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Original article

Synthesis and *in vitro* urease inhibitory activity of *N*,*N*′-disubstituted thioureas

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ABSTRACT

Thiourea derivatives (**1**–**38**) were synthesized and evaluated for their urease inhibition potential. The synthetic compounds showed a varying degree of *in vitro* urease inhibition with IC_{50} values 5.53 ± 0.02 −91.50 ± 0.08 μM, most of which are superior to the standard thiourea (IC_{50} = 21.00 ± 0.11 μM). In order to ensure the mode of inhibition of these compounds, the kinetic study of the most active compounds has been carried out. Most of these inhibitors were found to be mixed-type of inhibitors, except compounds **13** and **30** which were competitive, while compound **19** was identified as non-competitive inhibitor with *Ki* values between 8.6 and 19.29 μM.

1. Introduction

Urease (urea amidohydrolase EC 3.5.1.5) is a nickel-containing enzyme that catalyzes the hydrolysis of urea to ammonia and CO_2 or carbamate [1]. A variety of ureases are found in bacteria, fungi, higher plants and in soil as a soil enzyme [2] Activity of urease (E.C 3.5.1.5) has been shown to be an important virulence determinant in the pathogenesis of many clinical conditions which are detrimental for human and animal health as well as for agriculture. In agriculture, high urease activity causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization [3]. Moreover, it induces plant damage primarily by depriving plants of their essential nutrients and secondarily by ammonia toxicity, increasing the pH of the soil [4].

Urease has been implicated to play a role in the pathogenesis of many bacteria such as *Helicobacter pylori*, *Proteus mirabilis* and *Brucella abortus* [5]. Urease has been identified as an immunogenic modulator in several pathogen-induced inflammatory reactions [6]. It is also known to be a major cause of pathologies induced by *Helicobacter pylori* which allows the bacteria to survive at the low pH of the stomach during colonization and therefore plays an important role in the pathogenesis of gastric and peptic ulcers which may lead to cancer, Since urease is not only damaging for humans, but also for animals and in agriculture, various strategies based on urease inhibition were considered as treatment approaches for infections caused by urease-producing bacteria [7,8].

Moreover, *H. pylori* are recognized as a major risk factor for recurrent gastroduodenal inflammatory diseases and gastric adenocarcinoma [9]. Ureases play an important role in protecting the *H. pylori* from the effects of gastric acid [10]. Medically, ureases are important virulence factors implicated in the pathogenesis of many clinical conditions such as pyelonephritis, hepatic coma, peptic ulceration, and the formation of infection-induced urinary stones and reactive arthritis [11].

Thiourea is an organic compound with the molecular formula CSN_2H_4. It is a versatile reagent in synthetic chemistry. Thioureas are related to thioamides and can be prepared from ammonium thiocyanate, but more commonly by the reaction of phenyl isothiocyanate with alkyl or aryl amines. They are important building blocks in the synthesis of heterocycles [12]. Thioureas manifest important multiple biological effects and are the basis for target-

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oriented synthesis. Moreover, ureas and thioureas evaluate their plant growth-regulating activity mainly on the herbicidal, root growth inhibitory and stimulatory and cytokinin-like activities [13]. Thioureas and ureas (symmetrical or unsymmetrical) have attracted much attention as drug candidates against a variety of diseases due to their bioactivities and broad spectrum as pesticides and in pharmacological activities [14a]. A variety of thiourea derivatives and their metal complexes exhibit analgesic, anti-inflammatory [15], anticancer, antitumor [16], antiplatelet [17] antimalarial [18], and antimicrobial activities [19,20]. Thiourea derivatives also possess anti-HCV [21], anti-HIV, antituberculosis, and antileukemic activity [22]. Fluorinated thioureas constitute a novel class of potent influenza virus neuraminidase inhibitors [23].

In continuation of our research work on urea and thiourea derivatives as potential lead compounds in our drug discovery program and keeping in mind the urease inhibitory activity of thiourea [24], we have synthesized a variety of thioureas and screened them for their urease inhibitory properties.

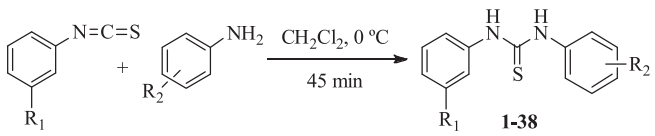
2. Results and discussion

2.1. Chemistry

We have previously reported the synthesis of unsymmetrically-substituted *N*,*N*′-diaryl thioureas and urea derivatives in excellent yields and their *in vitro* bioactivities determined as cytotoxic, phytotoxic, acetylcholinesterase, butyrylcholinesterase [14a], and anti-glycation activities [14b]. Along with that we have being working on sulfur containing compounds [25], as well as hydrazones in search lead molecules [26]. In the present study thirty-eight unsymmetrically *N*,*N*′-diaryl-substituted thioureas **1**–**38** were synthesized from commercially available phenyl and 3-chlorophenyl isothiocyanate with a variety of substituted anilines and heterocyclic amines in dichloromethane at 0 °C for 45 min according to literature procedures [27], as shown in Scheme 1 (Table 1). In all experiments, solid materials were formed which were filtered, triturated with 10 mL of dichloromethane and excess of hexane, dried under vacuum and recrystallized with methanol. The structures were determined using different spectroscopic methods like ^{1}H NMR, EI MS, IR and gave satisfactory CHN analysis. The compounds **1**, **8**, **10**, **15**, **25**–**32**, **34**, **37** are reported by various research groups [28].

2.2. In vitro urease inhibitory evaluation

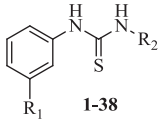
All the synthetic thioureas were screened for their urease inhibitory assays according to literature protocol [29]. Most of the 38 thiourease **1**–**38**, demonstrated good *in vitro* urease inhibitory properties having IC_{50} values in the range of 5.53 ± 0.02–82.70 ± 0.14 μM, whereas standard inhibitor, thiourea, has an IC_{50} value 21.00 ± 0.08 μM, respectively. Compounds **1**, **3**, **4**, **9**, **13**–**16**, **18**–**20**, **26**, and **30** exhibiting IC_{50} values 20.00 ± 0.02, 20.33, 20.06 ± 0.02, 20.90 ± 0.02, 11.03 ± 0.02, 20.10 ± 0.00, 19.10 ± 0.00, 16.30 ± 0.02, 15.03 ± 0.02, 18.60 ± 0.04, 15.90 ± 0.00, 18.30 ± 0.14,

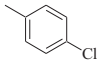
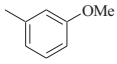
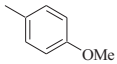
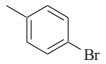
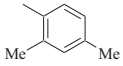
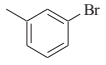
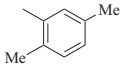
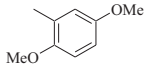
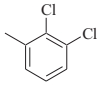
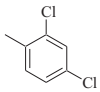
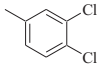
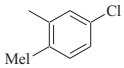
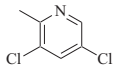
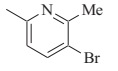


Synthetic Scheme of Arylthiourea **1-38**

Scheme 1. Synthetic Scheme of Arylthiourea **1**–**38**.

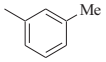
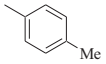
Table 1
Synthesis of symmetrical and unsymmetrical *N*,*N*′-disubstituted thioureas **1**–**38**.



Compound	R_1	R_2	Compound	R_1	R_2
1	Cl		**20**	Cl	
2	Cl		**21**	Cl	
3	Cl		**22**	Cl	
4	Cl		**23**	Cl	
5	Cl		**24**	Cl	
6	Cl		**25**	H	
7	Cl		**26**	H	
8	Cl		**27**	H	
9	Cl		**28**	H	
10	Cl		**29**	H	
11	Cl		**30**	H	
12	Cl		**31**	H	
13	Cl		**32**	H	
14	Cl		**33**	H	
15	Cl		**34**	H	
16	Cl		**35**	H	

(*continued on next page*)

Table 1 (*continued*)

Compound	R_1	R_2	Compound	R_1	R_2
17	Cl		**36**	H	
18	Cl		**37**	H	
19	Cl		**38**	H	

and 8.43 ± 0.02 μM, respectively, were found to be the most active members of this series and superior to the standard inhibitor, while **2**, **5**–**9**, **11**, **17**, **21**–**23**, **25**, **27**–**29**, **32**–**34**, **37** and **38** showed still good inhibiting activity against ureases. Compounds **10**, **12**, **24**, **35**, and **36** have shown minor or no inhibitory potential (Table 2).

N-(3-Chlorophenyl)-*N*′-(2-methoxyphenyl) thiourea (**4**) bearing a 2-methoxyphenyl residue at R_2 of the thiourea bridge was found to be the better active than standard (IC_{50} = 20.06 ± 0.02 μM). If the activity of compound **4** as compared to that of its analogs **5** and **6** (IC_{50} values 29.56 ± 0.07 and 24.10 ± 0.04 μM, respectively), then it appears that the position of the methoxy group at R_2 plays a crucial rule for urease inhibitory activity.

N-Phenyl-*N*′-(3-pyridinyl) thiourea (**30**), bearing a 3-pyridyl substituent at R_2 of the thiourea bridge, was the second most active compound (IC_{50} = 8.43 ± 0.02 μM) of the series. Comparing its activity with those of the isomers *N*-phenyl-*N*′-(2-pyridinyl)thiourea (**29**) (IC_{50} = 23.36 ± 0.02 μM), and *N*-phenyl-*N*′-(4-pyridinyl)thiourea (**31**) (IC_{50} = 43.93 ± 0.14 μM), then the inhibiting power decreases from 3-pyridinyl >2-pyridinyl >4-pyridinyl analogs. Increasing the distance of the 3-pyridinyl residue of **6** from the thiourea moiety by a methyl spacer causes a dramatic decrease of the inhibiting activity as demonstrated by *N*-phenyl-*N*′-(3-pyridinyl) thiourea (**37**) (IC_{50} = 33.50 ± 0.04 μM). Introduction of a 2-chloro atom into the pyridine ring of **30** as in *N*-(2-chloro-3-pyridinyl)-*N*′-phenylthiourea (**36**), causes most probably due to the space-filling and electronic property of the chlorine atom a complete loss of chelation with the enzyme's active site. This suggestion is also confirmed by the total inactivity found for *N*-(3-chlorophenyl)-*N*′-(2-chloro-3-pyridinyl) thiourea (**24**) with a close structure to *N*-(2-chloro-3-pyridinyl)-*N*′-phenylthiourea (**36**). A general trend in the decline of inhibiting activity is observed comparing the *N*-phenyl derivatives, *N*-phenyl-*N*′-(3-pyridinyl) thiourea (**30**) (IC_{50} = 8.43 ± 0.02 μM), *N*-phenyl-*N*′-(3-pyridinyl) thiourea (**37**) (IC_{50} = 33.50 ± 0.04 μM), and *N*-phenyl-*N*′-(4-pyridinylmethyl)thiourea (**38**) (IC_{50} = 32.60 ± 0.07 μM) with their chloro analogs *N*-(3-chlorophenyl)-*N*′-(3-pyridinyl)thiourea (**23**) (IC_{50} = 24.46 ± 0.02 μM), *N*-(3-chlorophenyl)-*N*′-(3-pyridinylmethyl) thiourea (**22**) (IC_{50} = 23.03 ± 0.02 μM), and *N*-(3-chlorophenyl)-*N*′-(4-pyridinylmethyl)thiourea (**21**) (IC_{50} = 22.70 ± 0.07 μM) demonstrating that the chlorine atom attached to the phenyl ring causes only a minor influence on the chelating efficiency of the inhibitors.

Compounds **20** (IC_{50} = 15.90 ± 0.00 μM) and **32** (IC_{50} = 25.30 ± 0.08 μM) with a 3-methyl residue attached to the pyridyl moiety were also found to be potent urease inhibitors. As mentioned above, the chlorine atom attached to the phenyl residue has only a minor effect on the inhibiting activity. *N*-(3,5-Dichloro-2-pyridinyl)-*N*′-phenylthiourea (**33**), another pyridine-containing compound, showed an IC_{50} value of 37.03 ± 0.07 μM, while *N*-(5-bromo-6-methyl-2-pyridinyl)-*N*′-phenylthiourea (**35**) (IC_{50} = 82.70 ± 0.14 μM) was found to be the least active compound among the present series.

N-(3-Chlorophenyl)-*N*′-(3,4-dichlorophenyl) thiourea (**13**) containing a dichlorophenyl residue at the thiourea bridge, showed a good urease inhibitory potential with an IC_{50} value 11.03 ± 0.02 μM), however, other dichlorophenyl-containing compounds, like **10**, **11**, and **12**, had IC_{50} values of 40.96 ± 0.02, 27.10 ± 0.04, and 42.20 ± 0.00 μM, respectively. This activity difference may be explained on the basis of the different positions of dichloro substituents on the phenyl ring. In compound **13**, the dichloro residues are present at *meta* and *para* position to the thiourea bridge which may be attend an orientation to block the active site of the urease. However, in compound **11**, the chloro substituents are at *meta* and *ortho* position of the phenyl ring, whereas, in compounds **10** and **12** the chloro atoms are attached at *ortho*, *ortho* and *ortho*, *para* positions, respectively. Comparing the dichloro substitutions and activity differences it appears that the *meta* chloro substituent is more influential on the urease inhibiting activity than the *ortho* and *para* substituents: Compound **13** (*meta*-, *para*-disubstituted) showed higher activity than compound **11** (*ortho*-, *meta*-disubstituted), and compounds **10** (IC_{50} = 40.96 ± 0.02 μM) and **12** (42.20 ± 0.00 μM), lacking a *meta*-chloro substituent, had the least urease inhibitory activity. A same pattern of activity was observed for the monochlorinated compounds **1**, **2**, and **3** exhibiting IC_{50} values of 20.00 ± 0.02, 22.20 ± 0.04, and 20.33 ± 0.00 μM in accordance to the foregoing discussions. Generally, it was observed that halogen substitution at *meta* position of the phenyl ring significantly enhances the urease inhibitory potential of unsymmetrically-substituted thioureas as demonstrated by the following comparison: *N*-(3-Bromophenyl)-*N*′-phenylthiourea (**26**) (IC_{50} = 18.30 ± 0.12 μM) >*N*-(4-bromophenyl)-*N*′-phenylthiourea (**25**). The methyl residue at *meta* position of the phenyl ring exerts an enhancing effect on the compound's inhibiting activity compared to the *ortho*- and *para*-phenyl analogs: Monomethyl substitution **18** (*N*-(3-chlorophenyl)-*N*′-(3-methylphenyl)thiourea (IC_{50} = 15.03 ± 0.02 μM) > **19** (*N*-(3-chlorophenyl)-*N*′-(4-methylphenyl)thiourea (IC_{50} = 18.60 ± 0.04 μM) > dimethyl substitution, like **9** (*N*-(3-chlorophenyl)-*N*′-(2,5-dimethylphenyl)thiourea (IC_{50} = 20.90 ± 0.02 μM) > **7** (*N*-(3-chlorophenyl)-*N*′-(2,4-dimethylphenyl)thiourea (IC_{50} = 24.10 ± 0.04 μM) > **8** (*N*-(3-chlorophenyl)-*N*′-(2,6-dimethylphenyl)thiourea (IC_{50} = 27.40 ± 0.09 μM).

The present studies demonstrate that substitutions by functional groups attached to the phenyl or heterocyclic ring R_2 to the thiourea bridge exert a decisive influence on the urease inhibitory

Table 2
In vitro urease inhibitory activity of compounds **1**–**38**.

Compounds	IC_{50} ± S.E.M.[a] (μM)	Compounds	IC_{50} ± S.E.M.[a] (μM)
1	20.00 ± 0.02	**20**	15.90 ± 0.00
2	22.20 ± 0.04	**21**	22.70 ± 0.07
3	20.33 ± 0.00	**22**	23.03 ± 0.02
4	20.06 ± 0.02	**23**	24.46 ± 0.02
5	24.46 ± 0.02	**24**	NA[b]
6	29.56 ± 0.07	**25**	34.26 ± 0.05
7	24.10 ± 0.04	**26**	18.30 ± 0.14
8	27.40 ± 0.09	**27**	24.30 ± 0.00
9	20.90 ± 0.02	**28**	29.93 ± 0.07
10	40.96 ± 0.02	**29**	23.36 ± 0.02
11	27.10 ± 0.04	**30**	8.43 ± 0.02
12	42.20 ± 0.00	**31**	43.93 ± 0.14
13	11.03 ± 0.02	**32**	25.30 ± 0.08
14	20.10 ± 0.00	**33**	37.03 ± 0.07
15	19.10 ± 0.00	**34**	32.36 ± 0.12
16	16.30 ± 0.02	**35**	82.70 ± 0.14
17	34.40 ± 0.04	**36**	NA[b]
18	15.03 ± 0.02	**37**	33.50 ± 0.04
19	18.60 ± 0.04	**38**	32.60 ± 0.07
Thioureas[c]	21.00 ± 0.11	–	–

[a] S.E.M. the standard error of the mean.
[b] Not active.
[c] Standard inhibitor of the enzyme urease.

Table 3
The inhibition and kinetics parameters of urease by thiourea derivatives.

Compounds	IC_{50} (μM)	K_m (mM)	K_m app (mM)	V_{max} (μmol/min)$^{-1}$	V_{max} app (μmol/min)$^{-1}$	*Ki* (μM) ± S.E.M	Type of inhibition
3	20.3	2.25	2.61	28.16	11.98	18.1 ± 0.02	Mixed
1	20.0	2.25	3.24	30.2	20.04	18.07 ± 0.013	Mixed
9	20.9	2.25	3	30.21	22.83	19.29 ± 0.026	Mixed
13	11.0	2.25	6	27.32	27.32	11.7 ± 0.031	Competitive
14	20.1	2.25	2.83	30.67	17.87	18.48 ± 0.014	Mixed
15	19.1	2.25	2.9	30.3	18.14	16.82 ± 0.024	Mixed
18	15.0	2.25	2.86	27.62	19.58	15.7 ± 0.020	Mixed
19	18.6	2.25	2.25	22.47	7.56	12.1 ± 0.013	Noncompetitive
26	18.3	2.25	2.64	30.39	16.44	15.58 ± 0.0233	Mixed
30	8.4	2.25	7.62	29.15	29.15	8.6 ± 0.024	Competitive
Standard (Thiourea)	21	2.25	7.38	19.12	19.2	20.01 ± 0.020	Competitive

Result represents as mean of triplicate ± standard error of mean (S.E.M.).

activity of this class of compounds. From the series, compound (**30**) (IC_{50} = 8.43 ± 0.02 μM) exhibit the strongest inhibiting power against the enzyme urease and it is striking that for structure, the lone pair electrons of the nitrogen of **30** in R_2 have a distance of three bond length from the thiourea center and seem to support the chelating efficiency of the inhibitor attacking the active site of the enzyme.

Kinetics studies

To check the inhibition mechanism of these compounds, the kinetics studies of the most active compounds (Compounds **1**, **3**, **9**, **13**, **14**, **15**, **18**, **19**, **26** and **30**) were performed. In the kinetics studies along with different concentration of test compound the substrates were also varied. These compounds inhibited the jack bean urease in a concentration-dependent manner with *Ki* values between 8.6 and 19.29 μM. Form the kinetics studies it was clear that most of these inhibitors were found to be mixed-type of inhibitors, except compounds **13**and **30** which were competitive, while compound **19** was identified as a non-competitive inhibitors Table 3. The type of inhibition was determined by Lineweaver–Burk plots, the reciprocal of the rate of the reaction were plotted against the reciprocal of substrate concentration to monitor the effect of inhibitor on both K_m and V_{max}. The *Ki* values were calculated by plotting the slope of each line in the Lineweaver–Burk plots against the different concentration of compounds. The *Ki* values were confirmed from Dixon plot by plotting the reciprocal of the rate of reaction against the different concentrations of compounds.

In mixed-type of inhibition, both K_m and V_{max} are affected. The high K_m and low V_{max} of these compounds indicated the mixed-type of inhibition (Fig. 1). In the presence of compound **13**, the V_{max} of jack bean urease was not affected, while the K_m increased which indicated a pure competitive type of inhibition (Fig. 2). In the presence of compound **19**, the K_m of enzyme was not affected, while the V_{max} of enzyme decreased thus indicated a non-competitive inhibition (Fig. 3).

3. Conclusion

Summarizingly, a series of substituted thiourea derivatives have been synthesized successfully in appreciable yields and evaluated for their *in vitro* urease inhibition potential. SAR studies carried out to investigate the role of substitutions by functional groups attached to the thiourea bridge which exert imperative influence on the urease inhibitory potential. It is also concluded that substituents having the lone pair of electrons and are appropriately oriented from the thiourea center and seem to support the chelating efficiency of the inhibitor attacking the active site of the enzyme exhibit the strongest inhibiting power against the enzyme urease. The easy, at low cost accessible thiourea series resulted in the discovery of several most efficient urease inhibitions which are

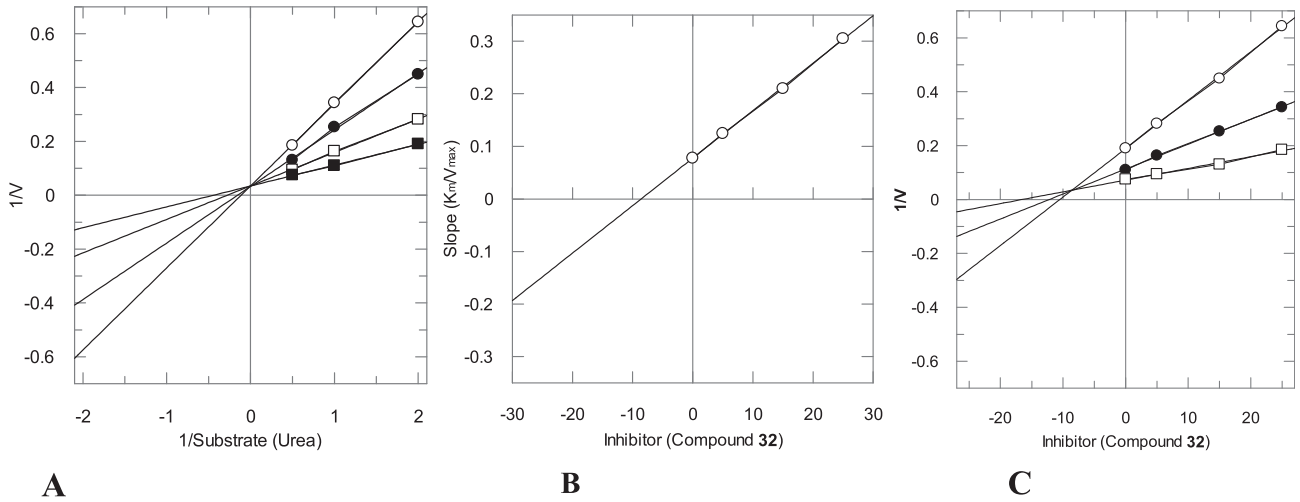


Fig. 1. The inhibition of urease by compound **30** (A) Lineweaver–Burk plot of reciprocal of rate of reaction (velocities) *vs* reciprocal of substrate (urea) in the absence (■), and in presence of 5 μM (□), 10 μM (●), and 15 μM (○) of compound **30**. (B) Secondary replot of Lineweaver–Burk plot between the slopes of each line on Lineweaver–Burk plot *vs* different concentrations of compound **30**. (C) Dixon plot of reciprocal of rate of reaction (velocities) *vs* different concentrations of compound **30**.

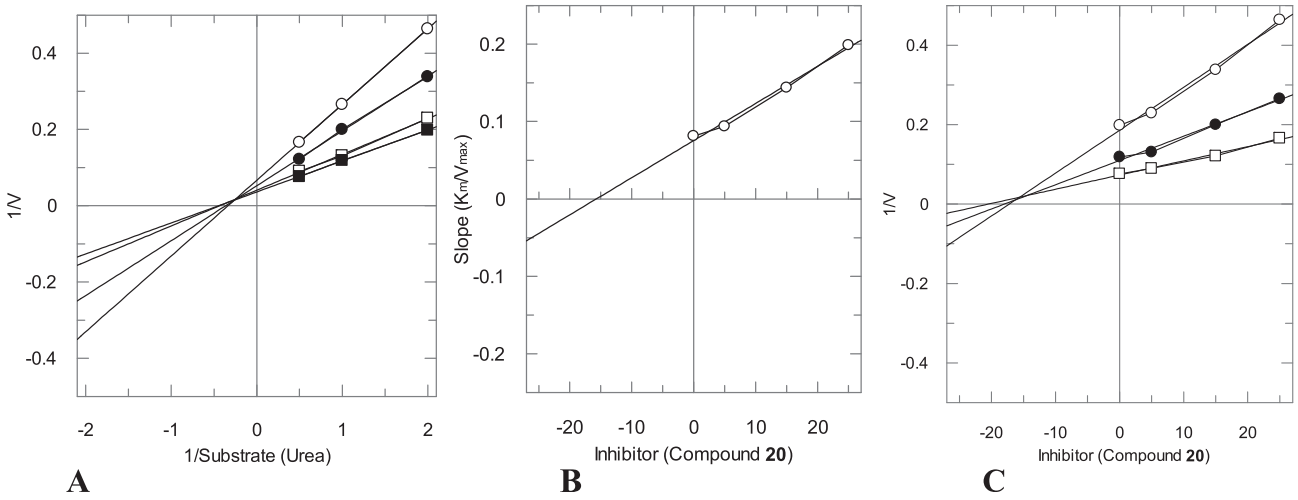


Fig. 2. The inhibition of urease by compound **18** (A) Lineweaver–Burk plot of reciprocal of rate of reaction (velocities) *vs* reciprocal of substrate (urea) in the absence (■), and in presence of 5 μM (□), 10 μM (●), and 15 μM (○) of compound **18**. (B) Secondary replot of Lineweaver–Burk plot between the slopes of each line on Lineweaver–Burk plot *vs* different concentrations of compound **18**. (C) Dixon plot of reciprocal of rate of reaction (velocities) *vs* different concentrations of compound **18**.

attractive compounds to be further developed for research and industrial applications.

4. General experimental

Melting points were determined on a Büchi 434 melting point apparatus and are uncorrected. NMR was performed on Bruker AV 300, 400, and 500 MHz instruments, respectively. CHN analyses were determined on a Carlo Erba Strumentazion-Mod-1106, Italy, instrument. Infrared (IR) spectra were recorded on a JASCO IR-A-302 spectrometer. Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A, Germany, spectrometer. Thin layer chromatography (TLC) was performed on precoated silica gel glass plates (Kieselgel 60, 254, E. Merck, Germany).

4.1. Urease assay and inhibition

The reaction mixtures, comprising 25 μL of enzyme (jack bean urease) solution and 55 μL of buffers containing 100 mM urea, were incubated with 5 μL of the test compounds (0.5 mM concentration) at 30 °C for 15 min in 96-well plates. For the kinetics assessment the urea concentrations were changed from 2 to 24 mM. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [26]. Briefly, 45 μL of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and, 70 μL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed by using SoftMaxPro software (molecular Device, USA). The entire assays were performed at pH 6.8. Percentage inhibition was calculated from the formula $100 - (OD_{test\ well}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor for urease.

4.2. General procedure for the synthesis of compounds *1*–*38*

3-Chlorophenyl isothiocyanate and phenyl isothiocyanate (1.9 mmol and 3.7 mmol) was dissolved in 10 mL dichloromethane

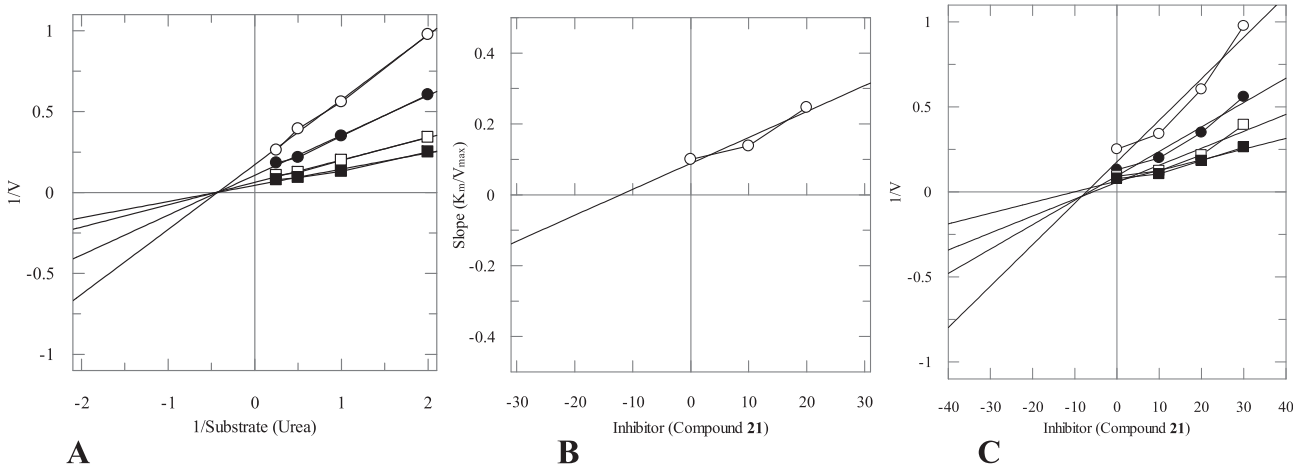


Fig. 3. The inhibition of urease by compound **19** (A) Lineweaver–Burk plot of reciprocal of rate of reaction (velocities) *vs* reciprocal of substrate (urea) in the absence (■), and in presence of 5 μM (□), 10 μM (●), and 15 μM (○) of compound **19**. (B) Secondary replot of Lineweaver–Burk plot between the slopes of each line on Lineweaver–Burk plot *vs* different concentrations of compound **19**. (C) Dixon plot of reciprocal of rate of reaction (velocities) *vs* different concentrations of compound **19**.

at 0 °C. Then aniline (2.7 mmol and 5.5 mmol), was added dropwise with constant stirring, and completion of reaction is checked by TLC analysis. After 45 min a white solid appeared, the resultant solid product was filtered, washed with hexane, triturated with 10 mL of dichloromethane and excess of hexane and dried under vacuum. Recrystallization with methanol afforded the desired solid thiourea **1**–**38**.

*4.2.1. 1,3-Bis(3-chlorophenyl)thiourea (**1**)*

Yield: 74%; M.p.: 161 °C R_f: 0.49 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3223, 3034, 1587, 1538, 1476, 1307, 1206, 1078 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 7.59 (t, 2H, H-2, 2′ J = 3.5 Hz), 7.35 (dt, 2H, H-4, 4′ J = 7.0, 3.0 Hz), 7.32 (t, 2H, H-5, 5′ J = 8.0 Hz), 7.17 (ddd, 2H, H-6, 6′, J = 7.5, 2.0, 2.0 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 177.14 (C═S), 137.50 (C—NHAr), 137.50 (C—NHAr), 133.80 (C—Cl), 133.80 (C—Cl) 130.20, 130.20, 128.10, 128.10, 126.50, 126.50, 124.20, 124.20; EI MS: m/z (rel. abund. %) 296 (5.0), 266 (36.0), 264 (100.0), 262 (100.0), 227 (6.0), 169 (36), 127 (37), 111 (42), 90 (21); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 52.54; H, 3.39; N, 9.43; Found: C, 52.57; H, 3.42; N, 9.47.

*4.2.2. 1-(2-Chlorophenyl)-3-(3-chlorophenyl)thiourea (**2**)*

Yield: 73%; M.p.: 162 °C R_f: 0.50 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3295, 3204, 1587, 1535, 1476, 1351, 1203, 1066 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.36 (s, 1H, NH), 8.87 (s, 1H, NH), 7.82 (br.t, 1H, H-2, J = 4.8 Hz), 7.78 (dd, 1H, H-6′, J = 8.1, 1.5 Hz), 7.51 (dd, 2H, H-4, 3′, J = 7.8, 1.5 Hz), 7.39 (br.t, 2H, H-5, 5′, J = 8.4 Hz), 7.30 (dt, 1H, H-4′, J = 7.8, 1.5 Hz), 7.21 (ddd, 1H, H-6, J = 8.1, 1.8, 1.5 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 178.10 (C═S), 138.20 (C—NHAr), 137.60 (C—NHAr), 134.10 (C—Cl), 133.20, 131.30, 131.20 (C—Cl), 130.60, 130.20, 128.10, 126.50, 124.60, 124.10; EI MS: m/z (rel. abund. %) 266 (14.0), 264 (71), 262 (100), 192 (10), 125 (12), 111 (13), 75 (14); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 52.54; H, 3.39; N, 9.43; Found: C, 52.56; H, 3.41; N, 9.45.

*4.2.3. 1-(3-Chlorophenyl)-3-(4-chlorophenyl)thiourea (**3**)*

Yield: 80%; M.p.: 163 °C °C R_f: 0.50 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3208, 3025, 1590, 1539, 1482, 1316, 1221, 1095 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.22 (s, 2NH), 7.72 (br.s, 1H, H-2), 7.56 (br.d, 2H, H-2′, 6′, J = 8.7 Hz), 7.46 (d, 1H, H-4, J = 8.1 Hz), 7.38 (d, 2H, H-3′, 5′, J = 8.7 Hz), 7.37 (t, 1H, H-5, J = 8.4 Hz), 7.19 (ddd, 1H, H-6, J = 6.0, 2.1, 1.8 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.50 (C═S), 138.30 (C—NHAr), 137.10 (C—NHAr), 134.60 (C—Cl), 133.20 (C—Cl), 131.30, 131.30, 130.20, 129.20, 129.20, 128.10, 126.70, 124.10; EI MS: m/z (rel. abund. %) 298 (M^+ + 1, 15), 297 (M^+, 5), 296 (21), 127 (31), 85 (63), 83 (100); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 52.54; H, 3.39; N, 9.43; Found: C, 52.58; H, 3.43; N, 9.47.

*4.2.4. 1-(3-Chlorophenyl)-3-(2-methoxyphenyl)thiourea (**4**)*

Yield: 78%; M.p.: 177 °C R_f: 0.45 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3266, 3192, 3008, 1590, 1507, 1464, 1363, 1282, 1202, 1117, 1024 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 7.58 (t, 1H, H-2, J = 4.0 Hz), 7.34 (dt, 1H, H-4′, J = 7.8, 1.5 Hz), 7.30 (t, 1H, H-5′, J = 8.0 Hz), 7.26 (t, 1H, H-5, J = 8.0 Hz), 7.16 (ddd, 1H, H-4, J = 7.5, 2.0, 1.5 Hz), 7.09 (t, 1H, H-3′, J = 4.5 Hz), 6.95 (dd, 1H, H-6′, J = 8.0, 2.0 Hz), 6.77 (ddd, 1H, H-6, J = 6.0, 2.5, 2.5 Hz), 4.83 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 178.530 (C═S), 154.20 (C—O), 138.10 (C—NHAr), 134.20 (C—Cl), 130.20, 129.10, 128.10, 126.60, 125.10; (C—NHAr), 124.80, 124.40, 121.80, 113.00, 55.80; EI MS: m/z (rel. abund. %) 292 (M^+, 4.0), 169 (100.0), 165 (42.0), 123 (85.2), 111 (56.4), 94 (52.3); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 57.43; H, 4.48; N, 9.57; Found: C, 57.45; H, 4.50; N, 9.59.

*4.2.5. 1-(3-Chlorophenyl)-3-(3-methoxyphenyl)thiourea (**5**)*

Yield: 79%; M.p.: 168 °C R_f: 0.45 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3253, 3139, 2949, 1593, 1542, 1469, 1350, 1161, 1041 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 7.73 (t, 1H, H-2′, J = 3.5 Hz), 7.59 (t, 1H, H-2, J = 4.0 Hz), 7.40 (ddd, 1H, H-4′, J = 8.0, 2.0, 1.5 Hz), 7.35 (dt, 1H, H-4, J = 8.0, 1.5 Hz), 7.32 (t, 2H, H-5′, 6′, J = 8.0 Hz), 7.26 (t, 1H, H-5, J = 8.0 Hz), 7.17 (ddd, 1H, H-6, $J_{6,5}$ = 7.5, 2.0, 1.5 Hz), 4.85 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.10 (C═S), 160.30 (C—O), 138.70 (C—NHAr), 138.10 (C—NHAr), 134.50 (C—Cl), 130.60, 130.10, 126.70, 124.50, 118.90, 117.00, 110.30, 55.60; EI MS: m/z (rel. abund. %) 292 (M^+, 4.0), 169 (100.0), 165 (42.0), 123 (85.2), 111 (56.4), 94 (52.3); EI MS: m/z (rel. abund. %) 215 (32), 171 (79), 169 (51), 111 (36), 91 (48), 75 (47); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 57.43; H, 4.48; N, 9.57; Found: C, 57.46; H, 4.51; N, 9.60.

*4.2.6. N-(3-Chlorophenyl)-N′-(4-methoxyphenyl)thiourea (**6**)*

Yield: 80%; M.p.: 169 °C R_f: 0.49 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3334, 3160, 3031, 1593, 1545, 1480, 1301, 1248, 1165, 1097, 1037 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 7.56 (t, 1H, H-2, J = 4.0 Hz), 7.32 (dt, 1H, H-4, J = 8.5, 2.0 Hz), 7.29 (t, 1H, H-5, J = 8.0 Hz), 7.27 (d, 2H, H-3′, 5′, J = 9.0 Hz), 7.16 (ddd, 1H, H-6, J = 7.5, 1.5, 2.0 Hz), 6.93 (d, 2H, H-2′, 6′, J = 7.0 Hz), 3.79 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 178.10 (C═S), 158.90 (C—O), 138.60 (C—NHAr), 134.40 (C—Cl), 131.10 (C—NHAr), 130.50, 128.40, 127.60, 127.60, 126.90, 124.60, 114.40, 114.40, 55.70; EI MS: m/z (rel. abund. %) 292 (M^+, 36), 259 (8.0), 171 (16), 169 (42), 165 (35), 140 (67), 127 (28), 111 (28), 108 (100.0), 83 (58); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 57.43; H, 4.48; N, 9.57; Found: C, 57.46; H, 4.51; N, 9.60.

*4.2.7. 1-(3-Chlorophenyl)-3-(2,4-dimethylphenyl)thiourea (**7**)*

Yield: 75%; M.p.: 193 °C R_f: 0.59 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3207, 3038, 1593, 1520, 1474, 1341, 1087 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 9.65 (s, 1H, NH), 9.42 (s, 1H, NH), 7.71 (br.s, 1H, H-2), 7.40 (d, 1H, H-4, J = 8.0 Hz), 7.33 (t, 1H, H-5, J = 8.0 Hz), 7.14 (d, 1H, H-6′, J = 8.0 Hz), 7.10 (d, 1H, H-5′, J = 8.0 Hz), 7.05 (s, 1H, H-3′), 7.00 (d, 1H, H-6, J = 8.0 Hz), 2.26 (s, 3H, CH_3), 2.18 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.50 (C═S), 143.90 (C—CH_3), 139.90 (C—CH_3), 138.60 (C—NHAr), 134.50 (C—Cl), 132.80 (C—NHAr), 131.30, 130.50, 128.50, 126.80, 126.40, 124.50, 119.50, 21.50, 18.50; EI MS: m/z (rel. abund. %) 290 (M^+, 3), 153 (4), 121 (22), 111 (40), 83 (100), 55 (33); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 61.95; H, 5.20; N, 9.63; Found: C, 60.57; H, 5.46; N, 9.57.

*4.2.8. 1-(3-Chlorophenyl)-3-(2,6-dimethylphenyl)thiourea (**8**)*

Yield: 74%; M.p.: 195 °C R_f: 0.59 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3331, 3152, 2976, 1591, 1533, 1491, 1349, 1088 cm^{-1}; ^1H NMR (500 MHz, DMSO): δ 9.77 (s, 1H, NH), 9.60 (s, 1H, NH), 7.68 (br.s, 1H, H-2), 7.39 (t, 1H, H-5, J = 8.4 Hz), 7.31 (br.d, 2H, H-4, 6, J = 8.4 Hz), 7.15 (br.d, 1H, H-4′, J = 7.8 Hz), 6.91 (d, 2H, H-3′, 5′, J = 8.7 Hz), 2.49 (s, 6H, 2CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.50 (C═S), 138.50 (C—NHAr), 135.70 (C—CH_3), 135.50 (C—NHAr), 135.40 (C—CH_3), 134.70 (C—Cl), 130.50, 128.20, 127.80, 127.80, 127.10, 126.80, 124.70, 18.60, 18.60; EI MS: m/z (rel. abund. %) 290 (M^+, 90), 275 (100), 257 (24), 121 (15), 83 (77); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 61.95; H, 5.20; N, 9.63; Found: C, 61.97; H, 5.22; N, 9.65.

*4.2.9. 1-(3-Chlorophenyl)-3-(2,5-dimethylphenyl)thiourea (**9**)*

Yield: 77%; M.p.: 194 °C R_f: 0.58 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3206, 3007, 2916, 1593, 1524, 1475, 1344, 1131, 1083 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 9.69 (s, 1H, NH), 9.45 (s, 1H, NH), 7.69 (s, 1H, H-2), 7.39 (d, 1H, H-4, J = 8.4 Hz), 7.33 (t, 1H, H-5, J = 8.0 Hz), 7.15 (s, 1H, H-6′), 7.12 (d, 1H, H-3′, J = 8.0 Hz), 7.03 (s, 1H, H-4′), 6.99 (d, 1H, H-6, J = 8.0 Hz), 2.25 (s, 3H, CH_3), 2.17 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.70 (C═S), 138.60 (C—NHAr), 135.8 (C—CH_3), 135.70 (C—NHAr), 133.40 (C—CH_3), 134.50 (C—Cl), 130.30, 129.20, 128.30, 127.00, 124.90, 124.60, 117.60, 23.20, 18.00; EI MS: m/z (rel. abund. %) 290 (M^+, 90), 275 (100), 257 (24), 121 (15), 83 (77); EI MS: m/z (rel. abund. %) 290 (M^+, 23), 275 (12), 163 (17), 138

(41), 130 (27), 121 (100), 120 (48), 111 (14), 106 (46), 77 (43); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 61.95; H, 5.20; N, 9.63; Found: C, 61.97; H, 5.22; N, 9.65.

*4.2.10. 1-(3-Chlorophenyl)-3-(2,6-dichlorophenyl)thiourea (**10**)*

Yield: 70%; M.p.: 193 °C R_f: 0.43 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3218, 3005, 1591, 1524, 1433, 1331, 1286, 1244, 1085 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 10.12 (s, 1H, NH), 9.58 (s, 1H, NH), 7.70 (s, 1H, H-2), 7.53 (d, 2H, H-3′, 5′, J = 8.0 Hz), 7.43 (d, 1H, H-4, J = 8.4 Hz), 7.38 (m, 2H, H-4′,5), 7.20 (d, 1H, H-6, J = 8.0 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.60 (C═S), 138.50 (C—NHAr), 138.20 (C—Cl), 138.20 (C—Cl), 134.60 (C—Cl), 130.80 (C—NHAr), 130.50, 128.40, 128.30, 128.30, 126.80, 126.40, 124.50; EI MS: *m*/*z* (rel. abund. %) 300 (32), 298 (98), 296 (100), 125 (11), 111 (9), 75 (10); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 47.08; H, 2.74; N, 8.45; Found: C, 47.10; H, 2.76; N, 8.47.

*4.2.11. 1-(3-Chlorophenyl)-3-(2,3-dichlorophenyl)thiourea (**11**)*

Yield: 74%; M.p.: 171 °C R_f: 0.44 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3219, 1588, 1533, 1501, 1360, 1200, 1154 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.48 (s, 1H, NH), 8.98 (s, 1H, NH), 7.80 (t, 1H, H-2, J = 3.9 Hz), 7.74 (dd, 1H, H-6′ J = 8.1, 1.5 Hz), 7.50 (dd, 2H, H-4, 4′, J = 8.1, 1.5 Hz), 7.39 (t, 2H, H-5, 5′, J = 8.1 Hz), 7.22 (ddd, 1H, H-6, J = 7.8, 1.8, 1.8 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.70 (C═S), 138.60 (C—NHAr), 137.40 (C—NHAr), 136.30 (C—Cl), 134.70 (C—Cl), 132.60 (C—Cl), 132.10, 130.50, 130.30, 128.6, 128.4, 126.80, 124.50; EI MS: *m*/*z* (rel. abund. %) 300 (4), 298 (17), 297 (72), 295 (100), 203 (12), 169 (11), 161 (16), 127 (17); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 47.08; H, 2.74; N, 8.45; Found: C, 47.10; H, 2.76; N, 8.47.

*4.2.12. 1-(3-Chlorophenyl)-3-(2,4-dichlorophenyl)thiourea (**12**)*

Yield: 73%; M.p.: 182 °C R_f: 0.45 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3296, 3197, 1587, 1532, 1499, 1354, 1202, 1098 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 7.80 (d, 1H, H-6′, J = 8.7 Hz), 7.79 (t, 1H, H-2, J = 3.9 Hz), 7.56 (d, 1H, H-3′, J = 2.4 Hz), 7.49 (ddd, 1H, H-4, J = 8.1, 1.8, 1.2 Hz), 7.41 (dd, 1H, H-5′, J = 8.4, 2.1 Hz), 7.39 (t, 1H, H-5, J = 8.1 Hz), 7.22 (ddd, 1H, H-6, J = 8.1, 1.8, 1.8 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.90 (C═S), 138.60 (C—NHAr), 134.60 (C—Cl), 134.10 (C—NHAr), 133.90 (C—Cl), 132.70, 131.60 (C—Cl), 130.70, 130.40, 128.40, 126.80, 124.50, 121.20; EI MS: *m*/*z* (rel. abund. %) 299 (16), 297 (75), 295 (100), 203 (16), 169 (21), 160 (48), 133 (16), 127 (46), 111 (35), 75 (43); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 47.08; H, 2.74; N, 8.45; Found: C, 47.10; H, 2.76; N, 8.47.

*4.2.13. 1-(3-Chlorophenyl)-3-(3,4-dichlorophenyl)thiourea (**13**)*

Yield: 75%; M.p.: 171 °C R_f: 0.47 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3175, 3009, 1586, 1537, 1472, 1328, 1245, 1128, 1098 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 7.77 (d, 1H, H-2′, J = 2.5 Hz), 7.59 (t, 1H, H-2, J = 4.0 Hz), 7.46 (d, 1H, H-5′, J = 8.5 Hz), 7.38 (dd, 1H, H-6′, J = 8.5, 2.5 Hz), 7.35 (dt, 1H, H-4, J = 8.0, 3.5 Hz), 7.32 (t, 1H, H-5, J = 8.1 Hz), 7.18 (ddd, 1H, H-6, $J_{6,5}$ = 8.0, 2.0, 1.5, Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.80 (C═S), 138.60 (C—NHAr), 136.60 (C—NHAr), 135.30, 134.60 (C—Cl), 131.30 (C—Cl), 130.40, 129.40 (C—Cl), 129.10, 128.40, 126.80, 124.70, 121.20; EI MS: *m*/*z* (rel. abund. %) 331 (M^+, 3), 203 (35), 169 (32), 163 (27), 161 (43), 145 (19), 129 (34), 127 (100), 111 (39), 92 (21), 75 (36); Anal. calcd for $C_{13}H_{10}Cl_2N_2S$: C, 47.08; H, 2.74; N, 8.45; Found: C, 47.11; H, 2.77; N, 8.48.

*4.2.14. 1-(5-Chloro-2-methylphenyl)-3-(3-chlorophenyl)thiourea (**14**)*

Yield: 80%; M.p.: 185 °C R_f: 0.49 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3253, 3034, 2838, 1593, 1518, 1475, 1333, 1241, 1081 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.18 (s, 1H, NH), 8.87 (s, 1H, NH), 7.74 (m, 1H, H-2), 7.47 (s, 1H, H-4′), 7.44 (dd, 1H, H-4, J = 6.3, 3.3 Hz), 7.36 (t, 1H, H-5, J = 8.1 Hz), 7.29 (d, 1H, H-4′, J = 8.1 Hz), 7.22 (d, 1H, H-6′, J = 8.4 Hz), 7.18 (dd, 1H, H-6, J = 7.8, 1.8 Hz), 2.29 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.90 (C═S), 138.60 (C—NHAr), 137.40 (C—NHAr), 134.70 (C—CH_3), 134.60 (C—Cl), 131.80 (C—Cl), 130.80, 130.40, 128.40, 127.00, 124.80, 124.50, 113.80, 17.90; EI MS: *m*/*z* (rel. abund. %) 331 (M^+, 3), 203 (35), 169 (32), 163 (27), 161 (43), 145 (19), 129 (34), 127 (100), 111 (39), 92 (21), 75 (36); EI MS: *m*/*z* (rel. abund. %) 312 (M^+ + 1, 8), 311 (M^+, 11), 183 (12), 169 (12), 148 (23), 141 (58), 129 (31), 127 (100), 111 (27), 106 (45), 89 (28), 75 (28); Anal. calcd for $C_{14}H_{12}Cl_2N_2S$: C, 54.03; H, 3.89; N, 9.00; Found: C, 54.05; H, 3.91; N, 9.02.

*4.2.15. 1-(3-Chlorophenyl)-3-(4-fluorophenyl)thiourea (**15**)*

Yield: 79%; M.p.: 189 °C R_f: 0.43 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3224, 3015, 1556, 1509, 1475, 1336, 1235, 1072 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.15 (s, 1H, NH), 7.76 (m, 1H, H-2), 7.54 (m, 2H, H-3′, 5′), 7.45 (dd, 1H, H-4, J = 8.1, 4.8 Hz), 7.36 (t, 1H, H-5, J = 8.1 Hz), 7.19 (dd, 1H, H-6, J = 6.9, 2.7 Hz), 7.15 (dt, 2H, H-2′, 6′, $J_{2',F} = J_{6',F} =$, 8.7, 2.1 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.90 (C═S), 163.20 (d, J = 205 Hz, C—F), 138.40 (C—NHAr), 134.60 (C—Cl), 134.10 (C—NHAr), 131.10, 131.10, 130.30, 128.20, 126.80, 124.50, 115.70, 115.70; EI MS: *m*/*z* (rel. abund. %) 283 (M^+ + 2, 3), 282 (M^+ + 1, 18), 280 (M^+, 51), 247 (6), 153 (17), 127 (49), 111 (100), 95 (15); Anal. calcd for $C_{13}H_{10}ClFN_2S$: C, 55.62; H, 3.59; N, 9.98; Found: C, 55.65; H, 3.62; N, 10.01.

*4.2.16. 1-(3-Chlorophenyl)-3-(3,4-difluorophenyl)thiourea (**16**)*

Yield: 75%; M.p.: 184 °C R_f: 0.40 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3204, 3031, 1594, 1537, 1474, 1345, 1246, 1207, 1151, 1109 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.25 (s, 1H, NH), 7.72 (m, 1H, H-2), 7.67 (d, 1H, H-2′, $J_{2',F}$ = 8.1, 1.5 Hz), 7.44 (dd, 1H, H-4, $J_{4,5}$ = 8.1, 4.8 Hz), 7.38 (t, 1H, H-5, J = 8.1 Hz), 7.31 (t, 1H, H-5′, $J_{5'(F,6)}$ = , 7.50 Hz), 7.28 (t, 1H, H-6′, J = 5.1 Hz), 7.21 (dt, 1H, H-6, J = 8.1, 3.0 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.90 (C═S), 148.70 (d, J = 175 Hz, C—F), 143.80 (d, J = 165 Hz, C—F), 138.40 (C—NHAr), 134.60 (C—Cl), 134.20 (C—NHAr), 130.40, 128.20, 126.80, 126.50, 124.60, 116.40, 114.80; EI MS: *m*/*z* (rel. abund. %) 300 (M^+ + 2, 12), 299 (M^+ + 1, 7), 298 (M^+, 33), 171 (16), 129 (100), 127 (52), 113 (20), 111 (16), 75 (15); Anal. calcd for $C_{13}H_9ClF_2N_2S$: C, 52.27; H, 3.04; N, 9.38; Found: C, 52.29; H, 3.06; N, 9.40.

*4.2.17. 1-(3-Chlorophenyl)-3-(o-tolyl)thiourea (**17**)*

Yield: 77%; M.p.: 168 °C R_f: 0.59 (acetone/hexane, 3:7); IR (KBr): ν_{max} 1588, 2926, 1789, 1645, 1526, 1448, 1207 cm^{-1}; ^1H NMR (500 MHz, DMSO): δ 9.84 (s, 1H, NH), 9.60 (s, 1H, NH), 7.79 (s, 1H, H-2), 7.44 (d, 1H, H-4, J = 8.1 Hz), 7.33 (t, 1H, H-5, J = 8.1 Hz), 7.26 (t, 2H, H-4', 5′, J = 7.8 Hz), 7.20 (dd, 1H, H-3′, J = 7.5 Hz), 7.15 (d, 1H, H-6′, J = 7.5 Hz Hz), 7.10 (s, 1H, H-6), 2.22 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.80 (C═S), 138.40 (C—NHAr), 135.60 (C—NHAr), 136.40 (C—CH_3), 134.50 (C—Cl), 130.70, 130.30, 129.60, 128.20, 127.00, 126.80, 126.10, 124.50, 17.80; EI MS: *m*/*z* (rel. abund. %) 278 (M^+ + 2, 33), 277 (M^+ + 1, 21), 276 (M^+, 89), 261 (29), 243 (48), 242 (100), 169 (37), 149 (39), 127 (68), 111 (22), 107 (78), 91 (29); Anal. calcd for $C_{14}H_{13}ClN_2S$: C, 60.75; H, 4.73; N, 10.12; Found: C, 60.77; H, 4.75; N, 10.14.

*4.2.18. 1-(3-Chlorophenyl)-3-(m-tolyl)thiourea (**18**)*

Yield: 79%; M.p.: 155 °C R_f: R_f: 0.57 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3338, 3155, 2991, 1585, 1535, 1500, 1475, 1346, 1203, 1083 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.14 (s, 1H, NH), 9.06 (s, 1H, NH), 7.75 (t, 1H, H-2, J = 3.9 Hz), 7.46 (ddd, 1H, H-4, J = 8.1, 2.7, 1.8 Hz), 7.34 (m, 3H, H-5, 2′, 4′), 7.26 (dt, 1H, H-5′, J = 7.5, 2.0 Hz), 7.17 (ddd, 1H, H-6′, J = 7.8, 4.8, 2.1 Hz Hz), 7.02 (d, 1H, H-6, J = 7.2 Hz), 2.86 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.80 (C═S), 138.60 (C—CH_3), 138.40 (C—NHAr), 137.10 (C—NHAr), 134.50 (C—Cl—Cl), 130.30, 128.80, 128.2, 126.80, 125.20, 125.10, 124.50,

123.40, 21.2; EI MS: *m/z* (rel. abund. %) 278 (M^+ + 2, 25), 277 (M^+ + 1, 15), 276 (M^+, 65), 244 (67), 242 (100), 171 (41), 169 (100), 149 (38), 127 (63), 111 (37), 107 (100), 91 (44); Anal. calcd for $C_{14}H_{13}ClN_2S$: C, 60.75; H, 4.73; N, 10.12; Found: C, 60.77; H, 4.75; N, 10.14.

*4.2.19. 1-(3-Chlorophenyl)-3-(p-tolyl)thiourea (**19**)*

Yield: 80%; m.p.: 178 °C R_f: 0.59 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3210, 3072, 3000, 1591, 1545, 1478, 1324, 1223, 1093 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.12 (s, 1H, NH), 9.01 (s, 1H, NH), 7.77 (m, 1H, H-2), 7.47 (d, 1H, H-4, J = 8.1 Hz), 7.38 (m, 3H, H-5, 3′, 5′), 7.19 (d, 2H, H-2′, 6′, J = 7.8 Hz), 7.15 (dd, 1H, H-6, J = 6.6, 1.8 Hz), 2.31 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.80 (C═S), 138.50 (C—NHAr), 137.10 (C—CH_3), 135.40 (C—NHAr), 134.50 (C—Cl), 130.30, 129.20, 129.20, 128.20, 126.80, 126.30, 126.30, 124.50, 21.20; EI MS: *m/z* (rel. abund. %) 278 (M^+ + 2, 27), 277 (M^++1, 16), 276 (M^+, 70), 244 (36), 242 (100), 169 (32), 127 (42), 111 (18), 107 (84), 91 (52); Anal. calcd for $C_{14}H_{13}ClN_2S$: C, 60.75; H, 4.73; N, 10.12; Found: C, 60.77; H, 4.75; N, 10.14.

*4.2.20. 1-(3-Chlorophenyl)-3-(3-methylpyridin-2-yl)thiourea (**20**)*

Yield: 80%; M.p.: 178 °C R_f: 0.59 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3210, 3072, 3000, 1591, 1545, 1478, 1324, 1223, 1093 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.12 (s, 1H, NH), 9.01 (s, 1H, NH), 7.77 (m, 1H, H-2), 7.47 (d, 1H, H-4, J = 8.1 Hz), 7.38 (m, 3H, H-5, 3′, 5′), 7.19 (d, 2H, H-2′, 6′, J = 7.8 Hz), 7.15 (dd, 1H, H-6, J = 6.6, 1.8 Hz), 2.31 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.80 (C═S), 154.40 (C—NHAr), 145.60, 138.40 (C—NHAr), 137.30, 134.50 (C—Cl), 130.30, 128.20, 126.80, 124.50, 116.80 (C—CH_3), 113.70, 16.90; EI MS: *m/z* (rel. abund. %) 278 (M^+ + 2, 27), 277 (M^+ + 1, 16), 276 (M^+, 70), 244 (36), 242 (100), 169 (32), 127 (42), 111 (18), 107 (84), 91 (52); Anal. calcd for $C_{14}H_{13}ClN_2S$: C, 60.75; H, 4.73; N, 10.12; Found: C, 60.77; H, 4.75; N, 10.14.

*4.2.21. 1-(3-Chlorophenyl)-3-(pyridin-4-ylmethyl)thiourea (**21**)*

Yield: 80%; M.p.: 178 °C R_f: 0.59 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3210, 3072, 3000, 1591, 1545, 1478, 1324, 1223, 1093 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.12 (s, 1H, NH), 9.01 (s, 1H, NH), 7.77 (m, 1H, H-2), 7.47 (d, 1H, H-4, J = 8.1 Hz), 7.38 (m, 3H, H-5, 3′, 5′), 7.19 (d, 2H, H-2′, 6′, J = 7.8 Hz), 7.15 (dd, 1H, H-6, J = 6.6, 1.8 Hz), 2.31 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 179.40 (C═S), 149.70, 149.70, 147.50 (C—CH_2—NHAr), 138.40 (C—NHAr), 134.50 (C—Cl), 130.30, 128.20, 126.80, 124.50, 122.30, 122.30, 50.70; EI MS: *m/z* (rel. abund. %) 278 (M^+ + 2, 27), 277 (M^+ + 1, 16), 276 (M^+, 70), 244 (36), 242 (100), 169 (32), 127 (42), 111 (18), 107 (84), 91 (52); Anal. calcd for $C_{14}H_{13}ClN_2S$: C, 60.75; H, 4.73; N, 10.12; Found: C, 60.77; H, 4.75; N, 10.14.

*4.2.22. 1-(3-Chlorophenyl)-3-(pyridin-3-ylmethyl)thiourea (**22**)*

Yield: 74%; M.p.: 167 °C R_f: 0.32 (acetone/hexane, 4.5:5.5); IR (KBr): ν_{max} 3159, 2993, 1589, 1532, 1428, 1382, 1245, 1118, 1025 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.14 (s, 1H, NH), 8.59 (br.s, 1H, H-2′), 8.45 (dd, 1H, H-6′, J = 4.8, 1.5 Hz), 7.93 (s, 1H, NH), 7.81 (d, 1H, H-4′, J = 7.8 Hz), 7.68 (m, 1H, H-2), 7.39 (dt, 1H, H-4, J = 6.6, 3.6 Hz), 7.35 (t, 1H, H-5, J = 7.8 Hz), 7.32 (t, 1H, H-5′, J = 7.8 Hz), 7.17 (dt, 1H, H-6, J = 7.2, 3.6 Hz), 4.92 (s, 2H, CH_2); ^{13}C NMR (125 MHz, CD_3OD): δ 179.40 (C═S), 148.60, 147.20, 138.40 (C—NHAr), 135.50 (C—CH_2—NHAr), 135.40, 134.50 (C—Cl), 123.10, 126.80, 128.20, 130.30, 124.50, 50.70; EI MS: *m/z* (rel. abund. %) 279 (M^+ + 2, 12), 278 (M^+ + 1, 6), 277 (M^+, 30), 171 (31), 169 (87), 127 (56), 111 (32), 92 (100), 80 (37); Anal. calcd for $C_{13}H_{12}ClN_3S$: C, 56.21; H, 4.35; N, 15.13; Found: C, 56.23; H, 4.37; N, 15.15.

*4.2.23. 1-(3-Chlorophenyl)-3-(pyridin-3-yl)thiourea (**23**)*

Yield: 74%; M.p.: 167 °C R_f: 0.31 (acetone/hexane, 4.5:5.5); IR (KBr): ν_{max} 3433, 2995, 1588, 1550, 1477, 1426, 1074 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.41 (s, 1H, NH), 9.32 (s, 1H, NH), 8.65 (d, 1H, H-2′, J = 2.4 Hz), 8.37 (dd, 1H, H-6′, J = 4.8, 1.5 Hz), 8.04 (ddd, 1H, H-4′, J = 5.7 2.7, 2.4 Hz), 7.76 (m, 1H, H-2), 7.47 (d, 1H, H-4, J = 8.1 Hz), 7.39 (t, 1H, H-5, J = 8.1 Hz), 7.35 (t, 1H, H-5′, J = 4.8 Hz), 7.21 (ddd, 1H, H-6, J = 6.3, 1.8, 1.2 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.40 (C═S), 140.0, 138.70, 138.40 (C—NHAr), 136.20, 134.20 (C—NHAr), 134.50 (C—Cl) 130.30, 128.20, 127.50, 126.70, 124.50; EI MS: *m/z* (rel. abund. %) 263 (M^+, 3), 229 (4), 169 (66), 136 (93), 127 (100), 111 (41), 94 (71), 78 (84); Anal. calcd for $C_{13}H_{12}ClN_3S$: C, 56.21; H, 4.35; N, 15.13; Found: C, 56.23; H, 4.37; N, 15.15.

*4.2.24. 1-(3-Chlorophenyl)-3-(2-chloropyridin-3-yl)thiourea (**24**)*

Yield: 73%; M.p.: 232 °C R_f: 0.40 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3243, 3181, 2957, 1631, 1564, 1428, 1302, 1245, 1170, 1095 cm^{-1}; ^1H NMR (500 MHz, DMSO): δ 11.19 (s, 1H, NH), 9.52 (s, 1H, NH), 8.30 (dd, 1H, H-6′, J = 4.8, 1.2 Hz), 8.07 (t, 1H, H-2, J = 3.9 Hz), 8.00 (dd, 1H, H-4′, J = 8.1, 1.2 Hz), 7.66 (dd, 1H, H-4, J = 7.8, 1.2 Hz), 7.42 (t, 1H, H-5′, J = 4.8 Hz), 7.40 (t, 1H, H-5, J = 8.1 Hz), 7.13 (dt, 1H, H-6, J = 8.1, 1.8 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.80 (C═S), 145.70 (C—NHAr), 139.40 (C—Cl), 138.70, 138.40 (C—NHAr), 134.50 (C—Cl), 130.30, 128.20, 126.80, 124.50, 123.60, 123.50; EI MS: *m/z* (rel. abund. %) 263 (40), 262 (50), 261 (100), 260 (93), 234 (7); Anal. calcd for $C_{12}H_9Cl_2N_3S$: C, 48.33; H, 3.04; N, 14.09; found: C, 48.35; H, 3.06; N, 14.11.

*4.2.25. 1-(4-Bromophenyl)-3-phenylthiourea (**25**)*

Yield: 80%; M.p.: 195 °C R_f: 0.59 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3218, 2993, 1592, 1551, 1449, 1241, 1067, 1010 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.14 (s, 1H, NH), 9.06 (s, 1H, NH), 7.53 (m, 6H, H-2, 6, 2′, 3′, 5′, 6′), 7.38 (t, 2H, H-3, 5, J = 8.1 Hz), 7.19 (t, 1H, H-4, J = 7.5 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.60 (C═S), 138.40 (C—NHAr), 137.40 (C—NHAr), 131.80, 131.80, 131.60, 131.60, 129.10, 129.00, 128.30, 126.50, 126.40, 122.60 (C—Br); EI MS: *m/z* (rel. abund. %) 309 (M^+ + 2, 7), 308 (M^+ + 1, 46), 307 (M^+, 12), 306 (39), 274 (100), 272 (94), 173 (66), 171 (72), 93 (60); Anal. calcd for $C_{13}H_{11}BrN_2S$: C, 50.82; H, 3.61; N, 9.12; found: C, 50.84; H, 3.63; N, 9.14.

*4.2.26. 1-(3-Bromophenyl)-3-phenylthiourea (**26**)*

Yield: 77%; M.p.: 157 °C R_f: 0.49 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3341, 3215, 1591, 1534, 1426, 1359, 1194, 1067 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.19 (s, 1H, NH), 9.09 (s, 1H, NH), 7.90 (m, 1H, H-6′), 7.51 (dd, 3H, H-2, 6, 4′, J = 8.1, 5.7 Hz), 7.38 (br.t, 2H, H-3, 5, J = 8.1 Hz), 7.31 (t, 1H, H-6′, J = 3.9 Hz), 7.29 (t, 1H, H-5′, J = 7.8 Hz), 7.20 (t, 1H, H-4, J = 7.8 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.80 (C═S), 139.10 (C—NHAr), 138.40 (C—NHAr), 130.20, 129.00, 129.00, 128.40, 126.50, 127.50, 126.50, 125.50, 125.60, 123.30 (C—Br); EI MS: *m/z* (rel. abund. %) 309 (M^+ + 2, 3), 308 (M^+ + 1, 16), 307 (M^+, 7), 274 (70), 272 (76), 215 (97), 213 (100), 173 (56), 135 (60), 93 (80); Anal. calcd for $C_{13}H_{11}BrN_2S$: C, 50.82; H, 3.61; N, 9.12; found: C, 50.84; H, 3.63; N, 9.14.

*4.2.27. 1-(2,4-Difluorophenyl)-3-phenylthiourea (**27**)*

Yield: 71.8%; M.p.: 158 °C R_f: 0.49 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3309, 1594, 1449, 1430, 1366, 1260, 1137 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.25 (s, 1H, NH), 8.65 (s, 1H, NH), 7.73 (m, due to fluorine coupling 1H, H-3′), 7.55 (d, 2H, H-2, 6, J = 7.8 Hz), 7.38 (t, 2H, H-3, 5, J = 7.5 Hz), 7.21 (t, 1H, H-4, J = 7.5 Hz), 7.11 (m, due to fluorine coupling 2H, H-5′, 6′); ^{13}C NMR (125 MHz, CD_3OD): δ 179.90 (C═S), 169.30 (d, J = 190 Hz, C—F), 160.80 (d, J = 200 Hz, C—F), 138.40 (C—NHAr), 129.6, 129.00, 129.00, 128.30, 126.4, 126.4, 111.3, 115.80 (C—NHAr), 105.1; EI MS: *m/z* (rel. abund. %) 266

($M^+ + 2$, 5.7), 265 ($M^+ + 1$, 17.6), 264 (M^+, 81.8), 245 (11.3), 230 (75.2), 171 (11.8), 129 (100.0), 101 (13.3), 93 (46.7); Anal. calcd for $C_{13}H_{10}F_2N_2S$: C, 59.08; H, 3.81; N, 10.60; found: C, 59.10; H, 3.83; N, 10.62.

*4.2.28. 1-(2,5-Dimethoxyphenyl)-3-phenylthiourea (**28**)*

Yield: 78%; M.p.: 166 °C R_f: 0.50 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3345, 3161, 2973, 1593, 1549, 1446, 1371, 1281, 1207 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 10.16 (s, 1H, NH), 9.20 (s, 1H, NH), 7.80 (br.s, 1H, H-6′), 7.52 (d, 2H, H-2, 6, J = 7.5 Hz), 7.34 (t, 2H, H-3, 5, J = 7.8 Hz), 7.14 (br.t, 1H, H-4, J = 8.1 Hz), 6.96 (d, 1H, H-3′, J = 8.7 Hz), 6.67 (br.d, 1H, H-4′, J = 6.3 Hz), 3.77 (3H, OCH_3), 3.67 (3H, OCH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 180.00 (C═S), 153.10 (C—OCH_3), 146.70 (C—OCH_3), 138.40 (C—NHAr), 129.10, 129.00, 128.30, 126.40, 126.40, 126.30 (C—NHAr), 111.20, 110.50, 110.50, 55.90, 55.90; EI MS: m/z (rel. abund. %) 289 ($M^+ + 1$, 8), 288 (M^+, 46), 257 (100), 254 (82), 239 (62), 224 (39), 138 (82); Anal. calcd for $C_{15}H_{16}N_2O_2S$: C, 62.48; H, 5.59; N, 9.71; Found: C, 62.50; H, 5.61; N, 9.73.

*4.2.29. 1-Phenyl-3-(pyridin-2-yl)thiourea (**29**)*

Yield: 70%; M.p.: 202 °C R_f: 0.46 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3219, 3038, 1598, 1554, 1471, 1427, 1313 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 13.93 (s, 1H, NH), 9.68 (s, 1H, NH), 8.36 (dd, 1H, H-6′, J = 5.1, 1.2 Hz), 7.88 (dd, 1H, H-5′, J = 7.8, 2.1 Hz), 7.82 (d, 2H, H-2, 6, J = 7.8 Hz), 7.39 (t, 2H, H-3, 5, J = 8.1 Hz), 7.30 (d, 1H, H-3′, J = 8.4 Hz), 7.21 (t, 1H, H-4, J = 7.5 Hz Hz), 7.13 (dt, 1H, H-4′, J = 5.4, 1.8 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 180.00 (C═S), 153.80 (C—NHAr), 148.2, 138.40 (C—NHAr), 138.20, 129.1, 129.10, 128.30, 126.50, 126.50, 117.80, 113.10; EI MS: m/z (rel. abund. %) 231 ($M^+ + 2$, 6), 230 ($M^+ + 1$, 16), 229 (M^+, 95), 195 (100), 169 (18), 137 (21), 94 (91), 78 (77); Anal. calcd for $C_{12}H_{11}N_3S$: C, 62.86; H, 4.84; N, 18.33; Found: C, 62.89; H, 4.87; N, 18.36.

*4.2.30. 1-Phenyl-3-(pyridin-3-yl)thiourea (**30**)*

Yield: 73%; M.p.: 198 °C R_f: 0.47 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3149, 3034, 1586, 1533, 1448, 1378, 1230 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 9.28 (s, 1H, NH), 9.10 (s, 1H, NH), 8.64 (br.s, 1H, H-2′), 8.34 (dd, 1H, H-6′, J = 4.8, 2.1 Hz), 8.04 (ddd, 1H, H-4′, J = 6.9, 2.7, 2.4 Hz), 7.54 (d, 2H, H-2, 6, J = 8.1 Hz), 7.39 (t, 2H, H-3, 5, J = 7.8 Hz), 7.32 (br.dd, 1H, H-5′, J = 8.4, 3.6 Hz), 7.21 (t, 1H, H-4, J = 7.5 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 180.00 (C═S), 140.1, 138.40 (C—NHAr), 138.70, 136.20, 134.20 (C—NHAr), 129.10, 129.10, 128.30, 127.50, 126.40, 126.40; EI MS: m/z (rel. abund. %) 230 ($M^+ + 1$, 3), 229 (M^+, 18), 195 (100), 136 (95), 93 (85), 78 (53); Anal. calcd for $C_{12}H_{11}N_3S$: C, 62.86; H, 4.84; N, 18.33; found: C, 62.89; H, 4.87; N, 18.36.

*4.2.31. 1-Phenyl-3-(pyridin-4-yl)thiourea (**31**)*

Yield: 75%; M.p.: 202 °C R_f: 0.48 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3272, 3121, 1632, 1566, 1532, 1494, 1292 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 7.52 (t, 2H, H-2′, 6′, J = 8.0 Hz), 7.43 (br.s, 1H, H-3′), 7.36 (m, 5H, H-2, 3, 5, 5′, 6), 7.14 (t, 1H, H-4, J = 7.2 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 180.00 (C═S), 155.20 (C—NHAr), 150.20, 150.20, 138.50 (C—NHAr), 129.20, 129.20, 128.30, 126.40, 126.40, 109.10, 109.10; EI MS: m/z (rel. abund. %) 203 (17), 135 (27), 93 (23), 77 (27); Anal. calcd for $C_{12}H_{11}N_3S$: C, 62.86; H, 4.84; N, 18.33; Found: C, 62.89; H, 4.87; N, 18.36.

*4.2.32. 1-(3-Methylpyridin-2-yl)-3-phenylthiourea (**32**)*

Yield: 72%; M.p.: 188 °C R_f: 0.59 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3398, 3020, 2769, 1631, 1594, 1514, 1449, 1362 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6): δ 14.08 (s, 1H, NH), 8.24 (d, 1H, H-6′, J = 5.1 Hz), 7.82 (d, 2H, H-2, 6, J = 7.8 Hz), 7.55 (d, 1H, H-4′, J = 8.1 Hz), 7.40 (t, 2H, H-3, 5, J = 8.1 Hz), 7.22 (t, 1H, H-4, J = 7.5 Hz), 7.09 (dd, 1H, H-5′, J = 7.2, 2.0 Hz), 2.42 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 180.00 (C═S), 154.40 (C—NHAr), 145.70, 137.40 (C—NHAr), 137.30, 129.10, 129.10, 128.30, 126.40, 126.40, 116.80, 113.70, 16.70; EI MS: m/z (rel. abund. %) 245 ($M^+ + 2$, 5), 244 ($M^+ + 1$, 15), 243 (M^+, 88), 209 (63), 150 (29), 108 (100), 93 (43); Anal. calcd for $C_{13}H_{13}N_3S$: C, 64.17; H, 5.38; N, 17.27; Found: C, 64.19; H, 5.42; N, 17.29.

*4.2.33. 1-(3,5-Dichloropyridin-2-yl)-3-phenylthiourea (**33**)*

Yield: 70%; M.p.: 190 °C R_f: 0.47 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3469, 3294, 1632, 1576, 1475, 1392, 1238, 1125, 1041 cm^{-1}; ^1H NMR (300 MHz, Acetone-d_6): δ 13.05 (s, 1H, NH), 8.60 (s, 1H, NH), 8.41 (d, 1H, H-6′, J = 2.4 Hz), 8.18 (d, 1H, H-4′, J = 2.4 Hz), 7.78 (d, 2H, H-2, 6, J = 7.8 Hz), 7.42 (t, 2H, H-3, 5, J = 8.1 Hz), 7.26 (t, 1H, H-4, J = 7.5 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 180.00 (C═S), 159.30 (C—NHAr), 149.10, 140.20, 138.40 (C—NHAr), 129.10, 129.10, 128.30, 126.40, 126.40, 117.70 (C—Cl), 112.50 (C—Cl); EI MS: m/z (rel. abund. %) 300 ($M^+ + 2$, 5), 299 ($M^+ + 1$, 24), 298 (M^+, 7), 297 (33), 262 (100), 162 (77), 135 (25), 110 (14), 93 (22); Anal. calcd for $C_{12}H_9Cl_2N_3S$: C, 48.33; H,3.04; N, 14.09; Found: C, 48.35; H, 3.06; N, 14.11.

*4.2.34. 1-(Furan-2-ylmethyl)-3-phenylthiourea (**34**)*

Yield: 70%; M.p.: 174 °C R_f: 0.47 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3287, 3167, 3005, 1591, 1549, 1496, 1319, 1249, 1164 cm^{-1}; ^1H NMR (500 MHz, DMSO): δ 9.56 (s, 1H, NH), 8.05 (s, 1H, NH), 7.60 (br.s, 1H, H-5′), 7.44 (d, 2H, H-2, 6, J = 8.7 Hz), 7.33 (t, 2H, H-3, 5, J = 8.1 Hz), 7.12 (t, 1H, H-4, J = 7.2 Hz), 6.41 (br.t, 1H, H-4′, J = 3.0 Hz), 6.32 (br.d, 1H. H-3′, J = 3.3 Hz Hz), 4.71 (br.d, 2H, CH_2, J = 5.1 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.40 (C═S), 145.70 (C—NHAr), 142.20, 138.40 (C—NHAr), 129.10, 129.10, 128.30, 126.40, 126.40, 110.50, 110.30; EI MS: m/z (rel. abund. %) 233 ($M^+ + 1$, 8), 232 (M^+, 51), 203 (13), 119 (7), 93 (36), 81 (100); Anal. calcd for $C_{12}H_{12}N_2OS$: C, 62.04; H,5.21; N, 12.06; Found: C, 62.07; H,5.24; N, 12.09.

*4.2.35. 1-(5-Bromo-6-methylpyridin-2-yl)-3-phenylthiourea (**35**)*

Yield: 72%; M.p.: 239 °C R_f: 0.45 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3218, 3026, 1600, 1551, 1447, 1370, 1299, 1141, 1033 cm^{-1}; ^1H NMR (500 MHz, DMSO): δ 13.59 (s, 1H, NH), 10.92 (s, 1H, NH), 8.00 (d, 1H, H-4′, $J_{4',3'}$ = 9.0 Hz), 7.75 (d, 2H, H-2, 6, J = 7.8 Hz), 7.41 (t, 2H, H-3, 5, J = 7.8 Hz), 7.22 (t, 1H, H-4, J = 7.5 Hz), 7.06 (d, 1H. H-3′, J = 8.7 Hz), 2.49 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 180.00 (C═S), 159.90 (C—CH_3), 157.10 (C—NHAr), 147.40, 138.40 (C—NHAr), 129.00, 129.00, 128.30, 126.40, 126.40, 109.70 (C—Br), 108.00; EI MS: m/z (rel. abund. %) 325 ($M^+ + 2$, 17), 323 ($M^+ + 1$, 23), 268 (15), 188 (22), 172 (27), 151 (66), 136 (52), 123 (88), 118 (71), 109 (51), 93 (100); Anal. calcd for $C_{13}H_{12}BrN_3OS$: C, 48.46; H, 3.75; N, 13.04; found: C, 48.48; H, 3.77; N, 13.06.

*4.2.36. 1-(2-Chloropyridin-3-yl)-3-phenylthiourea (**36**)*

Yield: 71.8%; M.p.: 230 °C R_f: 0.40 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3156, 2992, 1593, 1546, 1408, 1235, 1202, 1092 cm^{-1}; ^1H NMR (500 MHz, DMSO): δ 10.25 (s, 1H, NH), 9.54 (s, 1H, NH), 8.26 (dd, 1H, H-4′, J = 4.5, 1.5 Hz), 8.10 (dd, 1H, H-6′, J = 7.8, 1.5 Hz), 7.53 (d, 2H, H-2, 6, J = 7.8 Hz), 7.45 (dd, 1H, H-5′, J = 4.5, 7.8 Hz), 7.38 (t, 2H, H-3, 5, J = 8.1 Hz), 7.18 (t, 1H, H-4, J = 7.5 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 180.00 (C═S), 145.70 (C—NHAr), 139.40 (C—Cl), 138.70, 138.40 (C—NHAr), 129.10, 129.10, 128.30, 126.40, 126.40, 123.70, 123.50; EI MS: m/z (rel. abund. %) 263 (M^+, 6), 262 (24), 228 (47), 226 (100), 128 (18), 92 (10); Anal. calcd for $C_{12}H_{10}ClN_3OS$: C, 54.65; H, 3.82; N, 15.93; Found: C, 54.67; H, 3.84; N, 15.95.

*4.2.37. 1-Phenyl-3-(pyridin-3-ylmethyl)thiourea (**37**)*

Yield: 80%; M.p.: 175 °C R_f: 0.43 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3159, 2993, 1589, 1533, 1428, 1295 cm^{-1}; ^1H NMR (300 MHz,

DMSO): δ 9.67 (s, 1H, NH), 8.54 (br.s, 1H, H-2′), 8.45 (dd, 1H, H-6′, $J = 1.5, 4.8$ Hz), 8.22 (br.s, 1H, NH), 7.75 (t, 1H, H-5′, $J = 6.3$ Hz), 7.40 (m, 5H, H-2, 3, 4, 5, 6), 7.14 (br.t, 1H, H-4′, $J = 7.2$ Hz), 4.76 (br.d, 2H, CH_2, $J = 5.7$ Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.40 (C═S), 148.60, 147.20 (C–NHAr), 138.40 (C–NHAr) 135.50, 135.40, 129.10, 129.10, 128.30, 126.40, 126.40, 123.10, EI MS: *m/z* (rel. abund. %) 245 ($M^+ + 2$, 6), 244 ($M^+ + 1$, 18), 243 (M^+, 100), 209 (15), 168 (9), 107 (18), 93 (78); Anal. calcd for $C_{13}H_{13}N_3S$: C, 64.17; H, 5.38; N, 17.27; Found: C, 64.20; H, 5.41; N, 17.30.

*4.2.38. 1-Phenyl-3-(pyridin-4-ylmethyl)thiourea (**38**)*

Yield: 78%; M.p.: 175 °C R_f: 0.43 (acetone/hexane, 3:7); IR (KBr): ν_{max} 3266, 2996, 1598, 1529, 1420, 1344, 1242 cm^{-1}; ^1H NMR (300 MHz, DMSO): δ 9.74 (s, 1H, NH), 8.50 (br.d, 2H, H-2′, 6′, $J = 4.4$ Hz), 8.22 (br.s, 1H, NH), 7.41 (d, 2H, H-2, 6, $J = 7.6$ Hz), 7.35 (t, 2H, H-3, 5, $J = 7.6$ Hz), 7.28 (d, 2H, H-3′, 5′, $J = 6.0$ Hz), 7.15 (t, 1H, H-4, $J = 7.2$ Hz), 4.76 (br.d, 2H, CH_2, $J = 5.6$ Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 179.40 (C═S), 149.80, 149.80, 147.50 (C–NHAr), 138.40 (C–NHAr), 128.30, 129.00, 129.00, 126.50, 126.50, 122.30, 122.30, 50.70; EI MS: *m/z* (rel. abund. %) 245 ($M^+ + 2$, 3.), 244 ($M^+ + 1$, 10), 243 (M^+, 58), 210 (8), 107 (13), 93 (100), 77 (20); Anal. calcd for $C_{13}H_{13}N_3S$: C, 64.17; H, 5.38; N, 17.27; Found: C, 64.20; H, 5.41; N, 17.30.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.001.

References

[1] B. Krajewska, W. Zaborska, Bioorg. Med. Chem. 15 (2007) 4144–4151.
[2] H.L.T. Mobley, R.P. Hausinger, Microbiol. Rev. 53 (1989) 85–108.
[3] Z. Amtul, N. Kausar, Atta-ur-Rahman, S.A. Kazmi, K.M. Khan, M.I. Choudhary, Bioorg. Med. Chem. 14 (2006) 6737–6744.
[4] G. Seneviratne, L.H.J. Van Holm, E.M.H.G.S. Ekanayake, Field Crops. Res. 68 (2000) 199–203.
[5] N. Bhagat, J.S. Virdi, Bio. Med. Cent. Microbiol. 9 (2009) 262–275.
[6] F.J. Sangari, A. Seoane, M.C. Rodríguez, J. Agüero, J.M.G. Lobo, Infect. Immun. 75 (2007) 774–780.
[7] H.L. Mobley, M.D. Island, R.P. Hausinger, Microbiol. Rev. 59 (1995) 451–480.
[8] Y.T. Lin, Y.I. Kwon, R.G. Labbe, K. Shetty, Appl. Environ. Microbiol. 71 (2005) 8558–8564.
[9] C. Hatzifoti, Y. Roussel, A.G. Harris, B.W. Wren, J.W. Morrow, M. Bajaj-Elliott, Helicobacter 11 (2006) 113–122.
[10] M. Clyne, B. Drumm, Infect. Immun. 64 (1996) 2817–2820.
[11] S.W. Ragsdale, J. Biol. Chem. 284 (2009) 18571–18575.
[12] B. Yin, Z. Liu, M. Yi, J. Zhang, Tetrahedron Lett. 49 (2008) 3687–3690.
[13] P.A. Yonova, G.M. Stoilkova, J. Plant. Growth Regul. 23 (2005) 280–291.
[14] (a) S. Begum, M.I. Choudhary, K.M. Khan, Nat. Prod. Res. 23 (2009) 1719–1730;
(b) K.M. Khan, S. Saeed, M. Ali, M. Gohar, J. Zahid, A. Khan, S. Perveen, M.I. Choudhary, Bioorg. Med. Chem. 17 (2009) 2447–2451.
[15] F. Zhao, J.H. Xiao, Y. Wang, S. Li, Chin. Chem. Lett. 20 (2009) 296–299.
[16] S.N. Manjula, N.M. Noolvi, K.V. Parihar, S.A.M. Reddy, V. Ramani, A.K. Gadad, G. Singh, N.G. Kutty, C.M. Rao, Eur. J. Med. Chem. 44 (2009) 2923–2929.
[17] H. Zhang, Y. Zhang, G. Wu, J. Zhou, W. Huang, X. Hu, Bioorg. Med. Chem. Lett. 19 (2009) 1740–1744.
[18] N. Sunduru, K. Srivastava, S. Rajakumar, S.K. Puri, J.K. Saxena, P.M.S. Chauhan, Bioorg. Med. Chem. Lett. 19 (2009) 2570–2573.
[19] Z. Zhong, R. Xing, S. Liu, L. Wang, S. Cai, P. Li, Carbohydr. Res. 343 (2008) 566–570.
[20] T. Han, J.-H. Cho, C.-H. Oh, Eur. J. Med. Chem. 41 (2006) 825–832.
[21] I. Kang, L. Wang, C. Lee, Y. Lee, Y. Chao, T. Hsu, J. Chern, Bioorg. Med. Chem. Lett. 19 (2009) 1950–1955.
[22] P.P. Dixit, V.J. Patil, P.S. Nair, S. Jain, N. Sinha, S.K. Arora, Eur. J. Med. Chem. 41 (2006) 423–428.
[23] A. Saeed, U. Shaheen, A. Hameed, S.Z.H. Naqvi, J. Fluor. Chem. 130 (2009) 1028–1034.
[24] K. Sivapriya, P. Suguna, A. Banerjee, V. Saravanan, D.N. Rao, S. Chandrasekaran, Bioorg. Med. Chem. 17 (2007) 6387–6391.
[25] (a) K.M. Khan, M. Taha, F. Naz, M. Khan, F. Rahim, Samreen, S. Perveen, M.I. Choudhary, Med. Chem. 7 (2011) 704–710;
(b) K.M. Khan, M. Taha, F. Rahim, M. Ali, W. Jamil, S. Perveen, M.I. Choudhary, Lett. Org. Chem. 7 (2010) 415–419;
(c) K.M. Khan, M. Taha, M. Ali, S. Perveen, Lett. Org. Chem. 6 (2009) 319–320;
(d) K.M. Khan, M. Ali, M. Taha, S. Perveen, M.I. Choudhary, W. Voelter, Lett. Org. Chem. 5 (2008) 432–434.
[26] K.M. Khan, Z. Shah, V.U. Ahmad, M. Khan, M. Taha, F. Rahim, H. Jahun, S. Perveen, M.I. Choudhary, Med. Chem. 7 (2011) 572–580;
(b) K.M. Khan, M. Khan, M. Ali, M. Taha, S. Rasheed, S. Perveen, M.I. Choudhary, Bioorg. Med. Chem. 17 (2009) 7795–8780;
(c) K.M. Khan, M. Taha, F. Rahim, M.I. Fakhri, W. Jamil, M. Khan, S. Rasheed, A. Karim, S. Perveen, M.I. choudhary, J. Pak. Chem. Soc. 35 (2013) 929;
(d) K.M. Khan, F. Rahim, N. Ambreen, M. Taha, M. Khan, H. Jahan, Najeebullah, A. Shaikh, S. Iqbal, S. Perveen, M.I. Choudhary, Med. Chem. 9 (2013) 588;
(e) M. Taha, M.S. Baharudin, N.H. Ismail, K.M. Khan, F.M. Jaafar, Samreen, S. Siddiqui, M.I. Choudhary, Bioorg. Med. Chem. Lett. 23 (2013) 3463;
(f) K.M. Khan, M. Taha, F. Naz, S. Ali, S. Perveen, M.I. Choudhary, Med. Chem. 8 (2012) 705–7010.
[27] (a) S.M.A. Hai, S. Perveen, R.A. Khan, K.M. Khan, N. Afza, Nat. Prod. Res. 17 (2003) 351;
(b) S. Perveen, S.M.A. Hai, R.A. Khan, K.M. Khan, N. Afza, T.B. Sarfaraz, Synth. Commun. 35 (2005) 1663–1674.
[28] (a) R. Akilan, S. Kandasamy, S. Kulandaivelu, J. Narayanasamy, R. Krishnamoorthy, F. Hoong Kun, Acta Cryst. C. C52 (1996) 134–136;
(b) M.N. Noolvi, H.M. Patel, M. Kaur, Eur. J. Med. Chem. 54 (2012) 447–462;
(c) B.N. Ph, Ng. D. Xuong, Ng. H. Nam, J. Chem. Soc. (1955) 1573–1578;
(d) A.S. Fouda, M.A. Diab, A. Hussien, J. Chem. 38 (2011) 11–31;
(e) G. Vasilev, P. Ionova, Pharmazie 33 (1978) 270–273;
(f) F. Fukujiro, H. Kunio, H. Teruhisa, M. Teruki, N. Yoshikuni, K. Kayoko, S. Tatsuzo, S. Chiichiro, H. Yoshitaro, et al., Yakugaku Zasshi 91 (1971) 159–165;
(g) B. Saeedan, M.I. Choudhary, K.M. Khan, Nat. Prod. Res. 23 (2009) 1719–1730;
(h) R. Tripathi, A. Chaturvedi, R.K. Upadhayay, Res. J. Chem. Sci. 2 (2012) 18–27;
(i) S. Aakarsh, D.P. Robert, J. Chem. Cryst. 37 (2007) 755–764;
(j) R. Herle, P. Shetty, S.D. Kini, Shetty, Port. Electrochim. Acta 29 (2011) 69–78;
(k) K. Albert, U. Missao, J. Heterocycle Chem. 28 (1991) 2057–2058;
(l) G. Turan-Zitouni, D.M. Sivaci, Z.A. Kaplancikli, A. Ozdemir, Farmaco 57 (2002) 569–572.
[29] K.M. Khan, S. Iqbal, M.A. Lodhi, G.M. Maharvi, Zia-Ullah, M.I. Choudhary, Atta-ur-Rahman, S. Perveen, Bioorg. Med. Chem. 12 (2004) 1963–1968.