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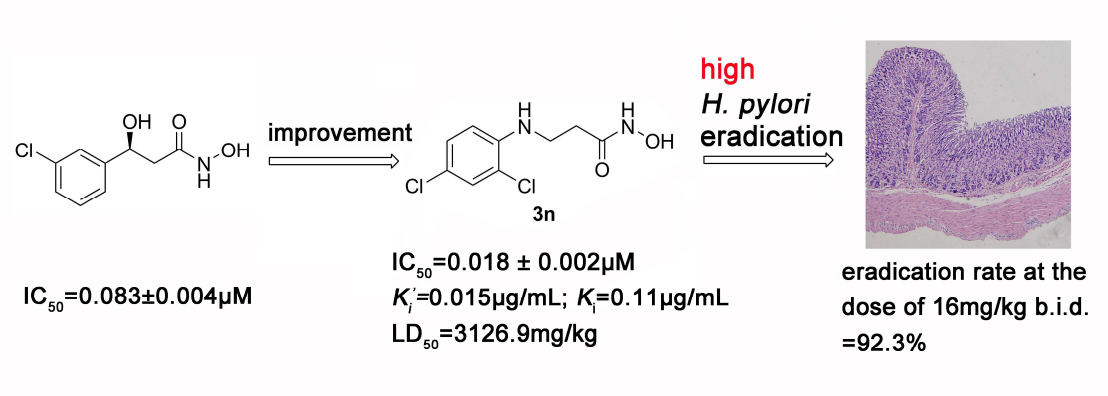

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[a]National Demonstration Center for Experimental Chemistry Education, Hunan Engineering Laboratory for Analyse and Drugs Development of Ethnomedicine in Wuling Mountains, Jishou University, Jishou 416000, P. R. China

[b]State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, P. R. China

Abstract: A novel series of aniline-containing hydroxamic acids were designed, synthesized and evaluated as anti-virulence agents for the treatment of gastritis and gastric ulcer caused by *Helicobacter pylori*. *In vitro* enzyme-based screen together with *in vivo* assays and structure−activity relationship (SAR) studies led to the discovery of three potent urease inhibitors 3-(3,5-dichlorophenylamino)-*N*-hydroxypropanamide (**3a**), 3-(2-chlorophenylamino)-*N*-hydroxypropanamide (**3d**) and 3-(2,4-dichlorophenylamino)-*N*-hydroxypropanamide (**3n**). Compounds **3a**, **3d** and **3n** showed excellent urease inhibition with IC_{50} values 0.043±0.005, 0.055 ± 0.008 and 0.018 ± 0.002 μM, and significantly depressed gastritis developing at the dose of 32 mg/kg b.i.d with eradication rates of *H. pylori* reaching 92.3, 84.6 and 100%, respectively. Preliminary safety studies (acute toxicity in mice) disclosed that **3a**, **3d** and **3n** was well-tolerated in KM mice with LD_{50}s of 2982.8, 3349.4 and 3126.9 mg/kg, respectively. Collectively, the data obtained in this study indicate that **3a**, **3d** and **3n**, in particular **3n**, could considered as promising candidates for the potential treatment of *H. pylori* caused gastritis and gastric ulcer, and hence merit further studies.

*Corresponding author. Fax: +86-743-8563911; E-mail address:xiaozhuping2005@163.com
*Corresponding author. Fax: +86-25-83592672; E-mail address: zhuhl@nju.edu.cn

Keywords: Arylamino containing hydroxamic acid; *H. pylori* urease inhibitor; Kinetics study; gastritis and gastric ulcer; therapeutic efficacy.

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1. Introduction:

Urease (EC 3.5.1.5), a nickel dependent metalloenzyme with an ability to catalyze the hydrolysis of urea to ammonia and carbamates,[1] exists in plants, fungi and bacteria,[2] being well known as an important virulence factor in the pathogenesis of several diseases.[3,4] *Helicobacter pylori*, a microaerophilic bacterium with high urease activity, is known to be one of the most successful human bacterial parasites in gastrointestinal track, and colonizes more than half of the human population.[5] On one hand, urease excreted by *H. pylori* buffers the pH to allow the bacterium survive in the acidic environment of the stomach;[6] on the other hand, urease serves as a virulence factor to cause about 15-20% of infected people developing into severe gastroduodenal pathologies, such as stomach and duodenal ulcers, adenocarcinomas and stomach lymphomas.[7,8] In 1991, Eaton demonstrated that urease-negative *H. pylori* mutants were unable to colonize the stomach of gnotobiotic piglets,[9] suggesting that urease activity is indispensable for survival of *H. pylori* in the gastric microenvironment. Therefore, the strategy based on urease inhibition is considered as a promising treatment for infections caused by urease-containing becteria.[10] In the past decades, the great medicinal potential for urease inhibitors attracts extensive attention from worldwide researchers,[11-14] and hundreds of structures such as hydroxamic acids, phosphorodiamidates, imidazoles, thiobarbituric acids, thioureas and flavonoids have been identified.[15-21] In mechanism, a hydroxamic acid strongly binds to nickel ions of the active site to form a strong complex with urease, and have therefore attracted much more attention.[22-23] Up to now, hydroxamic acids are the best recognized urease inhibitors, with a marketed drug acetohydroxamic acid (AHA) which was approved by U.S. Food and Drug Administration in May, 1983.

In the past several years, we focused our efforts on seeking novel urease inhibitors, and flavonoids,[21,24] deoxybenzoins,[25] deoxybenzoin oximes,[26] 1,2-diarylethanes,[27-28] furan-2(5*H*)-ones[29] and 3-arylpropionyldroxamic acids[30] were identified. 3-(3-Chlorophenyl)-3-hydroxypropionylhydroxamic acid (CPH), found as the most active with IC_{50} of 0.083±0.004 μM in our group, is a good lead for further modifications. Molecular docking of CPH revealed that phenyl moiety binds to the surface of urease, and hydroxamic acid moiety binds to both nickel ions in the active site. These two functional sections are linked by a carbon chain arm.[30] Meanwhile, consideration of CPH structurally containing a chiral centre and S-/R-isomers not showing significant difference in activity,[31] we replaced the chiral center COH with NH for synthetic convenience and structural diversity (Figure 1). A series of 2-(*N*-arylamino)acetohydroxamic acids (**2**) and 3-(*N*-arylamino)propionylhydroxamic acids (**3**) were designed and synthesized. Biological evaluations revealed that the modification led to a marked improvement in potency.

2. Results and dissussion

2.1. Chemistry

Direct replacement of the chiral center COH in the CPH skeleton with NH furnishes the first series (**2**), and the synthetic route illustrated in Scheme 1. *N*-Alkylation of the selected aniline **4** with ethyl bromoacetate offered compound **5**, which was amidated with $NH_2OH{\cdot}HCl$ to give 2-(*N*-arylamino)acetohydroxamic acids (**2**).[32]

Replacement of ethyl bromoacetate with ethyl 3-bromopropionate was beyond our expectations, and was suffered a very poor yield for preparing the corresponding *N*-alkylation intermediate (**6**). Therefore, **4** was converted to **6** by nucleophilic addition of **4** to ethyl acrylate in the presence of terfluoroacetic acid (TFA) (Scheme 2).

2.2. Inhibitory activity against cell-free urease

In the process of designing novel non-phosphoramide urease inhibitors, we prepared and evaluated a series of aryl-containing hydroxamic acids.[30,2] The structure-activity relationship (SAR) analysis and molecular dockings suggested that the arm linkage is important for the binding location of the aryl moiety. In light of this observation, our modification strategy first focused on the replacement of the branching point with NH, yielding the series **1** (**2a**-**2w**), as shown in Figure 1. Newly synthesized *β*-aminoacetohydroxamic acids (**2a**-**2w**) were initially evaluated for inhibition activity against extracted *H. pylori* urease, in parallel with AHA as a positive reference (Table 1). With a bromo group on the benzene ring, compounds **2k**, **2l** and **2v** showed significant inhibition activity with IC_{50} of 0.86±0.03, 2.01±0.05 and 12.3±0.9 μM, being 2- to 24-fold more potent than the reference compound AHA. Replacement of the bromine atom in **2k**, **2l** or **2v** with Cl, Me, F, NO_2 or OMe resulted in compounds (**2i**, **2j**, **2u**; **2s**; **2a**; **2n**; **2d**, **2t**, **2c**) less potency in the order of decreasing hydrophobicity, exhibiting IC_{50} values of 1.15-69.8μM. Interestingly, when both *meta*-positions were replaced by Cl, Me, F and OMe, the resulting compounds (**2m**, **2b**, **2r** and **2e**) showed high potency with IC_{50} values of 0.13-5.11μM. These observations suggest that hydrophobic substituents in benzene ring generally possess more favorable inhibitory activity. Molecular docking disclosed that benzene moiety establishes favorable hydrophobic interactions with backbone residues of urease (Figure 2A). Out of these compounds, **2m** was the most potent in this series, but a little less potent than the lead CPH.

In order to improve both the binding affinity and efficiency, **2m** was selected as a lead and we took a close look at urease-**2m** interactions. No significant hydrogen bonding interactions could be observed between NH of aniline moiety and near residues (Asn 168, Ala 169, Ala 365 and Cys 321), while this NH group is located near hydrophobic side chains of Ala 169

and Ala 365 (Figure 2A and 2B), indicating that a lipophilic CH_2 group could be inserted for possibly improving activity of compound **2m**. This hypothesis was further confirmed by the binding model of **3a** (Figure 2A). Based on this consideration, **3a** was designed and synthesized, showing 3-fold improvement of potency (IC_{50}=0.043μM).

Next, we designed molecules **3b**-**3q** to investigate the importance of the number of substituent and their positions on the phenyl ring, which were evaluated and summarized in Table 2. As expected, propionyl derivatives (**3b**-**3q**) generally show more potency than acetyl anaguoes (**2a**-**2w**) with a maximum of up to 3670-fold increase. This may be attributed to the intermolecular interactions built by NH and ethylene group. As an example, the most active compound **3n** interacts through its NH group with Cys321, His322 and Arg338 (Figure 3B), forming significant hydrogen bonding interactions which were not observed in **2m** binding model (Figure 2B). The binding complex of **3n**-urease was further stabilized by hydrophobic interactions of ethylene group with Ala169, Ala365 and Met366 (Figure 3B). In comparison with **3a**, the phenyl group of **3n** is closer to the hydrophobic areas of the active pocket (Figure 3A). Compounds **3c**-**3f**, bearing an alkoxy group or a halogen atom, displayed equal or improved potency compared to **3b**. In the mono-substitued series, **3d** carrying *o*-chloro was the most potency with IC_{50} of 0.055±0.08 μM. Introduction of additional chloro group at the other *ortho*-position or either of the two *meta*-positions in **3d** produces compounds (**3j**, **3h** and **3o**) with a moderate to marked loss in activity. However, introduction chloro groups at both *ortho*- and *para*-positions gave the most active compound **3n** (IC_{50}=0.018 ± 0.002μM) in all tested compounds, exhibiting an over 1500-fold potency increase compared to the positive control AHA.

2.3. Inhibitory activity against urease in intact cell

While the above data demonstrates activity in a cell-free system, in order to validate the

potential use of these compounds in proof of concept animal models, compounds with IC_{50}<1.0 μM (**2h**, **2k**, **2m**, **3a**, **3d**-**3f**, **3h**, **3i** and **3n**) were selected to be incubated with *H. pylori* for evaluating the efficiency in cell-based models by using AHA as positive control. The results are shown in Table 1 and 2. Assayed compounds suppressed urease in intact cell with IC_{50} values ranging from 2.23 to 48.1 μM, showing much higher potency than AHA (IC_{50}=172±7 μM). However, a 46- to 110-fold increase of IC_{50} values was observed in comparison with those against cell-free urease. The decrease of potency may be caused by the barrier of cell membrane that the compound must transfer and a metabolic loss of the compound in the presence of *H. pylori* cells. The most active compound, **3n** with 2,4-dichloro substitution pattern on the benzene ring, would be a promising urease inhibitor deserving further research as an antiulcer and infectious stone agent.

*2.4. Kinetics of urease inhibition by compounds **2m**, **3a** and **3n***

To gain information on the mechanism of inhibition, the potent inhibitor **2m**, **3a** and **3n** were selected for a kinetic study. Data are reported in Fig. 4-6 as plots by non-linear fitting of experimental data against Eq (1), and kinetic constants (K_i and K_i') were obtained by linear fitting of calculated values (*b* and *c*) *vs* [I] against Eq (2) and Eq (3) (Table 3). The present work establishes that inhibition of urease by arylamino containing hydroxamic acid is fully reversible, in agreement with our recent report.[30] As shown in Table 3, K_i is not equal to K_i', suggesting a dual-site binding mode. This may be attributed to a typical hydroxamic acid being presented as an equilibrium mixture of molecule and anion forms under physiological condition, which therefore supported that the novel hydroxamic acid acts as a mixed-type mechanism. Recently, based on this equilibrium hypothesis, our molecular docking model gave a possible explain for the mixed type inhibition mechanism of aryl derivative hydroxamic acids.[30] It is to be noted that Byung-Ha Oh et al. reported the co-crystal structure of *H. pylori* urease with AHA in 2001,[33] revealing that AHA is a specific inhibitor

of urease. In the past decades, kinetic studies disclosed that the aliphatic hydroxamic acids, including AHA, were noncompetitive inhibitors, while the aryl derivatives were of a mixed type.[34]

$$V = \frac{[S]}{b + c[S]} \quad (1)$$

$$b = \frac{K_m}{V_m}(1 + \frac{[I]}{K_i}) \quad (2)$$

$$c = \frac{1}{V_m}(1 + \frac{[I]}{K_i'}) \quad (3)$$

2.5. Acute Toxicity Study in KM Mice

An acute toxicity study of compounds **3a**, **3d** and **3n** was conducted *in vivo* in KM mice. The tested animals recieved a single oral dose of test compounds at 500, 1000, 2000, 3000 and 5000 mg/kg (suspended in 0.5% carboxymethylcellulose sodium (CMC-Na) solution). Compounds **3a**, **3d** and **3n** have been found to be compatible with potential *in vivo* studies showing LD_{50} of 2982.8, 3349.4 and 3126.9 mg/kg, respectively (Table 4). As the mice administered a single dose up to 1000 mg/kg of test compounds remained alive and appeared healthy during a subsequent 14 day observation. This acute toxicity study indicated that **3a**, **3d** and **3n** was well-tolerated in KM mice.

2.6. Compound evaluation in mice model of H. pylori

To further screen for compounds with potent urease inhibitory activity and tolerable toxicity *in vivo*, therapeutic efficacy was studied using an *H. pylori* infected mouse model. Compounds **3a**, **3d** and **3n** were therefore selected to be assayed and results are shown in Table 5 and Figure 7-8. Significant gastric mucosa injury was observed in untreated mice, while this was barely observable in treated mice. On the contrary, the market urease inhibitor,

AHA, only partially reduced the gastric mucosa injury (Figure 7-8). Oral **3n** treatment significantly reduced the survival of *H. pylori* with the eradication rate of up to 92.3% at 16 mg/kg, while compound **3a** and **3d** required a higher dose, 32 and 64 mg/kg, to achieve the same level of efficacy. In comparison with the excellent therapeutic efficacy of **3a**, **3d** and **3n**, the positive control AHA exhibited very low eradication rate (44.4%) at 32 mg/kg (Table 5). Together, our data clearly demonstrated that **3a**, **3d** and **3n** had potent activity against *H. pylori in vivo*.

3. Conculsions

In all, we have synthesized a series of aniline containing hydroxamic acids, which were tested for inhibitory activity against *H. pylori* urease. Generally, compounds with a three-carbon arm show higher potency than those with only two-carbon against *H. pylori* urease both in cell and in cell-free extracts. Many compounds show excellent activity against extracted *H. pylori* urease with micromolar to low nanomolar IC_{50} values, with top three active compounds (**3a**, **3d** and **3n**) show IC_{50}s of 43±5, 55 ±8 and 18 ± 2 nM. They also show good potency against *H. pylori* urease in intact cell with IC_{50} values having about 100-fold increase. Kinetic studies of three selected compounds (**2m**, **3d** and **3n**) suggested a mixed type of inhibition mechanism. Meanwhile, the molecular docking of these compounds supports their potency against *H. pylori* urease and confirms that they inhibited urease with a mixture of competitive and uncompetitive mechanism. Efficacy studies in *H. pylori*-infected KM mice demonstrated that compounds **3a**, **3d** and **3n** were markedly depressed gastritis developing at the dose of 32 mg/kg b.i.d with *H. pylori* eradication rate of 92.3, 84.6 and 100%, respectively. It is worth mentioning that the dose used in the *in vivo* anti-*H. pylori* infection experiment is much lower than LD_{50}s. Our findings indicate that these arylamino containing hydroxamic acids can potentially be applied for the treatment of gastritis and gastric ulcer caused by *H. pylori*.

4. Experimental section

4.1. Biology materials

Protease inhibitors (Complete, Mini, EDTA-free) were purchased from Roche Diagnostics GmbH (Mannheim, Germany) and Brucella broth was from Becton Dickinson and Company (Sparks, MD). Sheep serum was from Hyclone (Utah, American).

4.2. Bacteria

H. pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was grown in Brucella broth supplemented supplemented with 10% sheep sterile and defibrinated blood for 24 h at 37 °C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), as our previously described literatures.[24-27]

4.3. Preparation of H. pylori urease

For urease inhibition assays, 50 mL broth cultures (2.0×10^8 CFU/mL) were centrifuged (5000 g, 4 °C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *H. pylori* precipitation was stored at -80 °C for 8 h, and then was returned to room temperature, and after addition of 3 mL of distilled water and protease inhibitors, sonication was performed for 60 s. Following centrifugation (15,000×g, 4 °C), the supernatant was desalted through Sephadex G-25 column (PD-10 columns, Amersham Pharmacia Biotech, Uppsala, Sweden). The resultant crude urease solution was added to an equal volume of glycerol and stored at 4 °C until use in the experiment.

4.4. Measurement of urease inhibitory activity

The assay mixture, containing 25 mL (10U) of *H. pylori* urease which was replaced by 25 mL of cell suspension (4.0×10^7 CFU/mL) for the urease assay of intact cells and 25 mL of the

test compound, was pre-incubated for 1.5 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn.[35]

4.5. Kinetic study

Based on the indophenol method, the velocity of ammonia production (V) was measured in the presence of concentration gradients of urea ([S]) for every specific concentration of **2m**, **3a** and **3n** ([*I*]). Nonlinear fitting curves to data of V and [S] were used to determine the type of enzyme inhibition based on the general kinetics equation (Eq. 1). Consequently, resulted data of b (Eq. 2) or c (Eq. 3) and [*I*] were treated as a linear fitting to give the inhibitory constants K_i and K_i', which were determined from the intersection on the X-axis of the corresponding fitting line. All experiments were conducted in triplicate.

4.6. Protocol of docking study

Molecular docking of compounds **2m**, **3a** and **3n** into the structure of *H. pylori* urease complex structure was carried out using SYBYL-X version 2.1.1 software suite (Tripos, Inc., St. Louis, MO).[36] The X-ray structure of urease from *H. pylori* was downloaded from the Protein Data Bank (PDB code: 1e9y)[33] and was modified by adding hydrogen atoms and removing water as well as cocrystallized substrate (AHA). The active site was defined as all the amino acid residues confined within a 5 Å radius sphere centered about AHA, and the composite structure without original ligand was utilized as the in silico model for docking studies. Default parameters and values within the minimization dialogue were used except where otherwise mentioned. The docked conformations of ligands were evaluated and ranked using Surflex-Dock and four scoring functions implemented in the CSCORE software module within the SYBYL-X environment. The CSCORE module allowed consensus scoring

that integrated multiple well-known scoring functions such as ChemScore, D-Score, G-Score and PMF-Score to evaluate docked ligand conformations.

4.7. Acute toxicity experiment.

Male and female adult KM mice (18−22 g) were obtained from the Changsha Silaikejingda Animal Co. Ltd (Hunan, China) and housed under pathogen-free conditions with a 12 h light/dark cycle. Food and water were given ad libitum throughout the experiment. The *in vivo* acute toxicity of **3a**, **3d** or **3n** in KM mice was assessed by a single intragastrical injection at various doses (n=10, mice/group, 200 μL per mouse). Compound **3a**, **3d** or **3n** was dissolved in 0.5 % CMC-Na solution and the controls received injections of vehicle alone. The mortality was recorded for 14 days after administration. The 50% lethal dose (LD_{50}) was estimated by probit analysis using SPSS version 16.0.

4.8. Compound evaluation in mice model of H. pylori.

KM mice (18−22 g) were obtained from the Changsha Silaikejingda Animal Co. Ltd (Hunan, China) and housed under pathogen-free conditions with a 12 h light/dark cycle. Food and water were given ad libitum throughout the experiment. KM mice were received an intragastrical injection of $NaHCO_3$ solution (0.2 mol/L 0.25 mL) after were fasted for 4 h. Fifteen min later they were then intragastrically injected with *H. pylori* (0.4 mL,1 $\times 10^9$cfu/mL). They were infected with *H. pylori* once a day for a week. Three weeks later, *H. pylori* infected mice were randomly divided into fifteen groups (n=13): one model group, three **3a** groups (32 mg/kg b.i.d, 64 mg/kg b.i.d, 128 mg/kg b.i.d), three **3d** groups (32 mg/kg b.i.d, 64 mg/kg b.i.d, 128 mg/kg b.i.d), three **3n** groups (16 mg/kg b.i.d, 32 mg/kg b.i.d, 64 mg/kg b.i.d) and one acetohydroxamic acid group (32 mg/kg b.i.d). Compounds were dissolved in 0.5 % CMC-Na solution. Mice were intragastrically received 0.6 mL of

compound for two doses at 8:00 a.m. and 6:00 p.m. every day. The treatment lasted for 14 days. Mice were killed and each stomach was divided into two parts. One part was fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 3.0-μm-thick sections for analysis of gastric mucosal lesion. The tissue sections were deparaffinized, and hematoxylin and eosin (HE) stain was applied to the sections. The other part was homogenized for *H. pylori* determination according to a previous report.[37]

4.9. Chemistry

All chemicals (reagent grade) used were purchased from Aldrich (U.S.A.) and Sinopharm Chemical Reagent Co., Ltd (China). Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). EI mass spectra were obtained on Waters GCT mass spectrometer, and ^{1}H NMR spectra were recorded on a Bruker AV-400 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm (δ). Elemental analyses were performed on a Foss Heraeus CHN-O-Rapid instrument and were within ± 0.4% of the theoretical values.

*4.9.1. General procedure for the preparation of compounds **5***

To a solution of a selected aniline (5 mmol) in dry DMSO or acetone (25 mL) was added an equivalent of anhydrous K_2CO_3, which was heated to reflux. Then, 4.5 mmol of ethyl bromoacetate in dry DMSO or acetone (5 mL) was added dropwise over 30 min. The reaction mixture was refluxed for 3-8 h (monitored by TLC), and was poured into distilled water (20 mL). The mixture was extracted with EtOAc (3×50 mL), dried over$MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash silica chromatography to afford **5**.

*4.9.2. General procedure for preparation of compounds **6***

Anhydrous TFA (3-5 drops) was added into appropriately substituted aniline (10 mmol) and then heated to 100 °C. To this mixture was added 3 mL of ethyl acrylate (EA) within 10min, and the mixture was stirred at 100 °C for 24 h. After this period the EA was removed under vacuum. The resulted residue was purified by silica gel chromatography to yield compound **6**.

*4.9.3. General procedure for the preparation of compounds **2a-2w** and **3a-3q***

A mixture of sodium methoxide (10 mmol) and hydroxylamine hydrochloride (6 mmol) in anhydrous methanol was well stirred for 30 min. To this mixture was added compound **5** or **6** (3 mmol) was added, and the mixture was stirred at room temperature for 1-3h. The reaction was quenched with ice (30 g) and neutralized with dilute HCl, which was extracted with ethyl acetate (3 × 60 mL). The organic layers were collected, dried over sodium sulfate, filtered, and evaporated under vacuum. The residure was purified by flash silica chromatography (methanol-dichloromethane) to afford pure product (**2** or **3**) as a white solid.

*4.9.3.1. 2-(4-Fluorophenylamino)-N-hydroxyacetamide (**2a**)*

White power, 43%, mp 127-128 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.55 (d, *J*=6.0Hz, 2H); 5.81 (t, *J*=6.2 Hz, 1H); 6.55 (dd, *J*=8.9 Hz, *J*=4.4 Hz, 2H); 6.93 (t, *J*=8.9 Hz, 2H); 8.83 (s, 1H); 10.59 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 45.34; 113.53 (d, *J*=7.4Hz); 115.60 (d, *J*=21.9Hz); 145.49; 155.02 (d, *J*=231.2Hz); 167.37; EIMS *m/z* 184 (M^+). Anal. Calcd for $C_8H_9FN_2O_2$: C 52.17; H 4.93; F 10.32; N 15.21; found: C 52.23; H 4.92; F 10.31; N 15.22.

*4.9.3.2. 2-(3,5-Dimethylphenylamino)-N-hydroxyacetamide (**2b**)*

Light yellow power, 46%, mp 145-147°C, ^{1}H NMR(400 MHz, DMSO-d_6): 2.14(s, 6H); 3.54(d, *J*=6.1 Hz, 2H); 5.63(t, *J*=6.3 Hz, 1H); 6.18(s, 2H); 6.22(s, 1H); 8.80(s, 1H);10.52 (s,

1H); ^{13}C NMR(100 MHz, DMSO-d_6): 21.73; 45.04; 110.82; 118.78; 138.01; 148.75; 167.60; EIMS m/z 194 (M^+). Anal. Calcd for $C_{10}H_{14}N_2O_2$: C 61.84; H 7.27; N 14.42; found: C 61.78; H 7.28; N 14.44.

*4.9.3.3. N-Hydroxy-2-(2-methoxyphenylamino)acetamide (**2c**)*

White power, 41%, mp 145-146°C, ^{1}H NMR(400 MHz, DMSO-d_6): 3.62(d, *J*=5.9 Hz, 2H); 3.79(s, 3H); 5.13(t, *J*=6.0 Hz, 1H); 6.42(dd, *J*=7.8 Hz, *J*=1.5 Hz, 1H); 6.60(td, *J*=7.7 Hz, *J*=1.6 Hz, 1H); 6.78(td, *J*=7.6 Hz, *J*=1.3 Hz, 1H); 8.82(dd, *J*=7.9 Hz, *J*=1.3 Hz, 1H); 8.87(s, 1H); 10.61(s, 1H); ^{13}C NMR(100 MHz, DMSO-d_6): 44.61; 55.75; 109.97; 110.20; 116.83; 121.41; 137.94; 146.99; 167.32; EIMS m/z 196 (M^+). Anal. Calcd for $C_9H_{12}N_2O_3$: C 55.09; H 6.16; N 14.28; found: C 55.12; H 6.15; N 14.26.

*4.9.3.4. N-Hydroxy-2-(3-methoxyphenylamino)acetamide (**2d**)*

Light yellow power, 48%, mp 125-126°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.57 (d, *J*=6.1 Hz, 2H); 3.67 (s, 3H); 5.86 (t, *J*=6.2 Hz, 1H); 6.10-6.22 (m, 3H); 6.97 (d, *J*=8.0 Hz, 1H); 9.57 (bs, 2H); ^{13}C NMR(100 MHz, DMSO-d_6): 45.47; 55.80; 109.54; 111.08; 116.47; 121.85; 137.22; 145.88; 167.49; EIMS m/z 196 (M^+). Anal. Calcd for $C_9H_{12}N_2O_3$: C 55.09; H 6.16; N 14.28; found: C 55.12; H 6.15; N 14.26.

*4.9.3.5. 2-(3,5-Dimethoxyphenylamino)-N-hydroxyacetamide (**2e**)*

White power, 56%, mp 123-124°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.54 (d, *J*=6.1 Hz, 2H); 3.65 (s, 6H); 5.76 (s, 3H); 5.89 (t, *J*=6.2 Hz, 1H); 8.85 (s, 1H); 10.59 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 45.04; 56.13; 110.97; 118.43; 138.11; 148.72; 167.63; EIMS m/z 226 (M^+). Anal. Calcd for $C_{10}H_{14}N_2O_4$: C 53.09; H 6.24; N 12.38; found: C 53.06; H 6.25; N 12.39.

*4.9.3.6. 2-(2,3-Dichlorophenylamino)-N-hydroxyacetamide (**2f**)*

Light yellow power, 47%, mp 147-149°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.73 (d, *J*=5.8 Hz, 2H); 5.96 (t, *J*=5.3 Hz, 1H); 6.50 (d, *J*=8.2 Hz, 1H); 6.83 (d, *J*=7.6 Hz, 1H); 7.14 (t, *J*=8.1 Hz, 1H); 8.92 (s, 1H); 10.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 45.41; 109.36; 116.25; 117.80; 128.37; 132.29; 146.11; 168.02; EIMS m/z 234 (M^+). Anal. Calcd for $C_8H_8Cl_2N_2O_2$: C 40.88; H 3.43; Cl 30.16; N 11.92; found: C 40.91; H 3.43; Cl 30.13; N 11.93.

*4.9.3.7. 2-(2,4-Dichlorophenylamino)-N-hydroxyacetamide (**2g**)*

White power, 50%, mp 145-146°C, ^{1}H NMR(400 MHz, DMSO-d_6): 3.71 (d, *J*=5.8 Hz, 2H); 5.77 (t, *J*=6.0 Hz, 1H); 6.55 (t, *J*=8.9 Hz, 1H); 7.20 (dd, *J*=8.8 Hz, *J*=3.1 Hz, 1H); 7.38 (d, *J*=3.5 Hz, 1H); 9.86 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 44.34; 112.76; 118.97; 119.99; 128.21; 128.63; 143.38; 166.64; EIMS m/z 234 (M^+). Anal. Calcd for $C_8H_8Cl_2N_2O_2$: C 40.88; H 3.43; Cl 30.16; N 11.92; found: C 40.85; H 3.43; Cl 30.18; N 11.93.

*4.9.3.8. 2-(3,4-Dichlorophenylamino)-N-hydroxyacetamide (**2h**)*

Light yellow power, 55%, mp 116-118°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.60 (d, *J*=5.9 Hz, 2H); 6.45 (t, *J*=5.8 Hz, 1H); 6.55 (dd, *J*=8.8 Hz, *J*=2.7 Hz, 1H); 6.73 (d, *J*=2.4 Hz, 1H); 7.25 (d, *J*=8.8 Hz, 1H); 9.81 (bs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 44.93; 112.53; 118.75; 120.06; 128.29; 128.47; 143.62; 167.04; EIMS m/z 234 (M^+). Anal. Calcd for $C_8H_8Cl_2N_2O_2$: C 40.88; H 3.43; Cl 30.16; N 11.92; found: C 40.92; H 3.43; Cl 30.16; N 11.91.

*4.9.3.9. 2-(3-Chlorophenylamino)-N-hydroxyacetamide (**2i**)*

Light yellow power, 42%, mp 112-114°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.59 (d, *J*=6.0 Hz, 2H); 6.25 (t, *J*=5.8 Hz, 1H); 6.52 (d, *J*=8.0 Hz, 1H); 6.57 (d, *J*=8.0 Hz, 1H); 6.58 (s, 1H); 7.07 (t, *J*=8.2 Hz, 1H); 8.88 (s, 1H); 10.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 45.06;

110.54; 117.32; 119.08; 128.15; 129.70, 144.08, 166.88; EIMS m/z 200 (M^+). Anal. Calcd for $C_8H_9ClN_2O_2$: C 47.89; H 4.52; Cl 17.67; N 13.96; found: C 47.92; H 4.52; Cl 17.65; N 13.95.

*4.9.3.10. 2-(4-Chlorophenylamino)-N-hydroxyacetamide (**2j**)*

White power, 57%, mp 106-108°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.57 (d, *J*=6.1 Hz, 2H); 6.10 (t, *J*=6.0 Hz, 1H); 6.56 (d, *J*=8.8 Hz, 2H); 7.09 (d, *J*=8.8 Hz, 2H); 9.03 (s, 1H); 10.10 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 45.12; 107.55; 114.69; 131.80; 148.17; 167.06; EIMS m/z 200 (M^+). Anal. Calcd for $C_8H_9ClN_2O_2$: C 47.89; H 4.52; Cl 17.67; N 13.96; found: C 47.84; H 4.52; Cl 17.69; N 13.97.

*4.9.3.11. 2-(3-Bromophenylamino)-N-hydroxyacetamide (**2k**)*

White power, 48%, mp 102-103°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.58 (d, *J*=6.2 Hz, 2H); 6.27 (t, *J*=6.2 Hz, 1H); 6.55 (dd, *J*=8.3 Hz, *J*=2.2 Hz, 1H); 6.70 (dd, *J*=8.0 Hz, *J*=1.8 Hz, 1H); 6.71 (d, *J*=2.5 Hz, 1H); 7.01 (t, *J*=7.9 Hz, 1H); 9.55 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 44.93; 110.58; 117.21; 119.15; 128.09; 129.64, 144.18, 167.20; EIMS m/z 244 (M^+). Anal. Calcd for $C_8H_9BrN_2O_2$: C 39.21; H 3.70; Br, 32.60; N 11.43; found: C 39.25; H 3.70; Br, 32.56; N 11.44.

*4.9.3.12. 2-(4-Bromophenylamino)-N-hydroxyacetamide (**2l**)*

White power, 55%, mp 101-102°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.58 (d, *J*=6.0 Hz, 2H); 6.14 (t, *J*=6.2 Hz, 1H); 6.52 (d, *J*=8.6 Hz, 2H); 7.21 (d, *J*=8.7 Hz, 2H); 9.66 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 44.68; 107.36; 114.72; 131.74; 148.12; 167.14; EIMS m/z 244 (M^+). Anal. Calcd for $C_8H_9BrN_2O_2$: C 39.21; H 3.70; Br, 32.60; N 11.43; found: C 39.27; H 3.70; Br, 32.57; N 11.42.

*4.9.3.13. 2-(3,5-Dichlorophenylamino)-N-hydroxyacetamide (**2m**)*

Light yellow power, 60%, mp 121-122°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.62 (d, *J*=6.2 Hz, 2H); 6.56 (d, *J*=1.8 Hz, 2H); 6.62 (t, *J*=6.3 Hz, 1H); 6.64 (d, *J*=1.8 Hz, 1H); 8.90 (s, 1H); 10.60 (s, 1H); ^{13}C NMR(100 MHz, DMSO-d_6): 45.11; 110.78; 118.93; 138.10; 148.69; 167.82; EIMS m/z 234 (M^+). Anal. Calcd for $C_8H_8Cl_2N_2O_2$: C 40.88; H 3.43; Cl 30.16; N 11.92; found: C 40.84; H 3.43; Cl 30.19; N 11.93.

*4.9.3.14. N-Hydroxy-2-(3-nitrophenylamino)acetamide (**2n**)*

Yellow power, 45%, mp 119-121°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.71 (d, *J*=6.0 Hz, 2H); 6.72 (t, *J*=6.2Hz, 1H); 7.01 (dd, *J*=8.0 Hz, *J*=2.3 Hz, 1H); 7.28-7.38 (m, 2H); 7.40 (dd, *J*=7.7 Hz, *J*=2.1 Hz, 1H); 8.94 (s, 1H); 10.73 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 44.32; 105.95; 110.87; 119.21; 130.29; 149.18; 149.98; 166.77; EIMS m/z 211 (M^+). Anal. Calcd for $C_8H_9N_3O_4$: C 45.50; H 4.30; N 19.90; found: C 45.55; H 4.30; N 19.88.

*4.9.3.15. 2-(2,5-Dichlorophenylamino)-N-hydroxyacetamide (**2o**)*

Light yellow power, 47%, mp 141-142°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.74 (d, *J*=5.7 Hz, 2H); 5.94 (t, *J*=6.0 Hz, 1H); 6.56 (d, *J*=2.3 Hz, 1H); 6.64 (dd, *J*=8.4 Hz, *J*=2.3 Hz, 1H); 7.27 (d, *J*=8.4 Hz, 1H); 8.95 (s, 1H); 10.69 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 44.34; 112.76; 118.97; 119.99; 128.21; 128.63; 143.38; 166.64; EIMS m/z 234 (M^+). Anal. CalCd for $C_8H_8Cl_2N_2O_2$: C 40.88; H 3.43; Cl 30.16; N 11.92; found: C 40.91; H 3.43; Cl 30.14; N 11.90.

*4.9.3.16. N-Hydroxy-2-(phenylamino)acetamide (**2p**)*

White power, 46%, mp 124-125°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.58 (d, *J*=6.1 Hz, 2H);

6.85 (t, *J*=6.2 Hz, 1H); 6.34-6.78 (m, 3H); 7.08 (t, *J*=6.9 Hz, 2H); 9.80 (s, 2H); ^{13}C NMR(100 MHz, DMSO-d_6): 44.91; 112.80; 116.75; 129.25; 148.76; 167.53; EIMS m/z 166 (M^+). Anal. Calcd for $C_8H_{10}N_2O_2$: C 57.82; H 6.07; N 16.86; found: C 57.77; H 6.07; N 16.88.

*4.9.3.17. 2-(2,6-Dichlorophenylamino)-N-hydroxyacetamide(**2q**)*

White power, 51%, mp 131-132°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.88 (d, *J*=5.8 Hz, 2H); 5.30 (t, *J*=5.7 Hz, 1H); 6.84 (t, *J*=8.0 Hz, 1H); 7.32 (d, *J*=8.0 Hz, 2H); 8.94 (s, 1H); 10.60 (s, 1H); ^{13}C NMR(100 MHz, DMSO-d_6): 44.98; 110.89; 118.83; 138.27; 148.53; 167.19; EIMS m/z 234 (M^+). Anal. Calcd for $C_8H_8Cl_2N_2O_2$: C 40.88; H 3.43; Cl 30.16; N 11.92; found: C 40.85; H 3.43; Cl 30.18; N 11.93.

*4.9.3.18. 2-(3,5-Difluorophenylamino)-N-hydroxyacetamide (**2r**)*

White power, 43%, mp 138-140°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.60 (d, *J*=6.1 Hz, 2H); 6.22 (dd, *J*=10.8 Hz, *J*=2.3 Hz, 2H); 6.26 (dd, *J*=9.8 Hz, *J*=2.3 Hz, 1H); 6.62 (t, *J*=6.2 Hz, 1H); 8.90 (s, 1H); 10.64 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 43.07; 90.63 (t, *J*=26.1 Hz); 94.76 (d, *J*=28.1 Hz); 151.72 (t, *J*=13.4 Hz); 164.09 (dd, *J*=241.5 Hz; *J*=16.4 Hz); 167.19; EIMS m/z 202 (M^+). Anal. Calcd for $C_8H_8F_2N_2O_2$: C 47.53; H 3.99; F 18.80; N 13.86; found: C 47.57; H 3.99; F 18.78; N 13.85.

*4.9.3.19. N-Hydroxy-2-(p-tolylamino)acetamide (**2s**)*

Light yellow power, 50%, mp 139-140°C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.15 (s, 3H); 3.54 (d, *J*=6.1 Hz, 2H); 5.60 (t, *J*=6.2 Hz, 1H); 6.47 (dd, *J*=8.3 Hz, 2H); 6.89 (d, *J*=8.0 Hz, 2H); 8.80 (s, 1H); 10.53 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.99; 45.16; 107.31; 114.80; 131.71; 148.19; 167.05; EIMS m/z 180 (M^+). Anal. Calcd for $C_9H_{12}N_2O_2$: C 59.99; H 6.71; N 15.55; found: C 59.97; H 6.71; N 15.56.

*4.9.3.20. N-Hydroxy-2-(4-methoxyphenylamino)acetamide (**2t**)*

White power, 48%, mp 139-140°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.52 (d, *J*=6.2 Hz, 2H); 3.64 (s, 3H); 5.42 (t, *J*=6.3 Hz, 1H); 6.52 (d, *J*=8.9 Hz, 2H); 6.72 (d, *J*=8.9 Hz, 2H); 8.80 (s, 1H); 10.54 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 44.92; 55.14; 107.43; 114.60; 131.82; 148.06; 167.79; EIMS m/z 196 (M^+). Anal. Calcd for $C_9H_{12}N_2O_3$: C 55.09; H 6.16; N 14.28; found: C 55.06; H 6.17; N 14.29.

*4.9.3.21. 2-(2-Chlorophenylamino)-N-hydroxyacetamide (**2u**)*

White power, 57%, mp 121-123°C, ^{1}H NMR(400 MHz, DMSO-d_6): 3.69 (d, *J*=5.9 Hz, 2H); 5.56 (t, *J*=5.9 Hz, 1H); 6.55 (dd, *J*=8.2 Hz, *J*=2.5 Hz, 1H); 6.62 (td, *J*=7.6 Hz, *J*=1.5 Hz, 1H) 7.12 (td, *J*=7.7 Hz, *J*=1.5 Hz, 1H); 7.26 (dd, *J*=7.9 Hz, *J*=1.5 Hz, 1H); 8.89 (s, 1H); 10.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 44.36; 111.87; 117.56; 118.53; 128.43; 129.39, 144.14, 166.92; EIMS m/z 200 (M^+). Anal. Calcd for $C_8H_9ClN_2O_2$: C 47.89; H 4.52; Cl 17.67; N 13.96; found: C 47.92; H 4.52; Cl 17.66; N 13.95.

*4.9.3.22. 2-(2-Bromophenylamino)-N-hydroxyacetamide (**2v**)*

White power, 48%, mp 103-105°C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.69 (d, *J*=5.7 Hz, 2H); 5.42 (t, *J*=5.6 Hz, 1H); 6.53-6.59 (m, 2H); 7.18 (td, *J*=8.2 Hz, *J*=1.5 Hz, 1H); 7.42 (dd, *J*=7.8 Hz, *J*=1.5 Hz, 1H); 8.91 (s, 1H); 10.67 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 45.30; 112.05; 117.94; 118.89; 128.36; 129.32, 144.27, 167.11; EIMS m/z 244 (M^+). Anal. Calcd for $C_8H_9BrN_2O_2$: C 39.21; H 3.70; Br, 32.60; N 11.43; found: C 39.25; H 3.70; Br, 32.58; N 11.42.

*4.9.3.23. 2-(3,4-Dihydroquinolin-1(2H)-yl)-N-hydroxyacetamide (**2w**)*

White power, 53%, mp 132-134 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 1.89 (p, *J*=6.2 Hz, 2H); 2.68 (t, *J*=6.2 Hz, 2H); 3.34-3.37 (m, 2H); 3.76 (s, 2H); 6.42 (d, *J*=8.2 Hz, 1H); 6.50 (t, *J*=7.3 Hz, 1H); 6.88 (d, *J*=7.2 Hz, 1H); 6.93 (t, *J*=7.5 Hz, 1H); 8.81(s, 1H); 10.56 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 22.14; 28.07; 47.71; 49.08; 110.99; 115.78; 122.40; 127.46; 129.43; 144.98; 168.06; EIMS m/z 206 (M^+). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C 64.06; H 6.84; N 13.58; found: C 64.09; H 6.84; N 13.56.

*4.9.3.24. 3-(3,5-Dichlorophenylamino)-N-hydroxypropanamide (**3a**)*

White power, 52%, mp 131-132 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.29 (t, *J*=6.5 Hz, 2H); 3.48 (q, *J*=6.2 Hz, 2H); 6.56 (d, *J*=1.8 Hz, 2H); 6.62 (t, *J*=6.0 Hz, 1H); 6.64 (d, *J*=1.8 Hz, 1H); 8.85 (s, 1H); 10.54 (s, 1H); ^{13}C NMR(100 MHz, DMSO-d_6): 32.01; 44.95; 110.86; 118.87; 138.09; 148.68; 167.53; EIMS m/z 248 (M^+). Anal. Calcd for $C_9H_{10}Cl_2N_2O_2$: C, 43.40; H, 4.05; Cl, 28.47; N, 11.25; found: C, 43.43; H, 4.05; Cl, 28.46; N, 11.24.

*4.9.3.25. N-Hydroxy-3-(phenylamino)propanamide (**3b**)*

White power, 49%, mp 115-117 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.23 (t, *J*=7.1 Hz, 2H); 3.23 (q, *J*=6.7 Hz, 2H); 5.54 (t, *J*=5.9 Hz, 1H); 6.53 (t, *J*=7.3 Hz, 1H); 6.56 (d, *J*=7.8 Hz, 2H); 7.07 (t, *J*=7.8 Hz, 2H); 8.76 (s, 1H); 10.43 (s, 1H); ^{13}C NMR(100 MHz, DMSO-d_6): 32.77; 44.05; 112.60; 116.62; 129.39; 148.97; 168.01; EIMS m/z 180 (M^+). Anal. Calcd for $C_9H_{12}N_2O_2$: C 59.99; H 6.71; N 15.55; found: C 59.95; H 6.72; N 15.57.

*4.9.3.26. N-Hydroxy-3-(2-methoxyphenylamino)propanamide (**3c**)*

Light yellow power, 48%, mp 101-102 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.27 (t, *J*=6.6 Hz, 2H); 3.27 (q, *J*=6.4 Hz, 2H); 3.76 (s, 3H); 4.88 (t, *J*=5.9 Hz, 2H); 6.55(d, *J*=7.3 Hz, 1H); 6.56(d, *J*=7.0 Hz, 1H); 6.78 (t, *J*=8.0 Hz, 1H); 6.79 (t, *J*=7.9 Hz, 1H); 8.81 (s, 1H); 10.49 (s,

1H); ^{13}C NMR(100 MHz, DMSO-d_6): 32.44; 44.67; 55.71; 109.86; 110.27; 116.80; 121.45; 137.91; 146.90; 167.73; EIMS m/z 210 (M^+). Anal. Calcd for $C_{10}H_{14}N_2O_3$: C 57.13; H 6.71; N 13.33; found: C 57.11; H 6.72; N 13.34.

*4.9.3.27. 3-(2-Chlorophenylamino)-N-hydroxypropanamide (**3d**)*

Light yellow power, 42%, mp 101-102 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.30 (t, *J*=6.8 Hz, 2H); 3.35 (q, *J*=6.4 Hz, 2H); 5.39 (t, *J*=5.8 Hz, 1H); 6.60 (td, *J*=7.6 Hz, *J*=1.4 Hz, 1H); 6.74 (dd, *J*=8.2 Hz, *J*=1.4 Hz, 1H); 7.15 (td, *J*=7.7 Hz, *J*=1.5 Hz, 1H); 7.25 (dd, *J*=7.8 Hz, *J*=1.5 Hz, 1H); 8.82 (s, 1H); 10.51 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.18; 44.79; 111.80; 117.51; 118.59; 128.37; 129.42, 144.21, 167.12; EIMS m/z 214 (M^+). Anal. Calcd for $C_9H_{11}ClN_2O_2$: C 50.36; H 5.17; Cl 16.52; N 13.05; found: C 50.31; H 5.17; Cl 16.53; N 13.06.

*4.9.3.28. 3-(4-Fluorophenylamino)-N-hydroxypropanamide (**3e**)*

Light yellow power, 58%, mp: 100-102 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.22 (t, *J*=7.1 Hz, 2H); 3.20 (t, *J*=7.1 Hz, 2H); 5.50 (s, 1H); 6.55 (dd, *J*=9.0 Hz, *J*=4.3 Hz, 2H); 6.92 (t, *J*=8.8 Hz, 2H); 8.75 (s, 1H); 10.43 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 33.01; 45.09; 113.57 (d, *J*=7.2Hz); 115.66 (d, *J*=22.4Hz); 145.43; 155.07 (d, *J*=232.7Hz); 167.44; EIMS m/z 198 (M^+). Anal. Calcd for $C_9H_{11}FN_2O_2$: C 54.54; H 5.59; F 9.59; N 14.13; found: C 54.57; H 5.59; F 9.58; N 14.12.

*4.9.3.29. 3-(2-Bromophenylamino)-N-hydroxypropanamide (**3f**)*

Light yellow power, 53%, mp 121-123 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.30 (t, *J*=6.7 Hz, 2H); 3.34 (q, *J*=6.5 Hz, 2H); 5.27 (t, *J*=5.8 Hz, 1H); 6.54 (td, *J*=7.6 Hz, *J*=1.5 Hz, 1H); 6.72(dd, *J*=8.2 Hz, *J*=1.5 Hz, 1H); 7.19 (td, *J*=7.9 Hz, *J*=1.5 Hz, 1H); 7.41 (dd, *J*=7.8 Hz,

J=1.5 Hz, 1H); 8.82 (s, 1H); 10.52 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.07; 40.05; 109.18; 111.76; 117.78; 129.17; 132.71; 145.24; 168.18; EIMS m/z 258 (M^+). Anal. Calcd for $C_9H_{11}BrN_2O_2$: C 47.12; H 4.28; Br 30.84; N 10.81; found: C 47.16; H 4.28; Br 30.82; N 10.80.

*4.9.3.30. 3-(3,5-Dimethylphenylamino)-N-hydroxypropanamide (**3g**)*

White power, 55%, mp 127-128 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.14 (s, 6H); 2.21 (t, *J*=7.1 Hz, 2H); 3.21 (q, *J*=6.7 Hz, 2H); 5.35 (t, *J*=5.9 Hz, 1H); 6.18 (s, 2H); 6.19 (s, 1H); 8.74 (s, 1H); 10.42 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 21.75; 32.80; 39.86; 110.52; 118.27; 138.11; 148.93; 168.15; EIMS m/z 208 (M^+). Anal. Calcd for $C_{11}H_{16}N_2O_2$: C 63.44; H 7.74; N 13.45; found: C 63.48; H 7.73; N 13.43.

*4.9.3.31. 3-(2,5-Dichlorophenylamino)-N-hydroxypropanamide (**3h**)*

White power, 48%, mp: 123-124 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.28 (t, *J*=6.8 Hz, 2H); 3.36(q, *J*=6.7 Hz, 2H); 5.74 (t, *J*=5.8 Hz, 1H); 6.61 (dd, *J*=8.4 Hz, *J*=2.4 Hz, 1H); 6.76 (d, *J*=2.4 Hz, 1H); 7.26 (d, *J*=8.4 Hz, 1H); 8.82 (s, 1H); 10.52 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.15; 44.97; 112.71; 118.92; 119.98; 128.26; 128.55; 143.42; 166.99; EIMS m/z 248 (M^+). Anal. Calcd for $C_9H_{10}Cl_2N_2O_2$: C 43.40; H 4.05; Cl 28.47; N 11.25; found: C 43.43; H 4.05; Cl 28.45; N 11.24.

*4.9.3.32. 3-(3,4-Dichlorophenylamino)-N-hydroxypropanamide (**3i**)*

White power, 43%, mp 123-125 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.21 (t, *J*=6.9 Hz, 2H); 3.24 (q, *J*=6.6 Hz, 2H); 6.13 (t, *J*=5.9 Hz, 1H); 6.55 (dd, *J*=8.8 Hz, *J*=2.8 Hz, 1H); 6.74 (d, *J*=2.7 Hz, 1H); 7.25 (d *J*=8.7 Hz, 1H); 8.76 (s, 1H); 10.43 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.30; 45.11; 112.82; 118.93; 119.92; 128.27; 128.66; 143.34; 167.04; EIMS m/z

248 (M^+). Anal. Calcd for $C_9H_{10}Cl_2N_2O_2$: C 43.40; H 4.05; Cl 28.47; N 11.25; found: C 43.37; H 4.05; Cl 28.49; N 11.26.

*4.9.3.33. 3-(2,6-Dichlorophenylamino)-N-hydroxypropanamide (**3j**)*

White power, 47%, mp 103-105 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.23 (t, *J*=6.8 Hz, 2H); 3.48 (q, *J*=6.7 Hz, 2H); 4.86 (t, *J*=6.6 Hz, 1H); 6.87 (t, *J*=8.0 Hz, 1H); 7.34 (dd, *J*=8.0 Hz, *J*=1.5 Hz, 2H); 8.79 (s, 1H); 10.48 (s, 1H); ^{13}C NMR(100 MHz, DMSO-d_6): 32.06; 44.92; 110.95; 118.76; 138.23; 148.35; 167.20; EIMS m/z 248 (M^+). Anal. Calcd for $C_9H_{10}Cl_2N_2O_2$: C 43.40; H 4.05; Cl 28.47; N 11.25; found: C 43.45; H 4.05; Cl 28.45; N 11.24.

*4.9.3.34. N-Hydroxy-3-(4-nitrophenylamino) propanamide (**3k**)*

Yellow power, 42%, mp 159-160 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.28 (t, *J*=6.7 Hz, 2H); 3.40 (q, *J*=6.6 Hz, 2H); 6.65 (d, *J*=9.3 Hz, 2H); 7.35 (t, *J*=5.8 Hz, 1H); 7.99 (d, *J*=9.2 Hz, 2H); 8.82 (s, 1H); 10.49 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.27; 45.12; 107.44; 114.89; 134.21; 149.38; 167.14; EIMS m/z 225 (M^+). Anal. Calcd for $C_9H_{11}N_3O_4$: C 48.00; H 4.92; N 18.66; found: C 47.97; H 4.92; N 18.67.

*4.9.3.35. N-Hydroxy-3-(3-nitrophenylamino) propanamide (**3l**)*

Yellow power, 49%, mp 130-131 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.26 (t, *J*=6.1 Hz, 2H); 3.33 (q, *J*=6.2 Hz, 2H, δ 3.34 containing the HOD signal); 6.44 (t, *J*=5.8 Hz, 1H); 6.99 (d, *J*=2.3 Hz, 1H); 7.20-7.52 (m, 3H); 9.70 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.32; 39.45; 105.63; 110.26; 118.67; 130.39; 149.30; 149.97; 167.86; EIMS m/z 225 (M^+). Anal. Calcd for $C_9H_{11}N_3O_4$: C 48.00; H 4.92; N 18.66; found: C 48.04; H 4.92; N 18.65.

*4.9.3.36. N-Hydroxy-3-(2-nitrophenylamino) propanamide (**3m**)*

Red power, 51%, mp 144-145 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.40 (t, *J*=6.4 Hz, 2H); 3.59 (q, *J*=6.3 Hz, 2H); 6.70 (t, *J*=8.1 Hz, 1H); 7.08 (d, *J*=8.1 Hz, 1H); 7.55 (t, *J*=7.3 Hz, 1H); 8.07 (d, *J*=8.0 Hz, 1H); 8.27 (t, *J*=5.8 Hz, 1H); 9.00 (s, 1H); 10.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.19; 41.45; 105.90; 110.33; 118.72; 130.35; 149.37; 149.95; 167.28; EIMS m/z 225 (M^+). Anal. Calcd for $C_9H_{11}N_3O_4$: C 48.00; H 4.92; N 18.66; found: C 47.97; H 4.92; N 18.67.

*4.9.3.37. 3-(2,4-Dichlorophenylamino)-N-hydroxypropanamide (**3n**)*

White power, 40%, mp: 111-113 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.29 (t, *J*=6.9 Hz, 2H); 3.34 (q, *J*=6.5 Hz, 2H), 5.58 (t, *J*=6.6 Hz, 1H); 6.74 (d, *J*=8.8 Hz, 1H); 7.19 (dd, *J*=8.8 Hz, *J*=2.4 Hz, 1H); 7.35 (d, *J*=2.0 Hz, 1H); 8.84 (s, 1H); 10.52 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.05; 40.24; 112.50; 118.83; 119.52; 128.34; 128.67; 143.42; 168.10; EIMS m/z 248 (M^+). Anal. Calcd for $C_9H_{10}Cl_2N_2O_2$: C 43.40; H 4.05; Cl 28.47; N 11.25; found: C 43.45; H 4.05; Cl 28.45; N 11.24.

*4.9.3.38. 3-(2,3-Dichlorophenylamino)-N-hydroxypropanamide (**3o**)*

White power, 47%, mp 154-144 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.29 (t, *J*=6.0 Hz, 2H); 3.37 (q, *J*=6.0 Hz, 2H); 5.76 (t, *J*=5.6 Hz, 1H); 6.71 (d, *J*=8.3 Hz, 1H); 6.80 (d, *J*=7.9 Hz, 1H); 7.15 (t, *J*=8.1 Hz, 1H); 8.85 (s, 1H); 10.54 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.06; 40.05; 109.91; 116.00; 117.25; 128.94; 132.10; 146.06; 168.06; EIMS m/z 248 (M^+). Anal. Calcd for $C_9H_{10}Cl_2N_2O_2$: C 43.40; H 4.05; Cl 28.47; N 11.25; found: C 43.36; H 4.05; Cl 28.49; N 11.26.

*4.9.3.39. 3-(3,5-Difluorophenylamino)-N-hydroxypropanamide (**3p**)*

White power, 51%, mp 102-103 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.22 (t, *J*=6.3 Hz, 2H);

3.25 (q, *J*=6.5 Hz, 2H); 6.19 (t, *J*=8.2 Hz, 1H); 6.21 (d, *J*=9.6 Hz, 1H); 6.34 (t, *J*=6.2 Hz, 1H); 8.81 (s, 1H); 10.47 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 32.40; 39.45; 90.52 (t, *J*=26.7 Hz); 94.92 (d, *J*=27.7 Hz); 151.63 (t, *J*=13.9 Hz); 164.01 (dd, *J*=240.7 Hz; *J*=16.7 Hz); 167.82; EIMS m/z 216 (M^+). Anal. Calcd for $C_9H_{10}F_2N_2O_2$: C 50.00; H 4.66; F 17.58; N 12.96; found: C 50.06; H 4.66; F 17.56; N 12.95.

*4.9.3.40. 3-(3,4-Dihydroquinolin-1(2H)-yl)-N-hydroxypropanamide (**3q**)*

Light yellow power, 57%, mp 218-220 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 1.83 (quint, *J*=6.3 Hz, 2H); 2.20 (t, *J*=7.1 Hz, 2H); 2.65 (t, *J*=6.4 Hz, 2H); 3.21 (t, *J*=5.6 Hz, 2H); 3.47 (t, *J*=7.1 Hz, 2H); 6.47 (t, *J*=7.3 Hz, 1H); 6.58 (d, *J*=8.2 Hz, 1H); 6.86 (d, *J*=7.3 Hz, 1H); 6.96 (t, *J*=7.8 Hz, 1H); 8.76 (s, 1H); 10.46 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 22.19; 28.00; 29.72; 47.66; 49.05; 110.93; 115.84; 122.42; 127.40; 129.36; 144.93; 168.16; EIMS m/z 220 (M^+). Anal. Calcd for $C_{12}H_{16}N_2O_2$: C 65.43; H 7.32; N 12.72; found: C 65.46; H 7.32; N 12.71.

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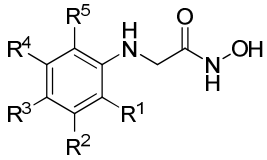
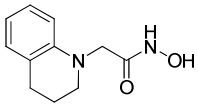
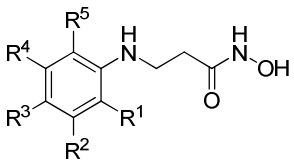
Table 1 Structure and inhibitory activity (IC_{50}) against *H. pylori* urease of **2a-2w**

Entry						IC_{50}(μM) against urease	
	R_1	R_2	R_3	R_4	R_5	Cell-free	intact cell[a]
2a	H	H	F	H	H	7.41±0.14	ND
2b	H	Me	H	Me	H	2.12±0.12	ND
2c	OMe	H	H	H	H	152±6	ND
2d	H	OMe	H	H	H	21.81±0.66	ND
2e	H	OMe	H	OMe	H	2.28±0.08	ND
2f	Cl	Cl	H	H	H	14.8±1.2	ND
2g	Cl	H	Cl	H	H	66.1±2.8	ND
2h	H	Cl	Cl	H	H	0.76±0.02	41.6±3.5
2i	H	Cl	H	H	H	1.15±0.03	ND
2j	H	H	Cl	H	H	2.47±0.12	ND
2k	H	Br	H	H	H	0.86±0.03	48.1±3.8
2l	H	H	Br	H	H	2.01±0.05	ND
2m	H	Cl	H	Cl	H	0.13±0.02	16.9±1.1
2n	H	NO_2	H	H	H	8.21±0.36	ND
2o	Cl	H	H	Cl	H	1.64±0.07	ND
2p	H	H	H	H	H	4.67±0.34	ND
2q	Cl	H	H	H	Cl	191±7	ND
2r	H	F	H	F	H	5.11±0.23	ND
2s	H	H	Me	H	H	3.91±0.17	ND
2t	H	H	OMe	H	H	69.8±4.1	ND
2u	Cl	H	H	H	H	58.9±3.3	ND
2v	Br	H	H	H	H	12.3±0.9	ND
2w						71.1±4.5	ND

AHA	--	27.9±1.5	172±7

[a]ND means No Determination.

Table 2 Structure and inhibitory activity (IC_{50}) against *H. pylori* urease of **3a-3q**

Entry						IC_{50} (μM)	
	R_1	R_2	R_3	R_4	R_5	Cell-free	intact cell[a]
3a	H	Cl	H	Cl	H	0.043±0.005	3.9±0.10
3b	H	H	H	H	H	1.22 ± 0.15	ND
3c	OMe	H	H	H	H	1.64 ± 0.18	ND
3d	Cl	H	H	H	H	0.055 ± 0.008	5.91 ± 0.14
3e	H	H	F	H	H	0.18 ± 0.02	17.9 ± 1.2
3f	Br	H	H	H	H	0.13 ± 0.02	14.5 ± 0.9
3g	H	Me	H	Me	H	2.93 ± 0.13	ND
3h	Cl	H	H	Cl	H	0.45 ± 0.09	29.5±1.8
3i	H	Cl	Cl	H	H	0.71 ± 0.05	38.9±2.7
3j	Cl	H	H	H	Cl	25 ± 3.2	ND
3k	H	H	NO_2	H	H	1.23 ± 0.17	ND
3l	H	NO_2	H	H	H	5.1 ± 0.41	ND
3m	NO_2	H	H	H	H	10.53 ± 1.21	ND
3n	Cl	H	Cl	H	H	0.018 ± 0.002	0.14 ±0.01
3o	Cl	Cl	H	H	H	3.83 ± 0.53	ND

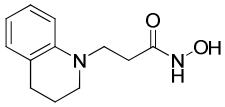
3p	H	F	H	F	H	1.52 ± 0.33	ND
3q						21.13 ± 0.75	ND
AHA			---			27.9±1.5	172±7

Table 3 Kinetic constants K_i and K_i' of **2m**, **3a** and **3n**

Entry	K_i (μg/mL)	K_i' (μg/mL)
2m	0.39	0.074
3a	0.39	0.038
3n	0.11	0.015

Table 4 Acute toxicities in mice

compound	LD_{50}(mg/kg)
3a	2982.8
3d	3349.4
3n	3126.9

Table 5 *In vivo* activities of selected compounds.

compound	dose (mg/kg b.i.d)	eradication rate (%)
3a	32	92.3
	64	100
	128	100
3d	32	84.6
	64	92.3
	128	100
3n	16	92.3
	32	100
	64	100
Acetohydroxamic acid	32	44.4

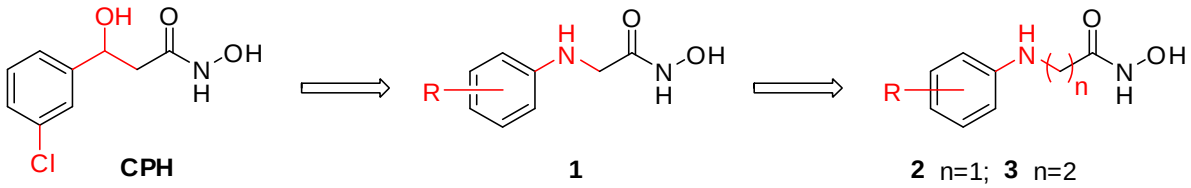


Figure 1 Design strategies for modification of the lead CPH

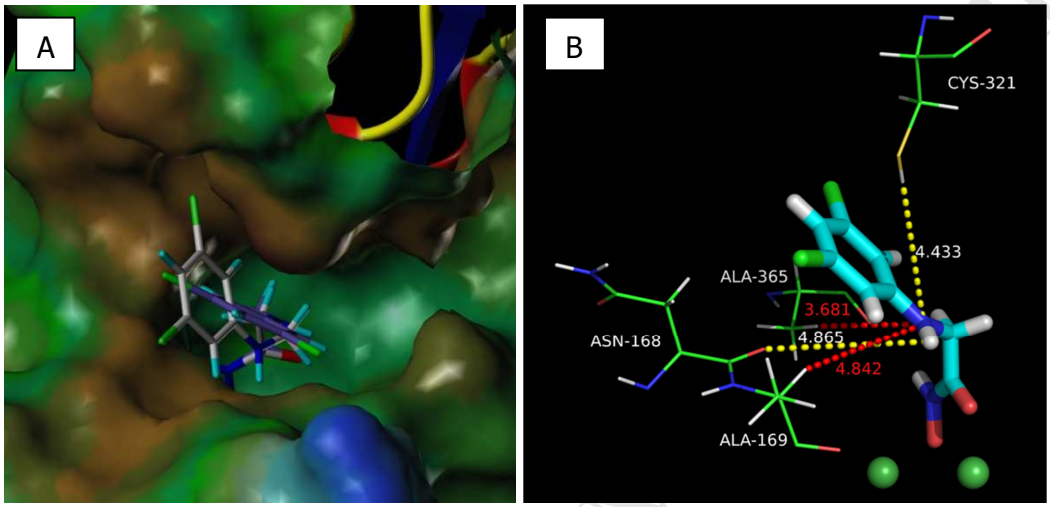


Figure 2 Predicted binding mode of ligand-urease (PDB code: 1E9Y). (A) Enzyme shown as surface, hydrophobic areas shown as brown and hydrophilic areas shown as blue; **2m** shown as dark purple, **3a** shown as white. (B) Enzyme shown as lines and the ligand **2m** shown as sticks, hydrogen bonds shown as yellow dashed lines and possible hydrophobic interactions shown as red dashed lines.

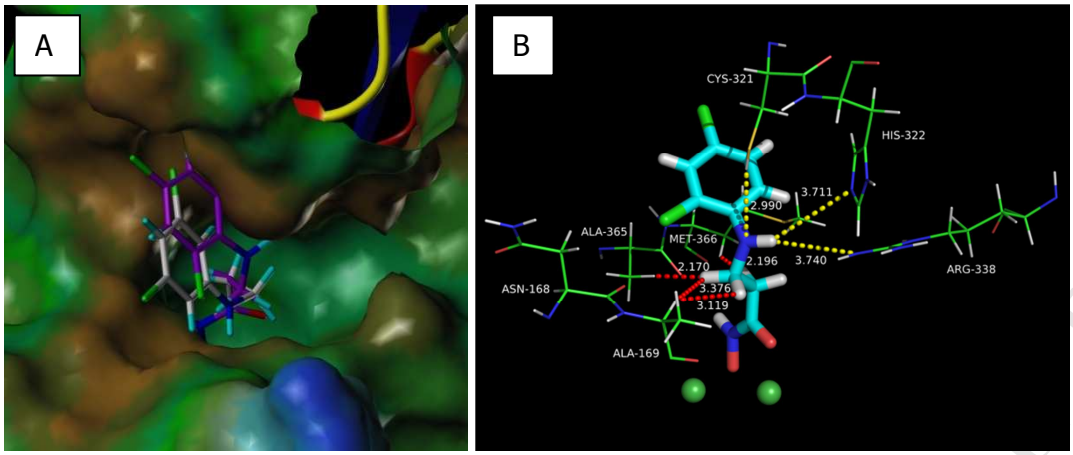


Figure 3 Predicted binding mode of of ligand-urease (PDB code: 1E9Y). (A) Enzyme shown as surface, hydrophobic areas shown as brown and hydrophilic areas shown as blue; **3a** shown as white, **3n** shown as purple. (B) Enzyme shown as lines and the ligand **3n** shown as sticks, hydrogen bonds shown as yellow dashed lines and hydrophobic contacts shown as red dashed lines.

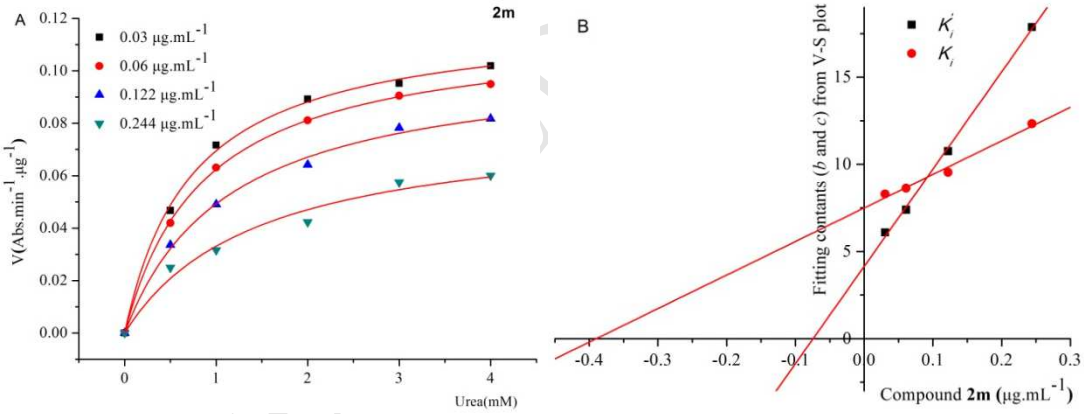


Figure 4 Kinetic plots of **2m**. (A) Velocity (V) was nonlinearly fitted against the concentrations of urea [S] in the presence of a specific concentration of compound **2m**; (B) The fitting constants (b) and (c) from the V-S plots were plotted against concentrations of compound **2m**.

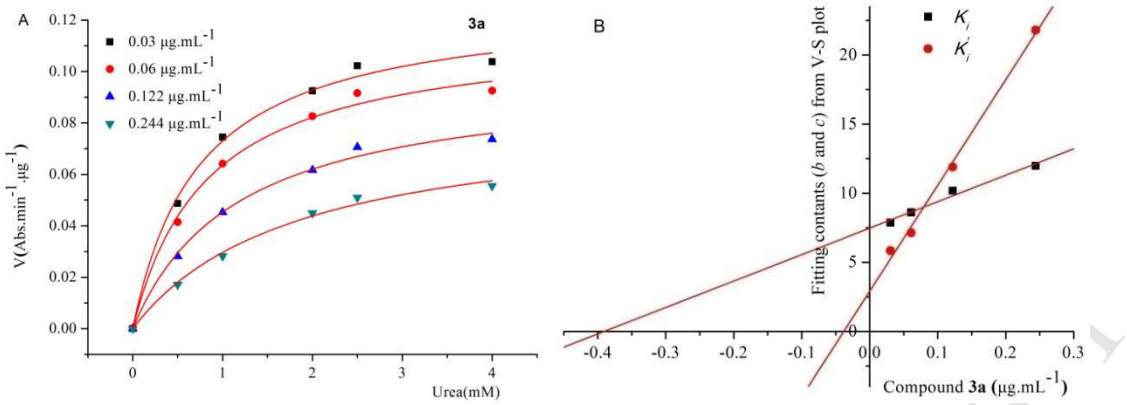


Figure 5 Kinetic plots of **3a**. (A) Velocity (V) was nonlinearly fitted against the concentrations of urea [S] in the presence of a specific concentration of compound **3a**; (B) The fitting constants (b) and (c) from the V-S plots were plotted against concentrations of compound **3a**.

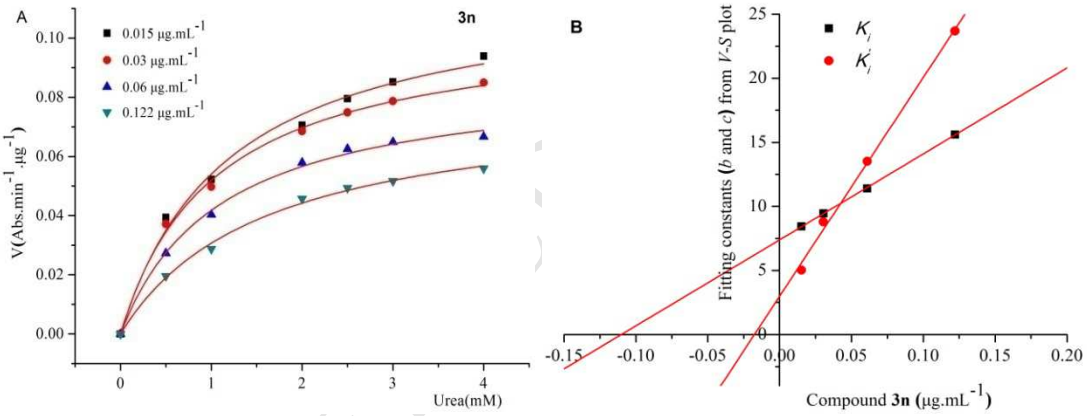


Figure 6 Kinetic plots of **3n**. (A) Velocity (V) was nonlinearly fitted against the concentrations of urea [S] in the presence of a specific concentration of compound **3n**; (B) The fitting constants (b) and (c) from the V-S plots were plotted against concentrations of compound **3n**.

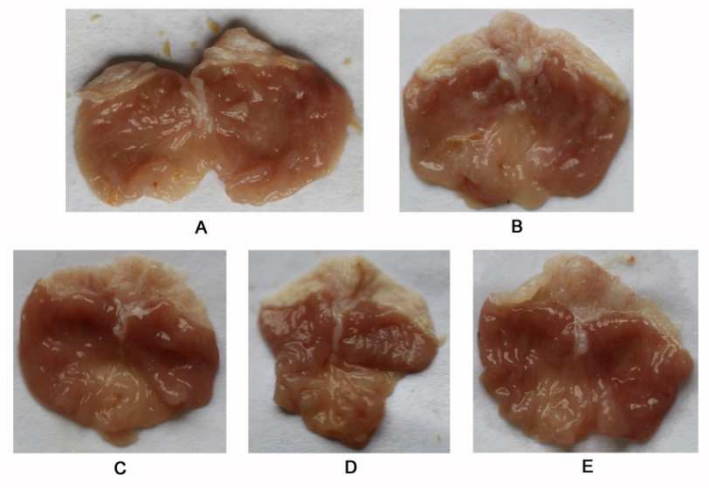


Figure 7 (A) Mouse stomach of *H. pylori*-infected (gastritis) mice model; (B) Mouse stomach after treatment with 32 mg/kg b.i.d of AHA; (C) Mouse stomach after treatment with 32 mg/kg b.i.d of **3a**; (D) Mouse stomach after treatment with 32 mg/kg b.i.d of **3d**; (E) Mouse stomach after treatment with 16 mg/kg b.i.d of **3n**.

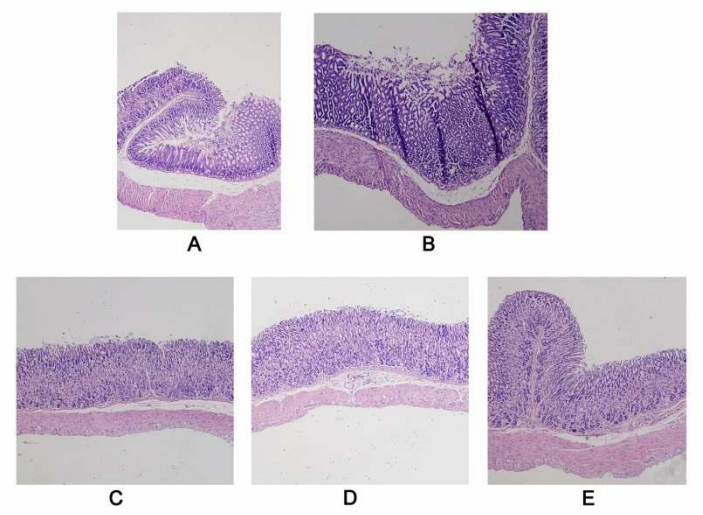
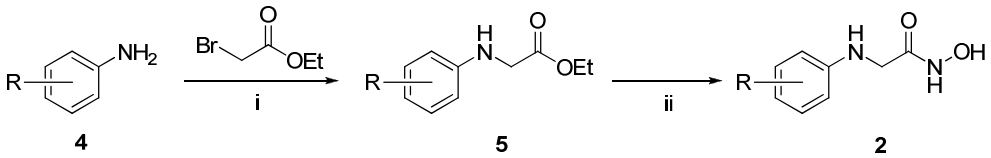
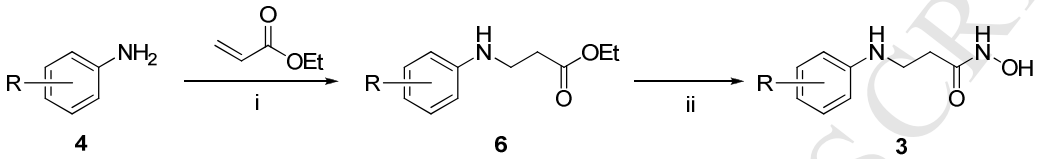


Figure 8 (A) Mouse stomach slices of *H. pylori*-infected (gastritis) mice model; (B) Mouse stomach slices after treatment with 32 mg/kg b.i.d of AHA; (C) Mouse stomach slices after treatment with 32 mg/kg b.i.d of **3a**; (D) Mouse stomach slices after treatment with 32 mg/kg b.i.d of **3d**; (E) Mouse stomach slices after treatment with 16 mg/kg b.i.d of **3n**.



Scheme 1. Synthesis of 2-(*N*-arylamino)acetohydroxamic acids (**2**). (i) K_2CO_3, DMSO, room temperature or acetone, reflux; (ii) $NH_2OH \cdot HCl$, MeONa, MeOH, room temperature.



Scheme 2. Synthesis of 3-(*N*-arylamino)propionylhydroxamic acids (**2**). (i) TFA, reflux; (ii) $NH_2OH \cdot HCl$, MeONa, MeOH, room temperature.

N-arylaminoacylhydroxamic acids were designed by structural improvement of 3-(3-chlorophenyl)-3-hydroxypropionylhydroxamic acid found in our group. > *N*-arylaminoacylhydroxamic acids were firstly reported as urease inhibitors. > Significant improvement of potency. > A dual site binding mode of these compounds was firstly disclosed.