European Journal of Medicinal Chemistry 49 (2012) 164–171

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and evaluation of anti-tubercular and antibacterial activities of new 4-(2,6-dichlorobenzyloxy)phenyl thiazole, oxazole and imidazole derivatives. Part 2

Xiaoyun Lu [a,b], Xiaobo Liu [a], Baojie Wan [c], Scott G. Franzblau [c], Lili Chen [d], Changlin Zhou [d], Qidong You [a,*]

[a] Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China
[b] Key Laboratory of Regenerative Biology and Institute of Chemical Biology, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, No. 190, Kaiyuan Avenue, Science Park, Guangzhou 510530, China
[c] Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA
[d] School of Life Science & Technology, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 24 October 2011
Received in revised form
4 January 2012
Accepted 5 January 2012
Available online 12 January 2012

Keywords:
4-(2,6-dichlorobenzyloxy)phenyl thiazole derivatives
Anti-tubercular activity
Antibacterial activity

ABSTRACT

A series of substituted 4-(2,6-dichlorobenzyloxy)phenyl thiazole, oxazole and imidazole derivatives were synthesized. The derivatives were screened for *in vitro* anti-tubercular activities against *Mycobacterium tuberculosis* H37Rv using the Microplate Alamar Blue Assay (MABA), and antibacterial activities with agar dilution method against clinical *S. aureus*, *E. coli*, *S. pneumoniae* and *penicilin-resistant S. pneumoniae*. Among 15 compounds, several thiazole derivatives exhibited good anti-tubercular activities with MIC values between 1 μM and 61.2 μM, and potent activities against *S. pneumoniae* with MIC values less than 0.134 μM. These studies suggest that the thiazole scaffold may serve as a new promising template for further elaboration as anti-tubercular and antibacterial drugs.

1. Introduction

Tuberculosis (TB), a highly contagious and air-borne disease caused by *Mycobacterium tuberculosis*, emerged with multi-drug resistant strains (MDR-TB) and acquired immune deficiency syndrome (AIDS) in recent years [1,2]. According to World Health Organization (WHO) [3], one third of the world's population is infected with *M. tuberculosis* and approximately 1.7 million deaths occured worldwide in 2009. In spite of the increasing worldwide incidence of TB, no new drugs have been brought to the market over the past four decades. Therefore, it is an imperative need to develop novel anti-tubercular drugs that can be equally effective against *M. tuberculosis* and MDR-TB, and also reduce the duration of therapy.

Our research group have recently disclosed that 2-acylated and 2-alkylated amino-5-(4-(benzyloxy)phenyl)thiophene-3-carboxylic acid, exemplified by compounds **1** and **2** (Fig. 1), were potent anti-tubercular agents [4]. Thiazole, oxazole and imidazole are indispensable structural units for medicinal chemists and have attracted continuing interest over the years as their varied biological activities, such as antibacterial [5–8], anti-tubercular [9,10], antifungal [11], anticancer [12,13] and anti-inflammatory [14], etc. In continuation of our research efforts to develop new therapeutics for treatment of TB, we envisioned the replacement of the thiophene nucleus by thiazole, oxazole and imidazole isostere to provide the general structure (Fig. 2). The major suggestion derived from previous study was that the compound containing *p*-chlorobenzamide group at thiophene ring was proved to be highly active in the amide series. Accordingly, with the aim to further explore the structure–activity relationships (SARs) of diazoles, which are presumed to be fundamental for anti-tubercular activity, a series of 4-(2,6-dichlorobenzyloxy)phenyl thiazole, oxazole and imidazole derivatives (**10a**–**f**, **11a**–**e**, **20**, **21**, **26** and **27**) were synthesized and evaluated for their anti-tubercular activity.

2. Chemistry

The synthetic strategies employed to obtain the target compounds are depicted in Schemes 1–3. For thiazole series, the key intermediate ethyl 2-amino-4-(4-(2,6-dichlorobenzyloxy)

* Corresponding author. Tel./fax: +86 25 83271351.
E-mail address: youqidong@gmail.com (Q. You).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2012.01.007

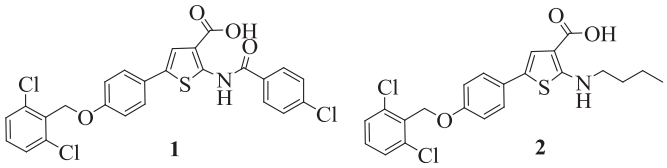


Fig. 1. The chemical structures of thiophene-based lead compounds **1** and **2**.

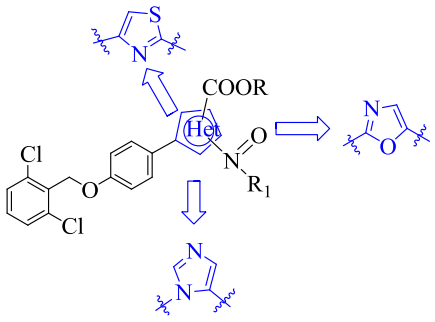


Fig. 2. The general structure designed by replacement of thiophene nucules by thiazole, oxazole and imidazole, respectively.

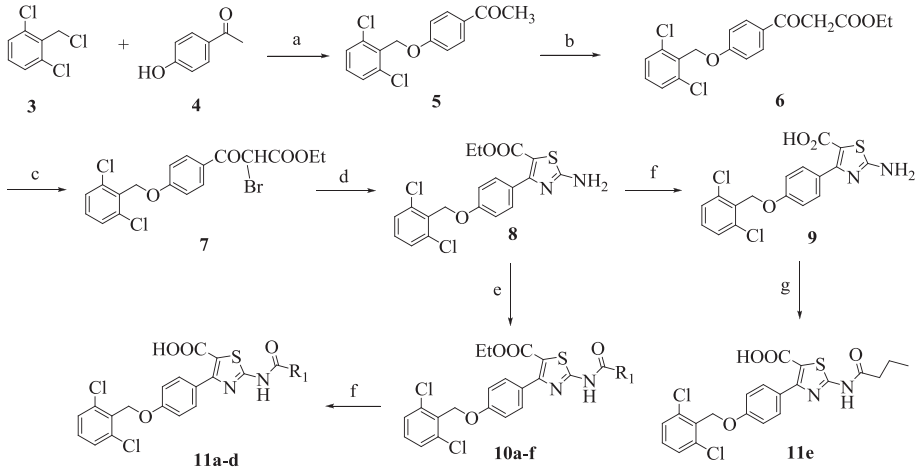
phenyl)thiazole-5-carboxylate **8** was prepared by cyclization of α-bromoketoesters **7** with thiourea [15]. Then, acylation of **8** with different acyl chloride in the presence of DMAP led to the desired esters **10a**–**f**. Hydrolysis of **10a**–**d** gave the corresponding carboxylic acids **11a**–**d**. Compound **11e** was obtained by reacting intermediate **9** with butyric anhydride due to the amide bond breakage under alkaline conditions. For oxazole series, the intermediate **19** was prepared by cyclization of 4-(2,6-dichlorobenzyloxy)benzoyl chloride **15** with ethyl aminocyanoacetate 4-toluenesulfonate **18** in NMP [16]. Acylation of **19** with *p*-chlorobenzoyl chloride afforded the ester **20**, which subsequently was hydrolyzed at reflux to give the corresponding acid **21**. For imidazole series, reaction of ethyl aminocyanoacetate, triethylorthoformate and 4-(2,6-dichlorobenzyloxy)aniline **24** in one-pot gave the intermediate **25** [17]. Acylation of **25** with *p*-chlorobenzoyl chloride afforded ester **26**, which subsequently was hydrolyzed at reflux to give the corresponding acid **27**.

3. Results and discussion

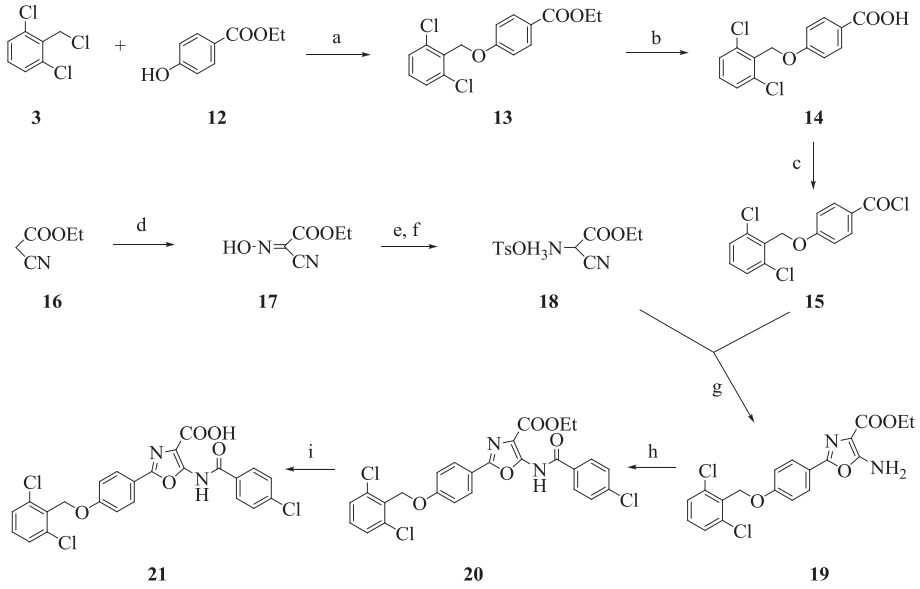
The title compounds along with the standard drugs (Isoniazid, Moxifloxacin and Gentamycin) for comparison were firstly evaluated for their activities against *M. tuberculosis* H37Rv (ATCC # 27294) by Microplate Alamar Blue Assay (MABA) [18,19] (Table 1). A closer look into the biological results of these compounds revealed that in thiazole series (**10a**–**f** and **11a**–**e**), esters **10a**–**f** (MIC = 1.0–61.2 μM) showed much better activities than acids **11a**–**e** (MIC = 45.4–128 μM). Among benzamides (**10a**–**d**), compound **10b** containing 4-chloro group was the most active compound (MIC = 1.0 μM), whereas other three compounds **10a**, **10c** and **10d** have were much less active. Compared to **10b**, compound **10c** showed a 25-fold reduction in potency (MIC = 25.4 μM), which suggested that the *ortho*-position of the phenyl ring was unfavorable for anti-tubercular activity. However, oxazole and imidazole derivatives (**20**, **21**, **26** and **27**) did not show considerable activity (MIC > 128 μM). The results clearly revealed that the thiazole scaffold had encouraging activity, while oxazole and imidazole nucleus did not show encouraging activity.

M. tuberculosis are unique to surround by a thick and waxy cell wall. Efficient anti-tubercular drugs should have a reasonable lipophilicity to penetrate the cell wall. To investigate the obvious activity difference between thiazole esters **10a**–**f** and acids **11a**–**e**, the logp values for the above compounds were calculated (Table 1). It may be explained that the esters posses preferable lipophilicity than acids and show optimal membrane permeability.

The results from the anti-tubercular activity of thiazole series (Table 1) prompted us to investigate their antibacterial activity against clinical *S. aureus*, *E. coli*, *S. pneumoniae*, *penicilin-resistant S. pneumoniae* (Table 2). The results revealed that compounds **10a**, **10d**–**f**, **11a**–**b** and **11d**–**e** exhibited potent activities against the Gram-positive clinical *S. pneumoniae* with MIC values better than the control drug ampicillin (MIC = 0.168 μM), but did not have activity against clinical *S. aureus*, *E. coli*, and penicilin-resistant *S. pneumoniae* (MIC > 128 μM). Compound **10b** exhibited the activity against clinical *S. pneumoniae* equal to the control drug gentamycin (MIC = 6.9 μM), while did not show any considerable activity against other three clinical strains (MIC > 128 μM). On the contrary, compounds comprising 2,4-dichloro substitution on phenyl ring (**10c** and **11c**) showed no inhibitory activity against all the tested clinical strains (MIC > 128 μM).



Scheme 1. Reagents and conditions: (a) K_2CO_3, KI, DMF, reflux for 12 h; (b) $CO(OEt)_2$, NaH, 1,4-dioxane, reflux for 4 h; (c) Br_2, CH_2Cl_2, 10 °C–rt, 1 h; (d) NH_2CSNH_2, C_2H_5OH; reflux for 2 h; rt for overnight; (e) RCOCl, DMAP, THF; rt; 10 h; (f) 1NNaOH, CH_3OH; rt; 3 days; (g) $(CH_3CH_2CH_2CO)_2O$, DMAP, THF; rt; 10 h.



Scheme 2. Reagents and conditions: (a) K_2CO_3, acetone; reflux for 24 h; (b) KOH, C_2H_5OH; reflux for 2 h; (c) $(COCl)_2$, CH_2Cl_2, DMF(Cat); reflux for 5 h; (d) $NaNO_2$, H_3PO_4, HCl, H_2O; 40–45 °C; rt for overnight; (e) $Na_2S_2O_4$, $NaHCO_3$, NaCl, H_2O; rt; 3 h; (f) TsOH, $(Et)_2O$, THF; (g) NMP; rt; overnight; (h) 4-ClPhCOCl, Et_3N, CH_2Cl_2; rt; 12 h; (i) 1NNaOH, C_2H_5OH; reflux for 2 h.

4. Conclusions

In conclusion, we have synthesized a series of 4-(2,6-dichlorobenzyloxy)phenyl thiazole, oxazole and imidazole derivatives, and studied their anti-tubercular and antibacterial activities. In particular, the thiazole **10b** carrying *p*-chlorobenzoyl substituent appeared to exhibit the highest anti-tubercular activity with MIC value as low as 1 μM. Such SAR results were in agreement with those reported from our previous studies [4]. The possible improvements in the activity can be further achieved by modifications the substituents on phenyl ring. Furthermore, thiazoles **10a**, **10d**–**f**, **11a**–**b** and **11d**–**e** appeared to exhibit potent antibacterial activity against *S. pneumoniae*. This new class of compounds is now being tested in more extensive *in vitro* studies to determine efficacy against *M. tuberculosis* and *S. pneumoniae*.

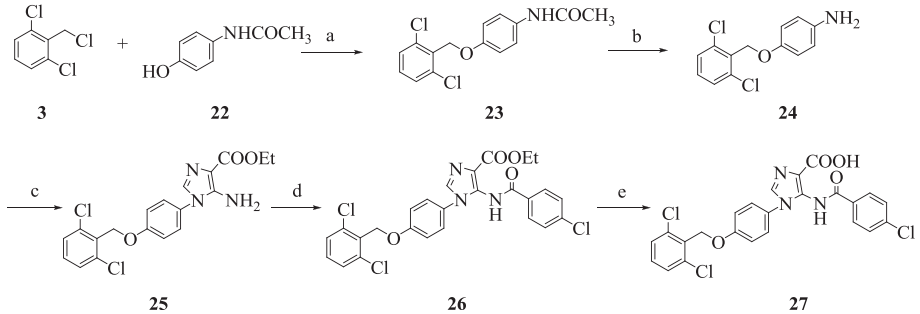
5. Experimental

5.1. Chemistry

Melting points were determined on a Mel-TEMP II melting point apparatus and are uncorrected. Infrared (IR) spectra (KBr) were recorded on a Nicolet Impact 410 instrument (KBr pellet). ^{1}H-NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants are in units of hertz (Hz). The spin multiplicities are indicated by the symbols s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). MS spectra were recorded on a Shimadzu GC–MS 2010 (EI) or a Mariner Mass Spectrum (ESI). Elemental analyses (C, H, N) were determined on on Carlo Erba 1106 elementary analysis apparatus and were within ±0.5% of the theoretical values. TLCs and preparative thin-layer chromatography were performed on silica gel GF/UV 254, and the chromatograms were performed on silica gel (100–200 mesh) visualized under UV light at 254 nm and 365 nm. All solvents were reagent grade and, when necessary, were purified and dried by standards methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate.

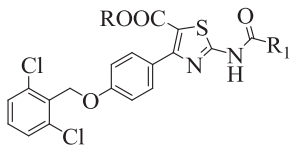
5.1.1. General procedure for the synthesis of compounds (*10a–f* and *11a–e*)

*5.1.1.1. 1-(4-(2,6-Dichlorobenzyloxy)phenyl)ethanone (**5**).* To a solution of 1-(4-hydroxyphenyl)ethanone **4** (6.8 g, 0.05 mol) and K_2CO_3 (13.8 g, 0.1 mol) in acetone (100 mL), 2.6-dichlorobenzyl chloride **3** (10.78 g, 0.055 mol) was added. The mixture was stirred vigorously, and heated to reflux for 24 h. On cooling the reaction mixture to room temperature without down, the mixture was concentrated

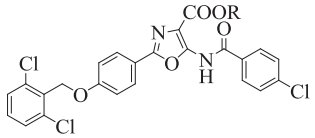


Scheme 3. Reagents and conditions: (a) K_2CO_3, acetone; reflux for 24 h; (b) KOH, C_2H_5OH; reflux for 16 h; (c) $NCC(NH_2)COOEt$, $(C_2H_5O)_3CH$, CH_3CN; rt; 3 h; (d) 4-ClPhCOCl, Et_3N, CH_2Cl_2; rt; 12 h; (e) 1NNaOH, C_2H_5OH; reflux for 2 h.

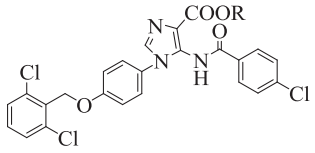
Table 1
In vitro anti-tubercular activities of compounds **10a**–**f**, **11a**–**e**, **20**, **21**, **26** and **27** against *M. Tuberculosis* H37Rv and their calculated clogP.



Compd.	R	R_1	MABA MIC (μM)	clog*P*[a]
10a	Et	Ph	61.2	6.25
11a	H	Ph	>128	5.78
10b	Et	4-Cl–Ph	1.0	6.50
11b	H	4-Cl–Ph	>128	5.94
10c	Et	2,4-Cl–Ph	25.4	6.81
11c	H	2,4-Cl–Ph	45.5	6.18
10d	Et	4–CH_3O–Ph	7.6	6.45
11d	H	4–CH_3O–Ph	>128	6.02
10e	Et	*n*–Pr	60.8	6.00
11e	H	*n*–Pr	> 128	5.74
10f	Et	Ethylene	7.2	5.36



Compd.	R	MABA MIC (μM)	clog*P*[a]
20	Et	> 128	6.52
21	H	> 128	6.06



compd	R	MABA MIC (μM)	clog*P*[a]
26	Et	> 128	5.74
27	H	> 128	5.28
Isoniazid	–	0.81	–
Moxifloxacin	–	0.50	–
PA824	–	0.44	–

[a] The clogP values were calculated using Pallas software.

and diluted with water (100 mL) and then extracted with AcOEt (2 × 50 mL). The organic layer was washed with 10% NaOH solution (50 mL), dried with Na_2SO_4 and then concentrated in vacuo to afford 1-(4-(2,6-dichlorobenzyloxy)phenyl)ethanone **5** (14.65 g, 99.3%) as a white solid. m.p.130–132 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 2.51 (3H, s, CH_3), 5.28 (2H, s, CH_2), 6.99 (2H, d, *J* = 8.7 Hz, ArH), 7.22 (1H, m, ArH), 7.32 (2H, d, *J* = 7.5 Hz, ArH), 7.91 (2H, d, *J* = 8.7 Hz, ArH). EI-MS (*m*/*z*): 294 $(M)^+$.

Table 2
In vitro anti-microbial activities of compounds **10a**–**f** and **11a**–**e**.

Compd.	MIC (μM)			
	S. aureus	*E. coli*	*S. pneumoniae*	*penicilin-resistant S. pneumoniae*
10a	>128	>128	≤0.119	>128
11a	>128	>128	≤0.125	>128
10b	>128	>128	7.12	>128
11b	>128	>128	≤0.117	>128
10c	>128	>128	>128	>128
11c	>128	>128	>128	>128
10d	>128	>128	≤0.112	>128
11d	>128	>128	≤0.118	>128
10e	>128	>128	≤0.127	>128
11e	>128	>128	≤0.134	>128
10f	>128	>128	≤0.131	>128
Ampicillin	2.7	10.8	0.168	10.8
Gentamycin	6.9	0.43	6.9	6.9

*5.1.1.2. Ethyl 3-(4-(2,6-dichlorobenzyloxy)phenyl)-3-oxopropanoate (**6**).* To a mixture of NaH/oil dispersion (0.528 g, 50% NaH by weight, calculated by 0.264 g of NaH, 11 mmol), diethyl carbonate (12 mL), and THF (15 mL) was added dropwise a solution of **5** (2.94 g, 10 mmol) in THF (15 mL). The mixture was heated at reflux for 4 h. The reaction mixture was poured into water, the pH was adjusted to 9, and mixture was extracted with methylene dichloride. The solvent was removed, and the residue was purified by flash silica gel chromatography (petroleum ether: ethyl acetate = 10 : 1) as the eluent to yield **6** (2.49 g, 67.84%) as yellow solid. m.p. 96–98 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.20 (3H, t, *J* = 7.2 Hz, CH_3), 3.89 (2H, s, $COCH_2$), 4.15 (2H, q, *J* = 7.2 Hz, $COOCH_2$), 5.28 (2H, s, CH_2O), 7.00 (2H, d, *J* = 8.7 Hz, ArH), 7.22 (1H, m, ArH), 7.32 (2H, d, *J* = 7.5 Hz, ArH), 7.90 (2H, d, *J* = 8.7 Hz, ArH). EI-MS (*m*/*z*): 366 $(M)^+$.

*5.1.1.3. Ethyl 2-bromo-3-(4-(2,6-dichlorobenzyloxy)phenyl)-3-oxopropanoate (**7**).* To a solution of **6** (1.83 g, 5 mmol) in CH_2Cl_2 (25 mL) was added a solution of Br_2 (0.69 g, 4.3 mmol) in CH_2Cl_2 (5 mL) in a dropwise manner at 10 °C. The reaction mixture was stirred further for an additional 1 h and then treated with 10% K_2CO_3 solution. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated in vacuo giving the crude bromide **7**, which was used subsequently in the next step. EI-MS (*m*/*z*): 444 $(M)^+$.

*5.1.1.4. Ethyl 2-amino-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylate (**8**).* To a solution of thiourea (0.38 g, 5 mmol) in absolute EtOH (25 mL) compound **7** was added dropwise. After the addition was completed, the solution was heated at reflux for 2 h and stirred at room temperature overnight (R_f = 0.4, PE: EA = 4:1). The mixture was then concentrated to dryness, and washed by concentrated NH_4OH. The solid was filtered and crystallized from EtOH to yield **8** (1.6 g, 75.83% two steps) as yellow solid. m.p. 171–173 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.20 (3H, t, *J* = 7.2 Hz, CH_3), 4.15 (2H, q, *J* = 7.2 Hz, $COOCH_2$), 5.25 (2H, s, CH_2O), 5.58 (2H, s, NH_2), 6.98 (2H, d, *J* = 8.4 Hz, ArH), 7.20 (1H, t, *J* = 4.8 Hz, ArH), 7.30 (2H, d, *J* = 8.1 Hz, ArH), 7.65 (2H, d, *J* = 8.7 Hz, ArH). EI-MS (*m*/*z*): 422 $(M)^+$.

*5.1.1.5. 2-Amino-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylic acid (**9**).* To a solution of **8** (420 mg, 1 mmol) in EtOH (40 mL) was added 1 N NaOH (5 mL, 5 mmol). The reaction mixture was stirred at room temperature for 3 days and after poured into water (30 mL) followed with 1 N AcOH to pH 4–5. The suspension solution was extracted with AcOEt (2 × 50 mL). The combined organic layers was dried over Na_2SO_4 and concentrated in vacuo to afford crude prouct. Recrystallization from AcOEt gave **9** (0.09 g, 22.8%) as a white solid. m.p. 224–227 °C (dec). ESI-MS (*m*/*z*): 393 $(M - H)^+$.

*5.1.2. General procedure for the synthesis of compounds **10a**–**f***

To a mixture of **8** (0.21 g, 0.5 mmol), DMAP (0.05 g, 0.4 mmol) and dry THF (10 mL), the solution of various chloride (4 mmol) in dry THF (10 mL) was added, while keeping the temperature at 0 °C. The whole mixture was stirred for 10 h at room temperature. Ammonia was added to the mixture to pH 8–9 and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine and dried with Na_2SO_4, then concentrated in vacuo. Purification of the crude products by column chromatography eluting with petroleum-ethyl acetate (1:1) gave **10a**–**f**.

*5.1.2.1. Ethyl 2-benzamido-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylate (**10a**).* Yield: 87.5%. White solid. m.p. 207–212 °C. IR (KBr): 3297, 1663, 1530, 1329, 1278, 1236, 1095, 1009 cm^{-1}.^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.34 (3H, t, J = 7.2 Hz, CH_3), 4.31 (2H, q, J = 7.2 Hz, $COOCH_2$), 5.29 (2H, s, CH_2O), 6.99 (2H, d, J = 8.7 Hz, ArH), 7.26 (1H, m, ArH), 7.38 (2H, d, J = 8.7 Hz, ArH), 7.47 (2H, t, J = 7.5 Hz, ArH), 7.59 (1H, t, J = 7.5 Hz, ArH), 7.69 (2H, d, J = 8.7 Hz, ArH), 7.87 (2H, d, J = 8.4 Hz, ArH), 10.50 (1H, s, NH). EI-MS (m/z): 526 $(M)^+$. Formula: $C_{26}H_{20}Cl_2N_2O_4S$. Anal (C%, H%, N%) Calcd: 59.21, 3.82, 5.31. Found: 59.24, 3.59, 5.26.

*5.1.2.2. Ethyl 2-(4-chlorobenzamido)-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylate (**10b**).* Yield: 64.3%. White solid. m.p. 259–262 °C (dec). IR(KBr): 3296, 1664, 1529, 1436, 1331, 1240, 1093, 1011 cm^{-1} ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.26 (3H, t, J = 7.2 Hz, CH_3), 4.23 (2H, q, J = 7.0 Hz, $COOCH_2$), 5.30 (2H, s, CH_2O), 7.14 (2H, d, J = 8.4 Hz, ArH), 7.49 (1H, m, ArH), 7.59 (2H, d, J = 7.5 Hz, ArH), 7.65 (2H, d, J = 8.4 Hz, ArH), 7.78 (2H, d, J = 8.7 Hz, ArH), 8.15 (2H, d, J = 8.4 Hz, ArH), 13.24 (1H, s, NH). ^{13}C NMR (300 MHz, DMSO-d_6): 14.02, 60.75, 64.95, 128.79, 130.27, 131.36–131.66, 136.05, 137.92, 159.02, 161.47. EI-MS (m/z): 560 $(M)^+$. Formula: $C_{26}H_{19}Cl_3N_2O_4S$. Anal (C%, H%, N%) Calcd: 55.58, 3.41, 4.99. Found: 55.41, 3.26, 4.83.

*5.1.2.3. Ethyl 2-(2,4-Dichlorobenzamido)-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylate (**10c**).* Yield: 84.2%. White solid. m.p. 177–183 °C. IR(KBr): 1692, 1532, 1437, 1332, 1297, 1243, 1098, 1006 cm^{-1} ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.26 (3H, t, J = 7.2 Hz, CH_3), 4.24 (2H, q, J = 7.2 Hz, $COOCH_2$), 5.30 (2H, s, CH_2O), 7.13 (2H, d, J = 8.7 Hz, ArH), 7.49 (1H, m, ArH), 7.59 (3H, m, ArH), 7.75 (3H, m, ArH), 7.82 (1H, s, ArH), 13.32 (1H, s, NH). EI-MS (m/z): 594 $(M)^+$. Formula: $C_{26}H_{18}Cl_4N_2O_4S\cdot 0.5H_2O$. Anal (C%, H%, N%) Calcd: 51.59, 3.16, 4.63. Found: 51.27, 3.39, 4.52.

*5.1.2.4. Ethyl 4-(4-(2,6-Dichlorobenzyloxy)phenyl)-2-(4-methoxybenzamido)thiazole-5-carboxylate (**10d**).* Yield: 71.9%. White solid. m.p. 239–242 °C (dec). IR (KBr): 3323, 1660, 1437, 1326, 1247, 1186, 1083, 1003 cm^{-1} ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.26 (3H, t, J = 7.2 Hz, $CH_2\underline{CH_3}$), 3.86 (3H, s, OCH_3), 4.23 (2H, q, J = 7.2 Hz, $COOCH_2$), 5.30 (2H, s, CH_2O), 7.12 (4H, m, ArH), 7.49 (1H, m, ArH), 7.59 (2H, d, J = 9.0 Hz, ArH), 7.78 (2H, d, J = 8.4 Hz, ArH), 8.15 (2H, d, J = 8.7 Hz, ArH), 12.98 (1H, s, NH). EI-MS (m/z): 556 $(M)^+$. Formula: $C_{27}H_{22}Cl_2N_2O_5S\cdot CH_3OH$. Anal (C%, H%, N%) Calcd: 57.05, 4.45, 4.75. Found: 57.42, 4.23, 4.31.

*5.1.2.5. Ethyl 2-butyramido-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylate (**10e**).* Yield: 65.0%. White solid. m.p. 178–182 °C. IR (KBr): 3262, 1693, 1670, 1530, 1488, 1437, 1372, 1327, 1240, 1152, 1094, 1007 cm^{-1} ^{1}H-NMR (300 MHz, DMSO-d_6) : δ 0.91 (3H, t, J = 7.2 Hz, $CH_2CH_2\underline{CH_3}$), 1.24 (3H, t, J = 7.2 Hz, $COOCH_2\underline{CH_3}$), 1.64 (2H, m, J = 7.2 Hz, $CH_2\underline{CH_2}CH_3$), 2.47 (2H, t, $\underline{CH_2}CH_2CH_3$), 4.21 (2H, q, J = 7.2 Hz, $COOCH_2$), 5.29 (2H, s, CH_2O), 7.12 (2H, d, J = 8.7 Hz, ArH), 7.49 (1H, m, ArH), 7.60 (2H, d, J = 7.5 Hz, ArH), 7.74 (2H, d, J = 8.7 Hz, ArH), 12.64 (1H, s, NH). EI-MS (m/z): 492 $(M)^+$. Formula: $C_{23}H_{22}Cl_2N_2O_4S$. Anal (C %, H%, N%) Calcd: 55.99, 4.49, 5.68. Found: 55.97, 4.39, 5.44.

*5.1.2.6. Ethyl 2-acrylamido-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylate (**10f**).* Yield: 12.2%. m.p. 200–203 °C. IR (KBr): 3237, 3190, 1683, 1657, 1609, 1533, 1436, 1329, 1243, 1175, 1088, 1009 cm^{-1} ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.34 (3H, t, J = 7.2 Hz, CH_3), 4.31 (2H, q, J = 7.2 Hz, $COOCH_2$), 5.30 (2H,s, CH_2O), 5.55 (2H, m, $CH{=}C\underline{H_2}$), 6.36 (1H, m, $C\underline{H}{=}CH_2$), 7.07 (2H, d, J = 8.7 Hz, ArH), 7.24 (1H, m, ArH), 7.39 (2H, d, J = 7.2 Hz, ArH), 7.76 (2H, d, J = 8.7 Hz, ArH), 11.24 (1H, s, NH). EI-MS (m/z): 476 $(M)^+$. Formula: $C_{22}H_{18}Cl_2N_2O_4S\cdot 0.25\ C_3H_8O_2$. Anal (C%, H%, N%) Calcd: 55.32, 4.04, 5.61. Found: 55.78, 4.34, 5.51.

*5.1.3. General procedure for the synthesis of compounds **11a–d***

To a solution of **10a–d** (1 mmol) in EtOH (40 mL) was added 1 N NaOH (15 mL, 15 mmol). The reaction mixture was stirred at room temperature for 3 days. The reaction mixture was poured into water (30 mL) followed with 1 N AcOH to pH 4–5. The suspension solution was extracted with AcOEt (50 mL × 3). The combined organic layers was dried with Na_2SO_4 and concentrated in vacuo to afford crude product. Recrystallization from AcOEt gave pure **11a–d**.

*5.1.3.1. 2-Benzamido-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylic acid (**11a**).* Yield: 46.1%. White solid. m.p. 280–282 °C (dec). IR (KBr): 3176, 3057, 1680, 1538, 1500, 1439, 1324, 1285, 1246, 1116, 1013 cm^{-1} ^{1}H-NMR (300 MHz, DMSO-d_6): δ 5.30 (2H,s, CH_2O), 7.13 (2H, d, J = 8.7 Hz, ArH), 7.49 (1H, m, ArH), 7.57 (4H, m, ArH), 7.67 (1H, t, J = 7.5 Hz, ArH), 7.80 (2H, d, J = 8.7 Hz, ArH), 8.14 (2H, d, J = 7.2 Hz, ArH), 13.04 (2H,s, NH, COOH). EI-MS (m/z): 497 $(M-H)^+$. Formula: $C_{24}H_{16}Cl_2N_2O_4S\cdot 2H_2O$. Anal (C%, H%, N%) Calcd: 53.84, 3.77, 5.23. Found: 53.90, 3.76,5.35.

*5.1.3.2. 2-(4-Chlorobenzamido)-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylic acid (**11b**).* Yield: 60.0%. White solid. m.p. 259–261 °C (dec). IR (KBr): 3175, 2958, 1681, 1603, 1536, 1495, 1436, 1324, 1286, 1248, 1148, 1094, 1010 cm^{-1} ^{1}H-NMR (300 MHz, DMSO-d_6) : δ 5.30 (2H, s, CH_2O), 7.13 (2H, d, J = 8.7 Hz, ArH), 7.49 (1H, m, ArH), 7.62 (4H, m, ArH), 7.79 (2H, d, J = 9.0 Hz, ArH), 8.15 (2H, d, J = 8.7 Hz, ArH), 13.11(2H, s, NH, COOH). EI-MS (m/z): 533 $(M-H)^+$. Formula: $C_{24}H_{15}Cl_3N_2O_4S$. Anal (C%, H%, N%) Calcd: 54.00, 2.83, 5.25. Found: 53.66, 2.64, 5.03.

*5.1.3.3. 2-(2,4-Dichlorobenzamido)-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylic acid (**11c**).* Yield: 73.9%. white solid. m.p. 212–215 °C. IR (KBr): 3417, 3187, 1708, 1671, 1531 , 1494, 1436, 1249, 1147, 1093, 1003 cm^{-1} ^{1}H-NMR (300 MHz, DMSO-d_6): δ 5.29 (2H, s, CH_2O), 7.11 (2H, d, J = 8.7 Hz, ArH), 7.49 (1H, m, ArH), 7.59 (3H, m, ArH), 7.77 (4H, m, ArH), 13.16 (2H, s, NH, COOH). EI-MS (m/z): 567 $(M-H)^+$. Formula: $C_{24}H_{14}Cl_4N_2O_4S\cdot 0.5H_2O$. Anal (C%, H%, N %) Calcd: 49.94, 2.62, 4.85. Found: 49.72, 2.97, 4.88.

*5.1.3.4. 4-(4-(2,6-Dichlorobenzyloxy)phenyl)-2-(4-methoxybenzamido)thiazole-5-carboxylic acid (**11d**).* Yield: 64.3%. White solid. m.p. 247–250 °C. IR (KBr): 3279, 2944, 1670, 1607, 1512, 1437, 1336, 1249, 1179, 1147, 1090, 1020 cm^{-1} ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.86 (3H, s, OCH_3), 5.30 (2H, s, CH_2O), 7.11 (4H, m, ArH), 7.49 (1H, m, ArH), 7.59 (2H, d, J = 7.5 Hz, ArH), 7.79 (2H, d, J = 8.4 Hz, ArH), 8.15 (2H, d, J = 8.7 Hz, ArH), 12.89 (2H, s, NH, COOH). EI-MS (m/z): 527 $(M-H)^+$. Formula: $C_{25}H_{18}Cl_2N_2O_5S\cdot CH_3OH$. Anal (C%, H%, N%) Calcd: 55.62, 3.95, 4.99. Found: 55.42, 3.54, 4.57.

*5.1.3.5. 2-Butyramido-4-(4-(2,6-dichlorobenzyloxy)phenyl)thiazole-5-carboxylic acid (**11e**).* To a mixture of **9** (0.05 g, 0.13 mmol), DMAP (0.05 g, 0.4 mmol) and dry THF (10 mL), the solution of butyric anhydride (0.16 g, 1 mmol) in dry THF (10 mL) was added, while keeping the temperature at 0 °C. The whole mixture was stirred for 10 h at room temperature. Water was added to the mixture and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine and dried with Na_2SO_4, then concentrated in vacuo. Purification of the crude products by column chromatography eluting with petroleum-ethyl acetate (2:1) gave **11e** a white solid (0.05 g, 84.7%). m.p. 255–258 °C. IR (KBr): 3414, 3160, 2961, 1699, 1573, 1533, 1438, 1326, 1246, 1178, 1141, 1092, 1013 cm^{-1} ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.91(3H, t, J = 7.2 Hz, $CH_2CH_2\underline{CH_3}$), 1.64 (2H, m, J = 7.2 Hz, $CH_2\underline{CH_2}CH_3$), 2.46 (2H, t, $\underline{CH_2}CH_2CH_3$), 5.29 (2H, s, CH_2O), 7.11 (2H, d, J = 8.7 Hz, ArH), 7.49 (1H, m, ArH), 7.59 (2H, d, J = 7.5 Hz, ArH), 7.74 (2H, d, J = 8.7 Hz, ArH), 12.51 (1H, s,

COOH), 12.89 (1H, s, NH). EI-MS (*m*/*z*): 463 $(M - H)^+$. Formula: $C_{21}H_{18}Cl_2N_2O_4S$. Anal (C%, H%, N%) Calcd: 54.20, H 3.90, 6.02. Found: 54.09, 3.76, 5.93.

*5.1.4. The procedure for synthesis of compounds **20** and **21***

*5.1.4.1. Ethyl 4-(2,6-dichlorobenzyloxy)benzoate (**13**).* To a solution of ethyl 4-hydroxybenzoate **12** (5.81 g, 0.035 mol) and K_2CO_3 (9.6 g, 0.07 mol) in acetone (80 mL), **3** (6.86 g, 0.035 mol) was added. The suspension was stirred vigorously, and heated to reflux for 24 h. On cooling the reaction mixture down to room temperature, the suspension was concentrated and diluted with water (100 mL) and then extracted with AcOEt (2 × 100 mL). The organic layers was washed with 10% NaOH solution (100 mL), dried with Na_2SO_4 and then concentrated in vacuo to afford **13** as a white solid (10.52 g, 92.20%). Mp 84–85 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.36–1.40 (t, J = 7.2 Hz, 3H, CH_3), 4.32–4.39 (q, J = 7.2 Hz, 2H, CH_2), 5.32 (s, 2H, CH_2), 7.02–7.05 (d, J = 8.7 Hz, 2H, Ar), 7.23–7.28 (t, J = 7.2 Hz, 1H, Ar), 7.36–7.38 (d, J = 7.2 Hz, 2H, Ar), 8.02–8.04 (d, J = 8.7 Hz, 2H, Ar); EI-MS (*m*/*z*): 324 $(M)^+$.

*5.1.4.2. 4-(2,6-Dichlorobenzyloxy)benzoic acid (**14**).* **13** (10.5 g, 0.032 mol) was dissolved in EtOH (80 mL) and water (10 mL). Potassium hydroxide (6.4 g, 0.16 mol) was added and the resulting mixture heated to reflux for 2 h. After cooling, the reaction mixture was diluted with water (300 mL) and acidified by HCl to pH 4–5. The solid was filtered, the filtrate washed with water and dried overnight to yield **14** as a white crystal (8.53 g, 89.78%). Mp 194–196 °C. EI-MS (*m*/*z*): 296 $(M)^+$.

*5.1.4.3. 4-(2,6-Dichlorobenzyloxy)benzoyl chloride (**15**).* **14** (4.87 g, 0.016 mol) was dissolved in dry dichloromethane (60 mL). Then thionyl chloride (5.5 mL, 0.065 mol) and DMF (2 drops) were added to the mixture. The reaction mixture heated to reflux for 5 h and concentrated to give yellow solid **15** used for the next step without further purification.

*5.1.4.4. Cyano-hydroxyimino-acetic acid ethyl eater (**17**).* A solution of sodium nitrite (5.73 g, 0.083 mol) in water (71 mL) was treated with ethyl cyanoacetate **16** (10 g, 0.083 mol). 85% orthiphosphoric acid (3.66 mL, 0.055 mol) was added dropwise, while keeping the temperature of the reaction mixture below 10 °C with the aid of an ice bath. At the end of the addition, the mixture was warmed to 40 °C and stirred for 1 h. The reaction was quenched at 45 °C with fuming HCl (7.4 mL, 0.088 mol), and the mixture was then left to cool to room temperature and at 0 °C overnight to complete precipitation. The solid was filtered, the filtrate washed with water and dried under high vacuum overnight to yield **17** as white crystals (7.73 g, 61.45%). Mp 126–127 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.39–1.44 (t, J = 7.2 Hz, 3H, CH_3), 4.41–4.49 (q, J = 7.2 Hz, 2H, CH_2), 10.49 (s, 1H, OH); EI-MS (*m*/*z*): 142 $(M)^+$.

*5.1.4.5. 1-(Cyano-2-ethoxy)-2-oxoethanominium 4-toluenesulfonate (**18**).* **17** (4.3 g, 0.03 mol) was dissolved in water (43 mL) and sat. aq $NaHCO_3$ solution (35 mL). $Na_2S_2O_4$ (15.6 g, 0.09 mol) was added and the resulting mixture stirred at 35 °C for 30 min and then stirred at room temperature for 3 h. The mixture was saturated with NaCl (20 g), extracted with CH_2Cl_2 (4 × 30 mL), the combined extracts dried (Na_2SO_4) and concentrated to give ethyl aminocyanoacetate as a red oil (1.0 g, 26.32%). EI-MS (*m*/*z*): 127 $(M)^+$.The crude product was dissolved in Et_2O (20 mL) and the solution treated with $TsOH \cdot H_2O$ (1.45 g, 0.0079 mol). After the addition of THF (10 mL) and cooling, the precipitate was removed by filtration, washed with cold Et_2O (20 mL), and dried under reduced pressure to provide **18** as a yellow solid (1.86 g, 93.47%). Mp 128–130 °C (lit [16]: 128–130 °C). ^{1}H-NMR (300 MHz, D_2O): δ 1.22–1.27 (t, J = 7.2 Hz, 3H, CH_3), 2.29 (s, 3H, CH_3), 4.31–4.33 (q, J = 7.2 Hz, 2H, CH_2), 7.25–7.28 (d, J = 8.4 Hz, 2H), 7.57–7.60 (d, J = 8.4 Hz, 2H).

*5.1.4.6. Ethyl 5-amino-2-(4-(2,6-dichlorobenzyloxy)phenyl)oxazole-4-carboxylate (**19**).* To a stirred solution of **18** (4 g, 0.016 mol) in 1-methyl-2-pyrrolidinone (60 mL) were added a solution of **15** in 1-methyl-2-pyrrolidinone (30 mL) at room temperature. The reaction mixture was stirred at 23–25 °C overnight (12 h–18 h) and then diluted with ethyl acetate (100 mL). This solution was washed with water (2 × 100 mL) and then with 10% sodium hydrogen carbonate. The organic layer was dried, the solvent was evaporated in vacua. Purification of the crude products by column chromatography eluting with petroleum-ethyl acetate (2:1) gave **19** (1.5 g, 23.43%) as a white solid. Mp 198–200 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.25–1.30 (t, J = 7.2 Hz, 3H, CH_3), 4.17–4.25 (q, J = 7.2 Hz, 2H, CH_2), 5.29 (s, 2H, CH_2), 7.17–7.20 (d, J = 8.7 Hz, 2H), 7.37(s, 2H, NH_2), 7.48–7.51 (t, J = 8.4 Hz, 1H, Ar), 7.57–7.60 (d, J = 7.2 Hz, 2H, Ar), 7.74–7.76 (d, J = 8.7 Hz, 2H); EI-MS (*m*/*z*): 406 $(M)^+$.

*5.1.4.7. Ethyl 5-(4-Chlorobenzamido)-2-(4-(2,6-dichlorobenzyloxy) phenyl)oxazole-4-carboxylate (**20**).* To a mixture of **19** (0.4 g, 1 mmol), dry Et_3N (3 mL) and dry CH_2Cl_2 (20 mL), 4-chlorobenzoyl chloride (0.34 g, 0.002 mol) was added, while keeping the temperature at 0 °C. The whole mixture was stirred for 12 h at room temperature. Water was added to the mixture and extracted with dichloromethane (15 mL × 2). The combined organic layers were washed with brine and dried with Na_2SO_4, then concentrated in vacuo. Purification of the crude products by column chromatography eluting with petroleum-ethyl acetate (8:1) gave **20** (0.21 g, 37.04%) as a yellow solid. m.p. 207–209 °C. IR (KBr, cm^{-1}): 3125, 1705 (CO), 1620 (–CONH), 1493, 1317, 1262(–C–O–C–), 1101, 1004, 842, 779; ^{1}H-NMR (300 MHz, $CDCl_3$): 1.44–1.49 (t, J = 7.2 Hz, 3H, CH_3), 4.45–4.52 (q, J = 7.2 Hz, 2H, CH_2), 5.34 (s, 2H, CH_2), 7.08–7.11 (d, J = 9.0 Hz, 2H, Ar), 7.24–7.29 (t, J = 8.4 Hz, 1H, Ar), 7.37–7.39 (d, J = 8.4 Hz, 2H, Ar), 7.50–7.52 (d, J = 8.4 Hz, 2H, Ar), 7.92–7.95 (d, J = 8.4 Hz, 2H, Ar), 8.09–8.12 (d, J = 9.0 Hz, 2H, Ar), 10.22 (s, 1H, NHCO); EI-MS (*m*/*z*): 544 $(M)^+$. Formula: $C_{26}H_{19}Cl_3N_2O_5 \cdot 2H_2O$; Anal (C%, H%, N%) 53.67, 3.98, 4.81; Calcd: 53.72, 3.84, 5.03.

*5.1.4.8. 5-(4-Chlorobenzamido)-2-(4-(2,6-dichlorobenzyloxy)phenyl) oxazole-4-carboxylic acid (**21**).* To a solution of **20** (103 mg, 0.18 mmol) in EtOH (20 mL) was added 1 N NaOH (2.2 mL, 2.3 mmol). The reaction mixture was heated to reflux for 2 h. After cooling to room temperature, the reaction mixture was poured into water (50 mL) followed with 1 N HCl to pH 4–5. The suspension solution was extracted with AcOEt (2 × 25 mL). The combined organic layers was dried with Na_2SO_4 and concentrated in vacuo to afford crude product. Recrystallization from AcOEt gave **21** (0.05 g, 53.76%) as a yellow solid. m.p. 191–194 °C. IR (KBr, cm^{-1}): 3364–3520 (–OH), 1709 (CO), 1625 (–CONH), 1252, 1137(–C–O–C–), 1001, 840, 771, 624; ^{1}H-NMR (300 MHz, DMSO): 5.33 (s, 2H, CH_2), 7.24–7.27 (d, J = 9.0 Hz, 2H, Ar), 7.50–7.52 (t, J = 8.4 Hz, 1H, Ar), 7.58–7.60 (d, J = 8.4 Hz, 2H, Ar), 7.64–7.66 (d, J = 8.4 Hz, 2H, Ar), 7.94–7.96 (d, J = 8.4 Hz, 2H, Ar), 8.00–8.03 (d, J = 9.0 Hz, 2H, Ar); EI-MS (*m*/*z*): 515 $(M - 1)^+$. Formula: $C_{24}H_{15}Cl_3N_2O_5$. Anal (C%, H%, N%) Calcd: 55.68, 2.92, 5.41. Found: 55.28, 2.97, 5.10.

*5.1.5. The procedure for synthesis of compounds **26** and **27***

*5.1.5.1. N-(4-(2,6-dichlorobenzyloxy)phenyl)acetamide (**23**).* To a solution of 4-acetamino phenol **22** (4.9 g, 0.03 mol) and K_2CO_3 (8.28 g, 0.06 mol) in acetone (80 mL), **3** (6.46 g, 0.033 mol) was added. The mixture was stirred vigorously, and heated to reflux for 24 h. On

cooling the reaction mixture down to room temperature, the mixture was concentrated and diluted with water (100 mL) and then extracted with EtOAc (2 × 100 mL). The organic layers was washed with 10% NaOH solution (100 mL), dried with Na_2SO_4 and then concentrated in vacuo to afford **23** (10.52 g, 92.20%) as a white solid. m.p. 185–187 °C. EI-MS (*m/z*): 309 $(M)^+$.

*5.1.5.2. 4-(2,6-Dichlorobenzyloxy)aniline (**24**).* To a solution of **23** (5.01 g, 0.016 mol) in 90% C_2H_5OH, potassium hydroxide (5.6 g, 0.1 mol) was added. The mixture heated to reflux for 16 h and concentrated in vacuo. Purification of the crude products by column chromatography eluting with petroleum-ethyl acetate (4:1) gave **24** (4 g, 93.46%) as red oil. EI-MS (*m/z*): 267 $(M)^+$.

*5.1.5.3. Ethyl 5-amino-1-(4-(2,6-dichlorobenzyloxy)phenyl)-1H-imidazole-4-carboxylate (**25**).* To a solution of ethyl 2-amino-2-cyanoacetate (1.27 g, 0.01 mol) in MeCN was added triethylorthoformate (1.48 g, 0.01 mol) and the resulting solution heated to 90 °C. After 45 min the reaction solution was cooled to room temperature and a solution of 4-(2,6-dichlorobenzyloxy)aniline (2.68 g, 0.01 mol) in MeCN was added. The reaction was stirred at room temperature for 3 h. The solid was filtered, the filtrate recrystallized from EtOH and dried under high vacuum overnight to yield **25** as white crystals (2.32 g, 57.14%). m.p. 218–220 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.39–1.44 (t, 3H, J = 7.2 Hz, CH_3), 4.34–4.41 (q, 2H, J = 7.2 Hz, CH_2), 5.33 (s, 2H, CH_2), 7.07–7.10 (d, 2H, J = 9.0 Hz, Ar), 7.24–7.27 (t, J = 7.8 Hz, 1H, Ar), 7.27–7.31 (d, J = 9.0 Hz, 2H, Ar), 7.37–7.40 (d, J = 7.8 Hz, 2H, Ar), 7.55 (s, 1H, H_2-imidazole); EI-MS (*m/z*): 405 $(M)^+$.

*5.1.5.4. Ethyl 5-(4-chlorobenzamido)-1-(4-(2,6-dichlorobenzyloxy) phenyl)-1H-imidazole-4-carboxylate (**26**).* To a mixture of **25** (0.4 g, 1 mmol), dry Et_3N (3 mL) and dry CH_2Cl_2 (20 mL), 4-chlorobenzoyl chloride (0.34 g, 2 mmol) was added, while keeping the temperature at 0 °C. The whole mixture was stirred for 12 h at room temperature. Water was added to the mixture and extracted with CH_2Cl_2 (15 mL × 2). The combined organic layers were washed with brine and dried with Na_2SO_4, then concentrated in vacuo. Purification of the crude products by column chromatography eluting with petroleum-ethyl acetate (4:1) gave **26** (0.23 g, 42.28%) as a white solid. m.p. 215–216 °C. IR (KBr, cm^{-1}): 3549, 3415, 1709 (COOEt), 1591, 1513, 1393, 1244 (–C–O–C–), 1099, 1006, 849; ^{1}H-NMR (300 MHz, $CDCl_3$): 1.13–1.18 (t, 3H, J = 7.2 Hz, CH_3), 4.17–4.22 (q, 2H, J = 7.2 Hz, CH_2), 5.24 (s, 2H, CH_2), 7.17–7.20 (d, 2H, J = 9 Hz), 7.40–7.43 (d, 2H, J = 9 Hz), 7.46–7.49 (t, 1H, J = 6.9 Hz, Ar), 7.54–7.57 (d, 2H, J = 6.9 Hz, Ar), 7.57–7.60 (d, 2H, J = 8.4 Hz), 7.86–7.89 (d, 2H, J = 8.4 Hz), 8.01 (s, 1H, H_2-imidazole); EI-MS (*m/z*): 543 $(M)^+$. Formula: $C_{26}H_{20}Cl_3N_3O_4 \cdot 0.5H_2O$; Anal (C%, H%, N%) Calcd: 56.39, 3.82, 7.59. Found: 56.36, 3.77, 7.47.

*5.1.5.5. 5-(4-Chlorobenzamido)-1-(4-(2,6-dichlorobenzyloxy)phenyl) -1H-imidazole-4-carboxylic acid (**27**).* To a solution of **26** (157 mg, 0.29 mmol) in EtOH (20 mL) was added 1 N NaOH (3.6 mL, 3.7 mmol). The reaction mixture was heated to reflux for 2 h. After cooling to room temperature, the reaction mixture was poured into water (50 mL) followed with 1 N HCl to pH 4–5. The suspension solution was extracted with AcOEt (2 x 25 mL). The combined organic layers was dried with Na_2SO_4 and concentrated in vacuo to afford crude product. Recrystallization from CH_2Cl_2 gave **27** (0.08 g, 53.33%) as a white solid. m.p. 165–167 °C. IR (KBr, cm^{-1}): 3499–3445 (–OH), 3109, 1689 (CO), 1594 (–CONH), 1513, 1244 (–C–O–C–), 1095, 1009, 841, 767; ^{1}H-NMR (300 MHz, DMSO-d_6): 5.24 (s, 2H, CH_2), 7.17–7.20 (d, 2H, J = 9 Hz), 7.40–7.43 (d, 2H, J = 9 Hz), 7.46–7.49 (t, 1H, J = 6.9 Hz, Ar), 7.54–7.57 (d, 2H, J = 6.9 Hz, Ar), 7.57–7.60 (d, 2H, J = 8.4 Hz), 7.86–7.89 (d, 2H, J = 8.4 Hz), 8.01 (s, 1H, H_2-imidazole), 10.34 (br, 1H, COOH); ^{13}C NMR (300 MHz, DMSO-d_6): 6518, 115.20, 126.18, 127.78, 128.60, 128.76, 129.62, 131.25, 131.69, 131.79, 136.02, 136.98, 158.33, 163.33, 165.83, 200.39, 204.28. EI-MS (*m/z*): 514 $(M - 1)^+$. Formula: $C_{26}H_{19}Cl_2NO_5S$. Anal (C%, H%, N%) Calcd: 55.78, 3.12, 8.13. Found: 55.85, 3.65, 7.71.

5.2. Biological assay

5.2.1. Anti-tubercular activity against H37RV (MABA)

Briefly, the test compound MICs against TB were assessed by the MABA using INH, Moxifloxacin and PA824 as positive controls. Compound stock solutions were prepared in DMSO at a concentration of 12.8 mM, and the final test concentrations ranged from 128 to 0.5 μM. Two-fold dilutions of compounds were prepared in Middlebrook 7H12 medium (7H9 broth containing 0.1% w/v casitone, 5.6 μg/mL palmitic acid, 5 mg/mL bovine serum albumin, 4 mg/mL catalase, filter-sterilized) in a volume of 100 μL in 96-well microplates (black viewplates). *M. tuberculosis* H37RV (100 μL inoculum of 2 × 105 cfu/mL) was added, yielding a final testing volume of 200 μL. The plates were incubated at 37 °C. On the 7th day of incubation 12.5 μL of 20% Tween 80 and 20 μL of Alamar Blue (Trek Diagnostic, Westlake, OH) were added to the test plate. After incubation at 37 °C for 16–24 h, fluorescence of the wells was measured (ex 530, em 590 nm). The MICs ware defined as the lowest concentration effecting a reduction in fluorescence of ≥90% relative to the mean of replicate bacteria-only controls.

5.2.2. Antibacterial activity against clinical strains

Agar dilution method was used to test the MIC of the test compounds against four *S. aureus*, *E. coli*, *S. pneumoniae* and *penicilin-resistant S. pneumoniae*, which was performed as described previously [20]. Briefly, a series of two-fold dilutions of the test compounds, ampicillin and gentamicin were prepared in molten Mueller-Hinton Agar (MHA) media, and kept at 37 °C to obtain the desired final concentrations. Media with concentrations ranging from 1280 to 0.125 μg/mL was obtained, and media without any drugs was made as control. Plates were inoculated with 1–2 μL culture containing 10^4 CFU/mL of the appropriate microorganism, and the plates were incubated at 37 °C. The MICs were the lowest concentration in solid media at which no growth was observed after 16–20 h.

Acknowledgements

The authors gratefully acknowledged financial support from the " Significant new drugs development" Science and Technology Major Projects of China (No 2009ZX09501-003) and the Innovation Program for the Postgraduates in China Pharmaceutical University, P.R.China. We also thank CompuDrug International Inc. for allowing us to use the Pallas software.

References

[1] R. Johnson, E.M. Streicher, G.E. Louw, R.M. Warren, P.D. van Helden, T.C. Victor, Curr. Issues Mol. Biol. 8 (2006) 97–112.
[2] J.T. Brooks, J.E. Kaplan, K.K. Holmes, C. Benson, A. Pau, H. Masur, Clin. Infect. Dis. 48 (2009) 609–611.
[3] Global Tuberculosis Control, Epidemiology, Strategy, Financing WHO Report 2009 (2009), http://www.who.int/tb/publications/global_report/2009/en/index.html.
[4] X.Y. Lu, B.J. Wan, S.G. Franzblau, Q.D. You, Eur. J. Med. Chem. 46 (2011) 3551–3563.
[5] L. Nagarapu, A. Satyender, B. Rajashaker, K. Srinivas, P. Rupa rani, K. Radhika, G. Subhashini, Bioorg. Med. Chem. Lett. 18 (2008) 1167–1171.
[6] P. Karegoudar, M.S. Karthikeyan, D.J. Prasad, M. Mahalinga, B.S. Holla, N.S. Kumari, Eur. J. Med. Chem. 43 (2008) 261–267.
[7] I. Mochalkin, J.R. Miller, L. Narasimhan, V. Thanabal, P. Erdman, P.B. Cox, J.V. Prasad, S. Lightle, M.D. Huband, C.K. Stover, ACS Chem. Biol. 4 (2009) 473–483.

[8] M.H. Moshafi, M. Sorkhi, S. Emami, M. Nakhjiri, A. Yahya-Meymandi, A.S. Negahbani, F. Siavoshi, M. Omrani, E. Alipour, M. Vosooghi, A. Shafiee, A. Foroumadi, Arch. Pharm. (Weinheim) 344 (2011) 178–183.
[9] Q. Al-Balas, N.G. Anthony, B. Al-Jaidi, A. Alnimr, G. Abbott, A.K. Brown, R.C. Taylor, G.S. Besra, T.D. McHugh, S.H. Gillespie, B.F. Johnston, S.P. Mackay, G.D. Coxon, PLoS One 4 (2009) e5617.
[10] M. Amini, L. Navidpour, A. Shafiee, DARU 16 (2008) 9–12.
[11] S.K. Bharti, G. Nath, R. Tilak, S.K. Singh, Eur. J. Med. Chem. 45 (2010) 651–660.
[12] R.N. Misra, H.Y. Xiao, D.K. Williams, K.S. Kim, S. Lu, K.A. Keller, J.G. Mulheron, R. Batorsky, J.S. Tokarski, J.S. Sack, S.D. Kimball, F.Y. Lee, K.R. Webster, Bioorg. Med. Chem. Lett. 14 (2004) 2973–2977.
[13] R. Romagnoli, P.G. Baraldi, M.D. Carrion, O. Cruz-Lopez, C.L. Cara, G. Basso, G. Viola, M. Khedr, J. Balzarini, S. Mahboobi, A. Sellmer, A. Brancale, E. Hamel, J. Med. Chem. 52 (2009) 5551–5555.
[14] L. Navidpour, H. Shadnia, H. Shafaroodi, M. Amini, A.R. Dehpour, A. Shafiee, Bioorg. Med. Chem. 15 (2007) 1976–1982.
[15] J.J. Baldwin, E.L. Engelhardt, R. Hirschmann, G.S. Ponticello, J.G. Atkinson, B.K. Wasson, C.S. Sweet, A. Scriabine, J. Med. Chem. 23 (1980) 65–70.
[16] H. Heitsch, A. Wagner, N. Yadav-Bhatnagar, C. Griffoul-Marteau, Synthesis (1996) 1325–1330.
[17] H. Liu, X.H. He, H.S. Choi, K.Y. Yang, D. Woodmansee, Z.C. Wang, A. Ellis-David, B.G. Wu, Y. He, N. Nguyen-Truc. (2006) WO2006047516.
[18] L. Collins, S.G. Franzblau, Antimicrob. Agents Chemother. 41 (1997) 1004–1009.
[19] K. Falzari, Z. Zhu, D. Pan, H. Liu, P. Hongmanee, S.G. Franzblau, Antimicrob. Agents Chemother. 49 (2005) 1447–1454.
[20] H.M. Zhou, J. Dou, J. Wang, L.L. Chen, H. Wang, W.D. Zhou, Y.M. Li, C.L. Zhou, Peptides 32 (2011) 1131–1138.