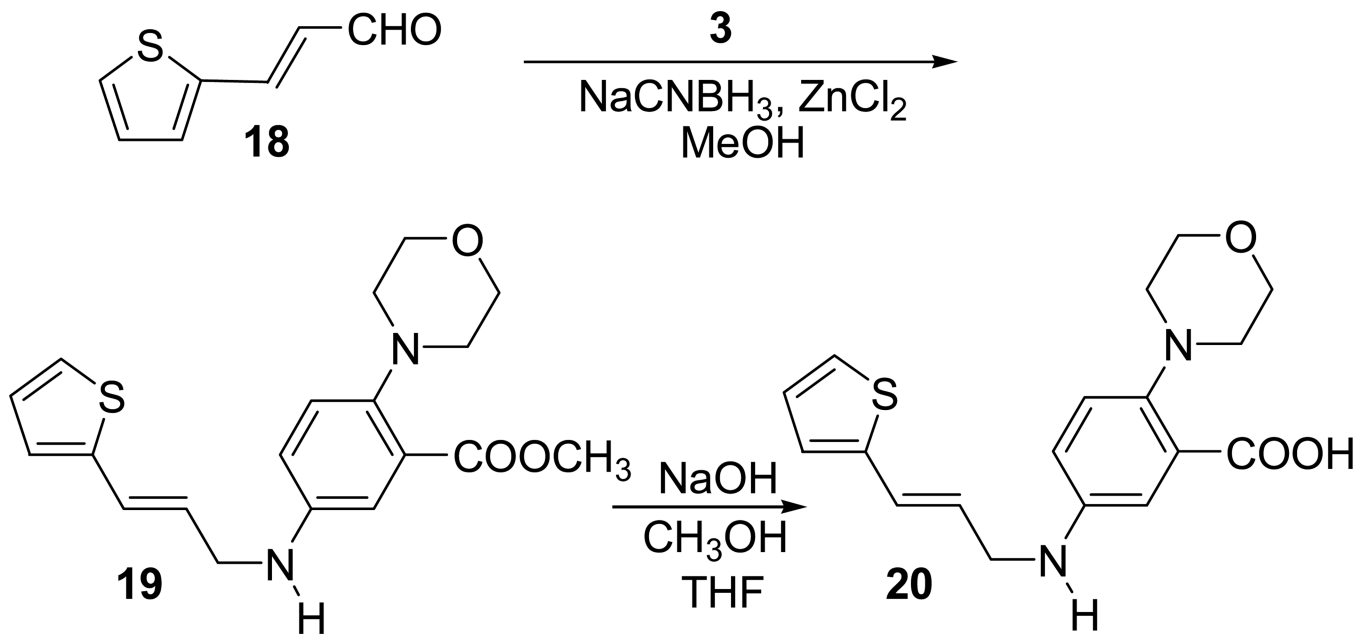

Scheme 5.
Synthesis of compound **20**

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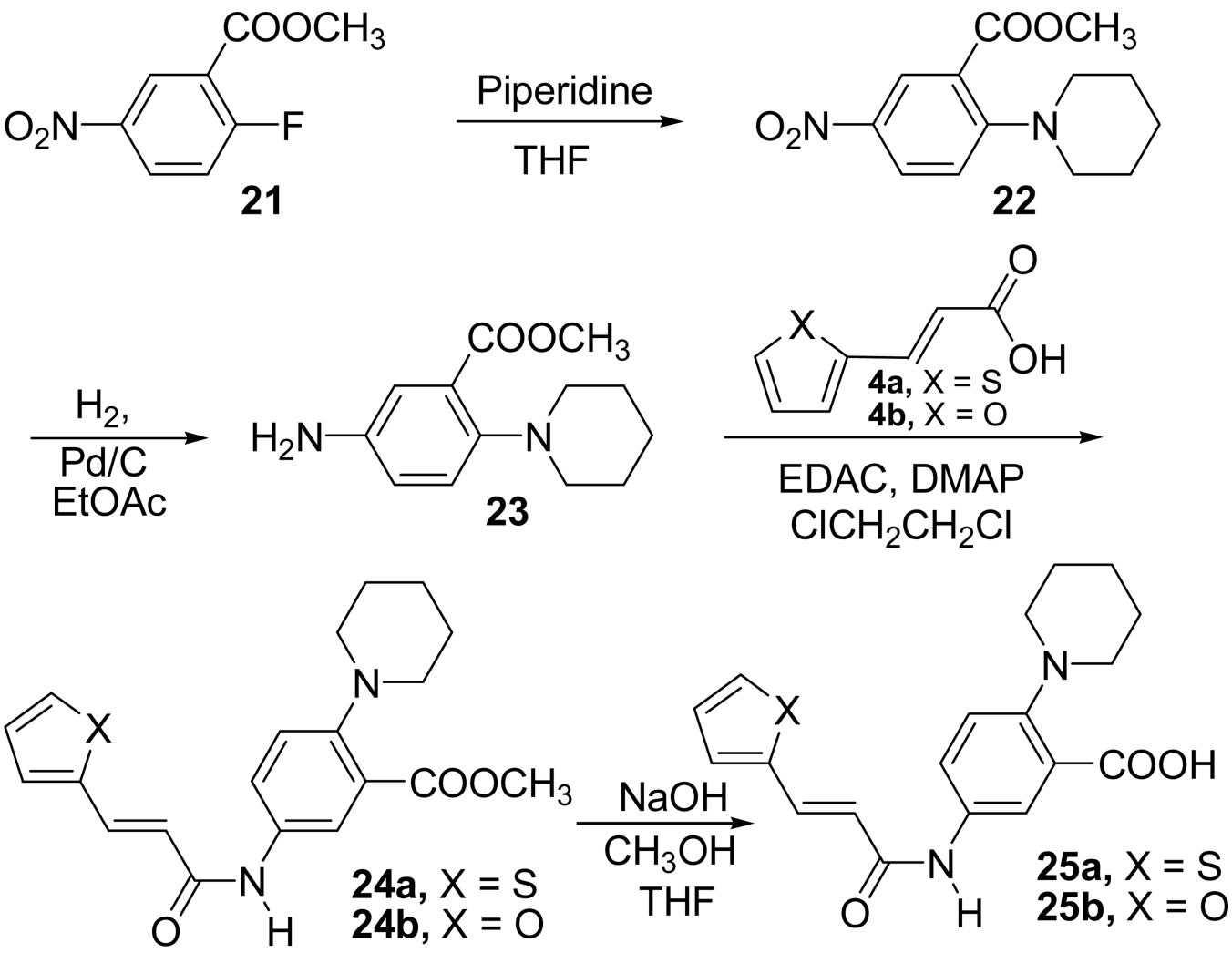

Scheme 6.
Synthesis of compounds **25a–b**

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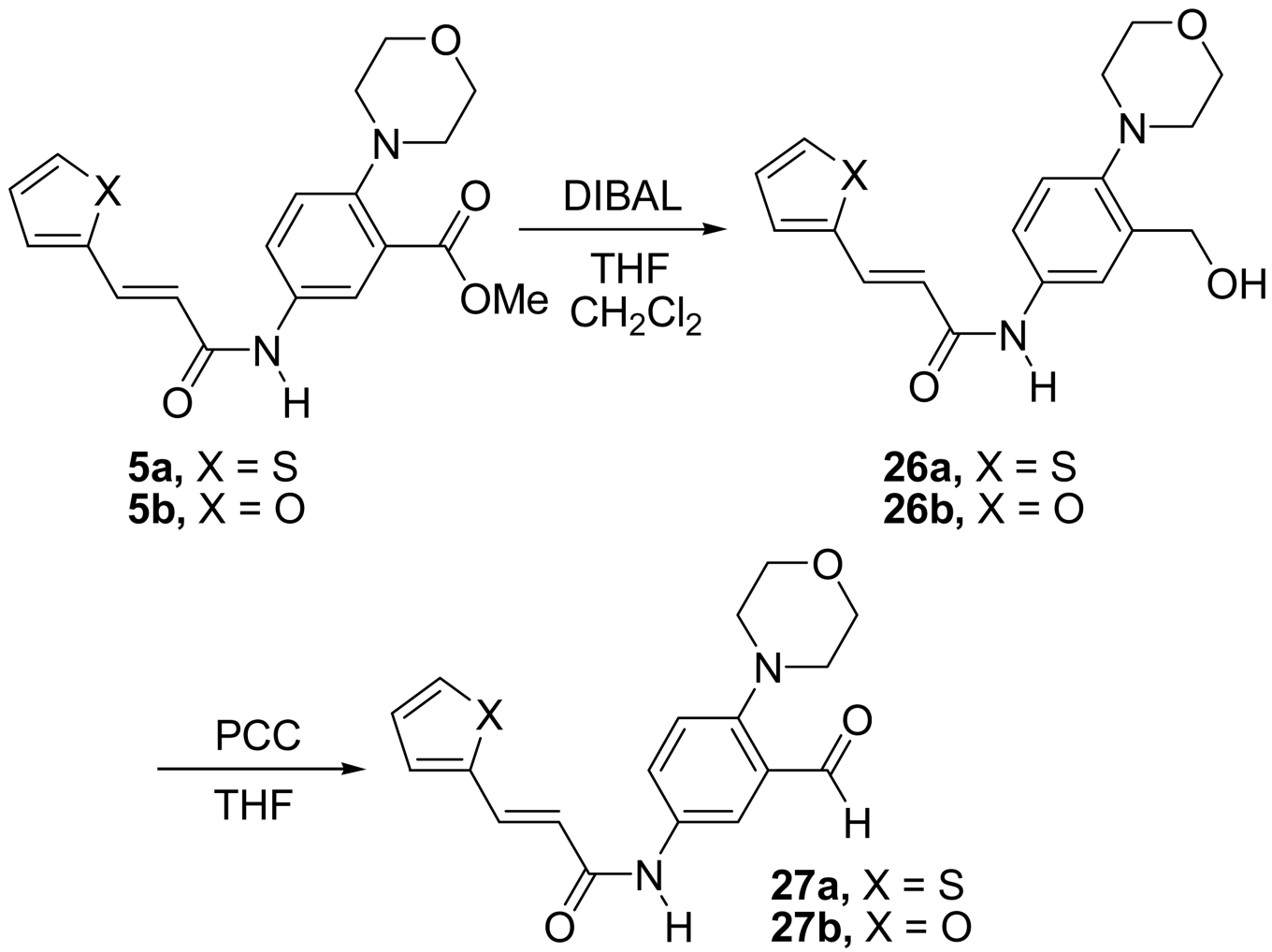

Scheme 7.
Synthesis of compounds **26a,b** and **27a,b**

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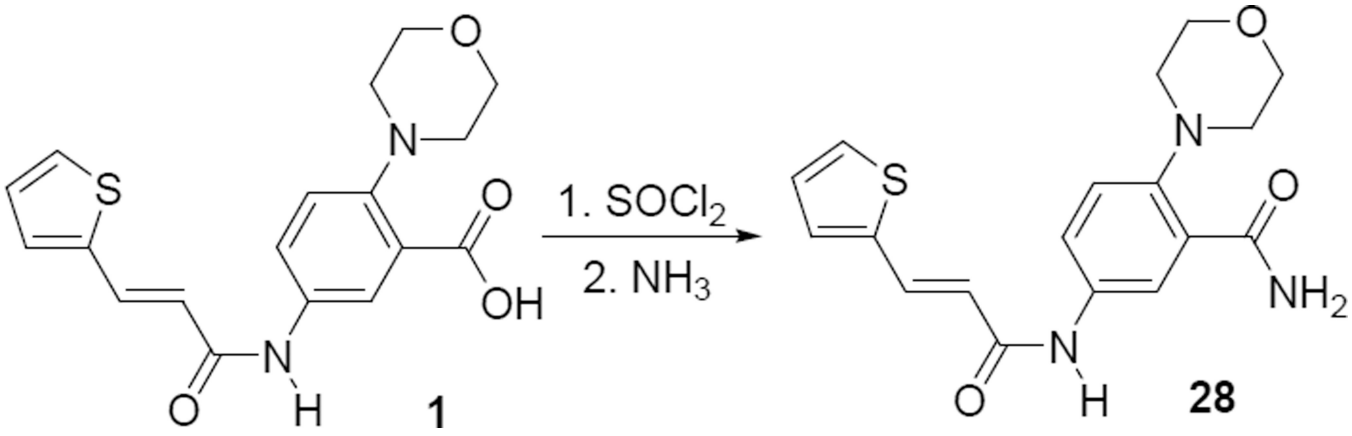

Scheme 8.
Synthesis of compound **28**

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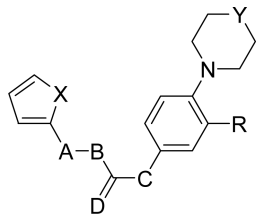
Table 1

S. aureus $SrtA_{\Delta 59}$ inhibition of synthesized derivatives of inhibitor **1**



No	X	A-B	Stereochemistry	C	D	Y	R	IC_{50}(μM)[a]
5a	S	CH=CH	E	NH	O	O	$COOCH_3$	71 ± 2.5
5b	O	CH=CH	E	NH	O	O	$COOCH_3$	58 ± 4.9
6	O	CH=CH	E	NH	O	O	COOH	181 ± 14
7	S	CH_2-CH_2	_[b]	NH	O	O	COOH	>600
8	S	CH_2-CH_2	_[b]	NH	O	O	$COOCH_3$	>600
9	O	CH_2-CH_2	_[b]	NH	O	O	COOH	>600
10	O	CH_2-CH_2	_[b]	NH	O	O	$COOCH_3$	>600
12	S	C≡C	_[b]	NH	O	O	$COOCH_3$	165 ± 9
13	S	C≡C	_[b]	NH	O	O	COOH	183 ± 12
14	S	CH=CH	Z	NH	O	O	$COOCH_3$	61 ± 4
15	S	CH=CH	Z	NH	O	O	COOH	154 ± 5
16a	S	CH=CH	E	NMe	O	O	$COOCH_3$	571 ± 43
16b	O	CH=CH	E	NMe	O	O	$COOCH_3$	514 ± 39
17a	S	CH=CH	E	NMe	O	O	COOH	>600
17b	O	CH=CH	E	NMe	O	O	COOH	>600
19	S	CH=CH	E	NH	HH	O	$COOCH_3$	249 ± 9
20	S	CH=CH	E	NH	HH	O	COOH	>600
24a	S	CH=CH	E	NH	O	CH_2	$COOCH_3$	92 ± 5
24b	O	CH=CH	E	NH	O	CH_2	$COOCH_3$	131 ± 14
25a	S	CH=CH	E	NH	O	CH_2	COOH	181 ± 12

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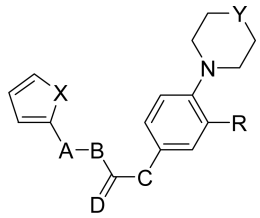

No	X	A-B	Stereochemistry	C	D	Y	R	IC_{50}(μM)[a]
25b	O	CH=CH	E	NH	O	CH_2	COOH	463 ± 19
26a	S	CH=CH	E	NH	O	O	CH_2OH	73 ± 2
26b	O	CH=CH	E	NH	O	O	CH_2OH	111 ± 5.7
27a	S	CH=CH	E	NH	O	O	CHO	77 ± 6.5
27b	O	CH=CH	E	NH	O	O	CHO	107 ± 8.5
28	S	CH=CH	E	NH	O	O	$CONH_2$	105 ± 3.7

[a] IC_{50} values were determined by a Fluorescent Resonance Energy Transfer (FRET) assay. Reported values are an average of 3–5 measurements.

[b] Not applicable

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