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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and antibacterial activities of some novel *N*,*N*′-disubstituted thiourea, 2-amino thiazole, and imidazole-2-thione derivatives

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Abstract In the search of bioactive molecules, a series of novel *N*-substituted thiourea derivatives **3**(**a**–**d**) are prepared by reaction of the α-amino pyridyl ketone hydrochloride (**2a**) with the corresponding aryl isothiocyanates. The synthesis of some new 2-amino thiazoles **4**(**a**–**d**) and imidazole-2-thiones **6**(**a**–**d**) were attempted by intramolecular cyclization reaction of the *N*,*N*′-disubstituted thioureas **3**(**a**–**d**) and their intermediate ketals **5**(**a**–**d**) in diluted aqueous acidic and strong acidic mediums. The structure of all newly synthesized compounds was established by analytical and spectral data. The antibacterial studies to all of the synthesized compounds against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis* bacteria with Gram-positive and negative strains, as MIC values are reported. Some of these compounds such as **3a**,**b**,**d** and **4b**,**d** exhibited a good to significant antibacterial activity. Also, all of new synthesized compounds **3**,**4**,**6**(**a**–**d**) were active against Gram-positive *S. aureus* bacterium. Thus, some of these compounds can emerge as a promising tool for further research work.

Keywords *N*,*N*′-Disubstituted thiourea · Imidazole · Thiazole · Microorganisms · Antibacterial activity

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Introduction

According to several recent researches, the continuous application of drugs has resulted in the evolution of resistant pathogenic microorganisms (Fluit *et al.*, 2001; National Nosocomial Infections Surveillance, 2004). One of the strategies to overcome this serious problem is the urgent synthetic development of drugs with new chemical structures. For this purpose, we joined in one single structure, two important biologically active scaffolds, the thiazole or imidazole and pyridine rings from thiourea.

It is well known that *N*,*N*′-disubstituted thioureas are important organic compounds because of their pharmacological activities (Tan *et al.*, 2011; Schroeder, 1995; Bhandari *et al.*, 2008; French *et al.*, 1970; Fanshewe and Epstein, 1991). Specially, some of their derivatives have been used in the anti-histamine drugs (Ganellin *et al.*, 1995). *N*,*N*′-(aroyl)thioureas are efficient ligands for the separation and refinement of platinum group metals (Koch, 2001). These compounds are not only a segment of biologically important but also a versatile intermediate for the synthesis of heterocyclic compounds such as 1,3-thiazoles, pyrimidines, 1,3-diazines, 1,3-quinazolines, and 1,2,4-triazines (Dodson and Carroll King, 1945; Foster and Snyder, 1963; Winckelmann and Larsen, 1986; Gopalsamy and Yang, 2000; Yang and Kaplan, 2001).

Thiazole and their derivatives have attracted continuing interest over the years because of their varied biological activities such as anti-bacterial (Tsuji and Ishikawa, 1994), anti-fungal (Wilson *et al.*, 2001), antitumor (Kumar *et al.*, 1993; Gouda *et al.*, 2012), anticonvulsant (Ergenc and Capan, 1994), antimalarial (Karade *et al.*, 2008), herbicidal, insecticidal, and anti-helminthic (Metzger, 1984). The fused and pendent 1,3-thiazoles are a ubiquitous feature of many pharmaceutical products and can be found in

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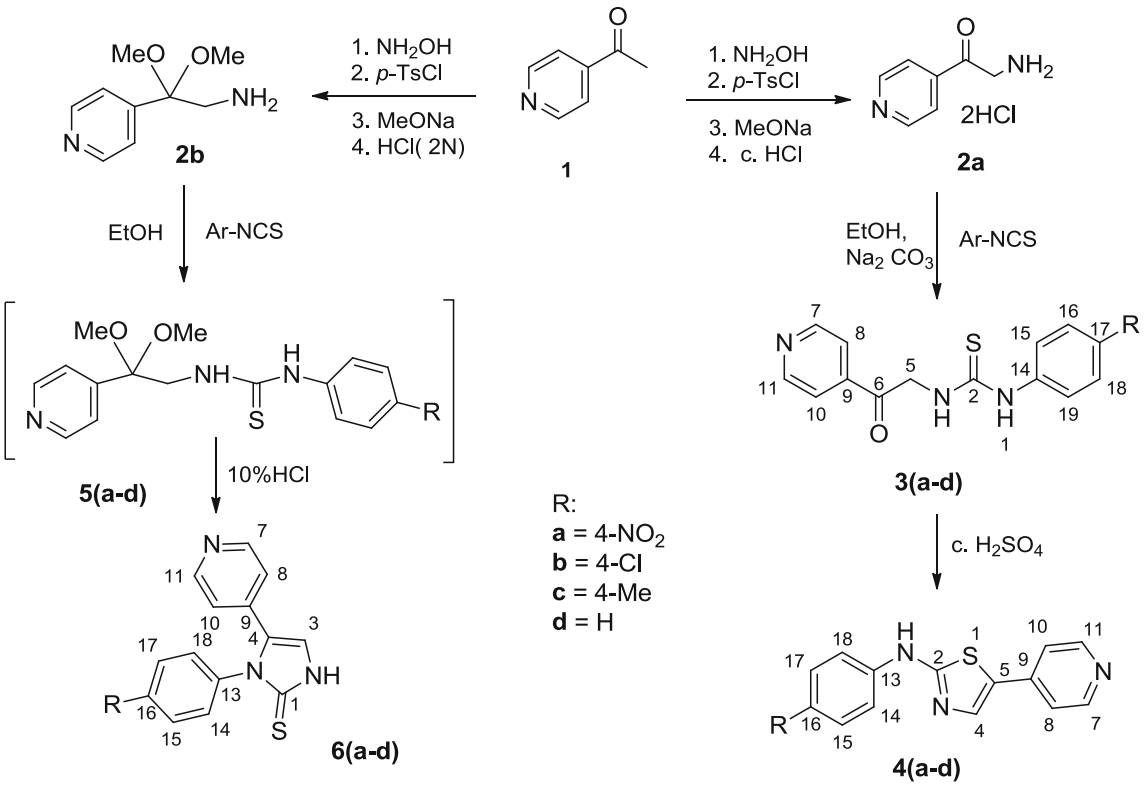

Scheme 1 The synthetic pathways of compounds **3**–**6**(**a**–**d**)

drugs that are useful in the treatment of hypertension (Patt *et al.*, 1992), schizophrenia (Jean *et al.*, 1990), inflammation (Haviv *et al.*, 1988; Clemence *et al.*, 1988), allergies (Hargrave *et al.*, 1983), and HIV (Bell *et al.*, 1995) infections. Also, the aminothiazoles are known to be ligands of estrogen receptors (Fink *et al.*, 1999).

Imidazoles are vastly distributed in nature and known as biocatalyst and biological ligands. These compounds play a vital role in life activities (Grimmett, 1984; Saeed and Batool, 2007). Based on several literature surveys, imidazole derivatives show a wide range of pharmacological activities (Shingalapur *et al.*, 2009; Sharma *et al.*, 2009; Olender *et al.*, 2009; Achar *et al.*, 2010). Furthermore, imidazole-2-thiones have been used as light-sensitive photographic materials (Saeki and Inagaki, 1985), and rubber antioxidants (Horsey and Patel, 1993). Some these derivatives, among methimazole, have been clinically used for the treatment of several diseases (Kruse *et al.*, 1990).

In view of these points and as part of ongoing studies on the synthesis and biological consideration of heterocycles (Kalhor *et al.*, 2011; Kalhor and Dadras, 2013; Mobinikhaledi *et al.*, 2010, 2012; Sharifzadeh *et al.*, 2013), we wish to report the synthesis of a new series of five-membered heterocyclic titled compounds, bearing the pyridine ring and their bactericidal activities.

Results and discussion

Synthesis

The synthetic routes for the preparation of the target compounds are outlined in Scheme 1. The required α-amino pyridyl ketone **2a** and acetal **2b** were prepared via reaction of 4-acetyl pyridine (**1**) with corresponding reagents according to low improvement Neber rearrangement (La Mattina, 1980; La Mattina and Suleske, 1985). The preparation of the thiourea derivatives **3a**–**d** was achieved by the reaction of the corresponding arylisothiocyanate with α-amino pyridyl ketone **2a**. Thiourea derivatives **3a**–**d** underwent an intramolecular cyclization under concentrated acidic conditions to produce 2-amino-1,3-thiazoles **4a**–**d** in excellent yields as the new synthetic pathway. Some of the major advantages of this practical procedure are use of available and inexpensive material, high yields, moderate temperature, easy workup and it can be as an efficient synthetic pathway for preparation of the 2-amino-1,3-thiazole rings which the research is ongoing in this regard.

The imidazole-2-thiones **6a**–**d** were afforded by refluxing a mixture corresponding aryl isothiocyanate and aminoacetal **2b** in ethanol to form an acetal-thiourea derivative as intermediate **5a**–**d** which was isolated and

Table 1 Antibacterial activities of chemical compounds **3–6(a–d)** (zone of inhibition in mm)

Compound	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. faecalis*
3a	8	10	15	25	10
3b	14	8	–[a]	18	18
3c	10	8	8	8	8
3d	14	15	8	18	10
4a	6	10	–	10	10
4b	20	–	8	29	17
4c	8	–	–	20	12
4d	10	–	18	31	10
6a	–	8	–	16	–
6b	18	7	–	18	–
6c	–	9	–	16	–
6d	–		–	20	–
Gentamicin[b]	26	23	24	22	25

Dimethyl sulfoxide (DMSO) only, control for compounds and references

[a] Not active

[b] Reference compound

Table 2 Minimum inhibitory concentration (MIC) of the selected compounds against microbial strains (μg/ml)

Compound	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. faecalis*
3a	–[a]	512	128	32	128
3b	64	–	256	256	64
3d	256	256	–	128	–
4b	64	–	–	32	128
4c	–	–	–	128	–
4d	512	–	128	16	512
6a	–	–	–	256	–
6b	128	–	–	256	–
6c	–	–	–	256	–
6d	–	512	–	128	–

Disc diffusion method used to determine the MICs (Wayne, 2011; Chand *et al.*, 1994). DMSO only, control for compounds

[a] Not tested

immediately heated in aqueous 10 %HCl via modified method of Marckwald (Matsuda *et al.*, 1997).

The structure of synthesized compounds was established by means of their IR, ^{1}H NMR, ^{13}C NMR spectra, and elemental analyses. The IR spectra of thiourea derivatives **3a–d** showed characteristic absorptions at 1202–1342, 1592–1653, and 3260–3474 cm^{-1} for the C=S, C=O, and NH stretching vibrations, respectively. ^{1}H NMR spectra of **3a–d** showed broad peaks at 5.63–11.64 ppm due to the resonance of CH_2–NH–CS and NH–CS protons, which disappeared upon D_2O addition. The ^{13}C NMR spectrum of compound **3a** showed ten signals, including a signal at 49 ppm for CH_2, a signal at 180 ppm for the C=S, and a signal at 188 ppm for the carbonyl carbon atoms.

The IR spectra of 2-amino thiazoles **4a–c** showed absorption bands at 3,241–3,467 cm^{-1} characteristic for the NH group. Also in the ^{1}H NMR spectra of all 1,3-thiazoles, the disappearance of CH_2NH signals of **4a–d**, the appearance of the NH broad signal at 10.65–10.92 ppm, and singlet signal at 8.04–8.15 ppm for aromatic hydrogen (C–H) the ring, confirm the formation of thiazole ring.

The ^{1}H NMR spectra of imidazoles **4a–d** showed singlet peaks at 10.85–11.62 ppm attributed to the resonance of the SH or NH protons, which disappeared upon D_2O addition. In the ^{13}C NMR spectra of compounds **6a–d**, the appearance of signals at the region between 166.3 and 167.9 ppm attributed to the carbon resonance of the C=N or C=S group in imidazole rings is in support of the expected thiol or thione structures. The resonance of all other protons and carbons appeared in the expected region of spectrums.

Antibacterial activities

Applying the agar plate diffusion technique (Wayne, 2011; Chand *et al.*, 1994), all of newly synthesized compounds were screened in vitro for antimicrobial activities against five pathogenic bacteria. The results of the bioassay are given in Table 1. A cursory view of the data indicates that some of the compounds **3a**,**b**,**d** and **4b**,**d** exhibit a moderate to high activity against four bacteria with Gram-positive and -negative strain. Also, all compounds are active against *Staphylococcus aureus* microbe. It is considerable that compounds **3a**, **4b**, and **4d** were found to be more active against *S. aureus* as Gram-positive bacterium than gentamicin, which is a known antimicrobial drug. Therefore, these compounds can have the potential to be good antibacterial candidate that the research is ongoing in this regard. We can also compare the inhibitory effects of compounds **3b–d** with their products, thiazoles **4b–d**, after cyclization. For instance, after cyclization of **3b** and **3d**, the inhibitory effect of the resulting thiazoles **4b** and **4d** are strikingly increased.

In addition, investigation of the minimum inhibitory concentration (MIC) values of the potent derivatives against five microorganisms was performed and the results are presented in Table 2. The compound **4d** indicates the highest bactericidal activity (16 μg/ml) against *S. aureus* bacterium. Also compounds **3a**,**b** and **4b** have a high activity (32–64 μg/ml) against three microorganisms. Just as was predicated, the compounds **6a–d** are not shown considerable bactericidal activity (Table 2).

Experimental

Melting points were determined using an electrothermal digital apparatus and are uncorrected. FT-IR spectra were obtained with a SHIMADZU-IR Prestige-21 spectrometer using KBr discs. The NMR spectra were recorded on a Bruker (500 MHz) spectrometer. Chemical shifts (ppm) are referenced to tetramethylsilane (TMS) as internal standard. Elemental analyses were performed with as elemental analyzer (Elemental, Vario EL III) at Arak University. Reactions were monitored by thin layer chromatography (TLC). The α-amino pyridyl ketone dihydrochloride **2a** and α-amino pyridyl acetal **2b** were prepared with low improvement following the previously reported procedure (La Mattina, 1980; La Mattina and Suleske, 1985).

General procedure for synthesis of the *N*,*N*′-disubstituted thioureas **3a**–**d** (Scheme 1)

A solution of α-amino pyridyl ketone **2a** (2 mmol) and sodium carbonate (1 mmol) in ethanol (15 ml) was stirred at room temperature for 10 min. To the reaction mixture, corresponding isothiocyanate was added, followed by refluxing for 9–10 h and monitored by TLC. After completing the reaction, 1 ml water was added and to reflux was continued for 1 h. The result was concentrated and cooled to room temperature to give the solid product **3a**–**d**, which was filtered, air dried, and recrystallized from ethanol.

*1-(4-Nitrophenyl)-3-(2-oxo-2-(pyridin-4-yl)ethyl)thiourea (**3a**)*

Yellowish needles (EtOH); this compound was prepared by refluxing for 10 h in 89 % yield; mp 200–201 °C; FT-IR (KBr) ν_{max} 3289 (N–H), 1653 (C=O), 1600, 1547 (C=N, C=C), 1506, 1328 (NO_2), 1202 (C=S), 1033, 844 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO–d_6): $\delta = 4.05$ (s, 2H, CH_2), 7.47 (d, 2H, $J = 6.00$ Hz, H–Ar), 7.82 (m, 2H, H–Ar), 8.13–8.24 (m, 2H, H–Ar), 8.62 (d, 2H, $J = 8.50$ Hz, H–Ar), 10.23 (s, 1H, NH), 11.64 (s, 1H, NH) ppm; ^{13}C-NMR (125 MHz, DMSO–d_6): $\delta = 49.4$ (CH_2, C-5), 120.6 (CH, C-8,10), 121.5 (CH, C-16,18), 122.5 (CH, C-15,19), 124.8 (C, C-9), 146.4 (C, C-17), 147.6 (C, C-14), 150.1 (CH, C-7,11), 180.5 (C=S), 188.1 (C=O) ppm; Anal. Calcd. for $C_{14}H_{12}N_4O_3S$: C, 53.16; H, 3.82; N, 17.71; S, 10.14; found: C, 53.37; H, 3.79; N, 17.68; S, 10.22 %.

*1-(4-Chlorophenyl)-3-(2-oxo-2-(pyridin-4-yl)ethyl)thiourea (**3b**)*

Yellowish needles (EtOH); this compound was prepared by refluxing for 10 h in 83 % yield; mp 191–192 °C; FT-IR (KBr) ν_{max} 3351, 3260 (N–H), 2939 (C–H), 1607(C=O), 1531, 1489, 1407 (C=N, C=C), 1342 (C=S), 1090 (C–Cl), 825 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): $\delta = 4.03$ (s, 2H, CH_2), 7.30 (d, 2H, $J = 8.65$ Hz, H–Ar), 7.43–7.46 (m, 4H, H-Aryl and H-Pyr), 8.61 (d, 2H, $J = 8.65$ Hz, H-Pyr), 9.70 (br, 1H, NH), 11.16 (br, 1H, NH) ppm; the NH protons disappeared on D_2O addition; ^{13}C-NMR (125 MHz, DMSO–d_6): $\delta = 49.3$ (CH_2, C-5), 122.5 (CH, C-8,10), 124.6 (CH, C-16,18), 128.3 (CH, C-15,19), 128.6 (C, C-17), 138.5 (C, C-14), 147.9 (C, C-9), 149.9 (CH, C-7,11), 181.2 (C=S), 186.1 (C=O) ppm; Anal. Calcd. for $C_{14}H_{12}ClN_3OS$: C, 54.99; H, 3.96; N, 13.74; S, 10.49; found: C, 55.12; H, 3.98; N, 13.69; S, 10.55 %.

*1-(2-Oxo-2-(pyridin-4-yl)ethyl)-3-(p-tolyl)thiourea (**3c**)*

Yellowish needles (EtOH); this compound was prepared by refluxing for 9 h in 84 % yield; mp 185–186 °C; FT-IR (KBr) ν_{max} 3383 (N–H), 2941 (C–H), 1592 (C=O), 1537, 1401, 1370 (C=N, C=C), 1307 (C=S), 1061, 643, 504 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 2.39$ (s, 3H, CH_3), 4.03 (s, 2H, CH_2), 5.63 (br, 1H, NH), 6.83 (d, 2H, $J = 8.15$ Hz, H–Ar), 7.16 (d, 2H, $J = 8.10$ Hz, H–Ar), 7.25–7.27 (m, 2H, H-Pyr), 7.65 (br, 1H, NH), 8.57 (d, 2H, $J = 8.69$, H-Pyr); ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 21.0$ (CH_3, C-20), 49.4 (CH_2, C-5), 100.6 (CH, C-8,10), 121.9 (CH, C-15,19), 125.2 (CH, C-16,18), 130.5(C, C-14), 132.8 (C, C-17), 137.6 (C, C-9), 149.5 (CH, C-7,11), 180.6 (C=S), 185.6 (C=O) ppm; Anal. Calcd. for $C_{15}H_{15}N_3OS$: C, 63.13; H, 5.30; N, 14.73; S, 11.24; found: C, 62.83; H, 5.31; N, 14.81; S, 11.10 %.

*1-(2-Oxo-2-(pyridin-4-yl)ethyl)-3-phenylthiourea (**3d**)*

Yellowish needles (EtOH); this compound was prepared by refluxing for 9 h in 86 % yield; mp 187–188 °C; FT-IR (KBr) ν_{max} 3474, 3181 (N–H), 3013, 2948 (C–H), 1593 (C=O), 1535, 1445, 1369 (C=N, C=C), 1207 (C=S), 684, 601 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 4.04$ (s, 2H, CH_2), 5.78 (br, 1H, NH), 6.97 (d, 2H, $J = 7.50$ Hz, H–Ar), 7.26–7.37 (m, 5H, H–Ar and H-Pyr), 8.25 (br, 1H, NH), 8.56 (d, 2H, $J = 8.62$ Hz, H-Pyr); ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 49.4$ (CH_2, C-5), 121.9 (CH, C-8,10), 124.7 (CH, C-15,19), 127.3 (C, C-17), 129.9 (CH, C-16,18), 135.7 (C, C-14), 148.2 (C, C-9), 149.7 (CH, C-7,11), 180.4 (C=S), 188.4 (C=O) ppm; Anal. Calcd. for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49; S, 11.82: found: C, 61.69; H, 4.86; N, 15.56; S, 11.73 %.

General procedure for synthesis of the 2-amino-thiazols **4a**–**d**

The *N*-thioureas **3a**–**d** (0.9 mmol) were added slowly to concentrated sulfuric acid (5–10 ml), which was stirred and

kept at 0 °C. The reaction mixture was stirred at room temperature for 4 h. It was then poured into ice-water (25 g) and neutralized with concentrated aqueous ammonia. The precipitate was filtered, washed with water (15 ml), dried, and recrystallized from DMF/H_2O to give pure **4a**–**d**.

*N-(4-Nitrophenyl)-5-(pyridin-4-yl)thiazol-2-amine (**4a**)*

Yellowish needles (DMF/H_2O); this compound was prepared by refluxing for 4 h in 100 % yield; mp 370–371 °C; FT-IR (KBr) ν_{max} 3281 (N–H), 1596, 1549, 1413 (C=N, C=C), 1501, 1330 (NO_2), 1103, 611 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO–d_6): $\delta = 7.57$ (d, 2H, $J = 6.20$ Hz, H–Ar), 7.88 (d, 2H, $J = 8.52$ Hz, H-Pyr), 8.15 (s, 1H, H-thiazole), 8.24 (d, 2H, $J = 8.1$ Hz, H–Ar), 8.55 (d, 2H, $J = 6.00$, H-Pyr), 10.90 (s, 1H, NH) ppm; ^{13}C-NMR (125 MHz, DMSO–d_6): $\delta = 117.1$ (CH, C-8,10), 120.0 (CH, C-14,18), 121.9 (C, C-5), 125.9 (CH, C-15,17), 138.8 (CH, C-4), 139.2 (C, C-16), 141.1 (C, C-9), 146.8 (C, C-13), 150.6 (CH, C-7,11), 163.3 (N=C–S thiazole) ppm; Anal. Calcd. for $C_{14}H_{10}N_4O_2S$: C, 56.37; H, 3.38; N, 18.78; S, 10.75: found: C, 56.07; H, 3.40; N, 18.65; S, 10.82 %.

*N-(4-Chlorophenyl)-5-(pyridin-4-yl)thiazol-2-amine (**4b**)*

Yellowish needles (DMF/H_2O); this compound was prepared by refluxing for 4 h in 92 % yield; mp 364–366 °C; FT-IR (KBr) ν_{max} 3241 (N–H), 3047 (C–H), 1629, 1599, 1546, 1494 (C=N, C=C), 1211, 1037 (C–S), 815 (C–Cl) cm^{-1}; ^{1}H-NMR (500 MHz, DMSO–d_6): $\delta = 7.38$ (d, 2H, $J = 8.50$ Hz, H–Ar), 7.51 (d, 2H, $J = 6.00$ Hz, H–Ar), 7.69 (d, 2H, $J = 8.50$ Hz, H-pry), 8.04 (s, 1H, H-thiazole), 8.51 (d, 2H, $J = 8.00$ Hz, H-Pyr), 10.70 (br, 1H, NH) ppm; the NH proton disappeared on D_2O addition; ^{13}C-NMR (125 MHz, DMSO–d_6): $\delta = 119.4$ (CH, C-8,10), 119.8 (CH, C-14,18), 124.5 (C, C-5), 125.8 (C, C-16), 129.3 (CH, C-15,17), 139.2 (CH, C-4), 139.4 (C, C-13), 139.7 (C, C-9), 150.4 (CH, C-7,11), 164.6 (N=C–S thiazole) ppm; Anal. Calcd. for $C_{14}H_{10}ClN_3S$: C, 58.43; H, 3.50; N, 14.60; S, 11.14: found: C, 58.62; H, 3.56; N, 14.81; S, 11.22 %.

*5-(Pyridin-4-yl)-N-(p-tolyl)thiazol-2-amine (**4c**)*

Yellowish needles (DMF/H_2O); this compound was prepared by refluxing for 4 h in 98 % yield; mp 355–356 °C; FT-IR (KBr) ν_{max} 3234 (N–H), 3081 (C–H), 1608, 1541, 1500 (C=N, C=C), 1210, 808, 619 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO–d_6): $\delta = 2.28$ (s, 3H, CH_3), 7.14–7.22 (m, 2H, H-Aryl), 7.49–7.54 (m, 3H, H–Ar), 8.01 (t, 1H, $J = 6.45$ Hz, H-Pyr), 8.07 (s, 1H, H-thiazole), 8.51 (d, 2H, $J = 8.70$ Hz, H-Pyr), 10.65 (s, 1H, NH) ppm; ^{13}C-NMR (125 MHz, DMSO–d_6): $\delta = 20.7$ (CH_3, C-19), 118.2 (CH, C-8,10), 119.9 (CH, C-14,18), 123.6 (C, C-5), 128.1 (CH, C-15,17), 129.9 (C, C-16), 130.9 (C, C-13), 134.2 (CH, C-4), 141.1 (C, C-9), 148.8 (CH, C-7,11), 165.2 (C-2, N=C–S thiazole) ppm; Anal. Calcd. for $C_{15}H_{13}N_3S$: C, 67.39; H, 4.90; N, 15.72; S, 11.99: found: C, 67.68; H, 4.91; N, 15.67; S, 11.88 %.

*N-Phenyl-5-(pyridin-4-yl)thiazol-2-amine (**4d**)*

Yellowish needles (DMF/H_2O); this compound was prepared by refluxing for 4 h in 85 % yield; mp 340–343 °C; FT-IR (KBr) ν_{max} 3467 (N–H), 3001, 2837 (C–H), 1631, 1503, 1463 (C=N, C=C), 1206, 1150, 1020 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO–d_6): $\delta = 7.07$–7.41 (m, 5H, H–Ar), 7.59 (d, 2H, $J = 7.50$ Hz, H-Pyr), 8.23 (s, 1H, H-thiazole), 8.57 (d, 2H, $J = 8.50$ Hz, H-Pyr) 10.92 (br, 1H, NH) ppm. EIMS (*m/z*, %): 253 (M^+, 25), 149 (10), 109 (10), 97 (20), 91 (10), 83 (25), 69 (80), 57 (100); Anal. Calcd. for $C_{14}H_{11}N_3S$: C, 66.38; H, 4.38; N, 16.59; S, 12.66: found: C, 66.08; H, 4.41; N, 16.60; S, 12.76 %.

General procedure for synthesis of the imidazole 2-thiones **6a**–**d**

A mixture of the appropriate aryl isothiocyanate (3 mmol) and pyridine amino acetal **2b** (3 mmol) in ethanol (10 ml) was refluxed for 4 h. After evaporating the solvent, intermediate compounds **5**(**a**–**d**) were formed which were dissolved in 10 % HCl (10 ml) and heating to reflux for 22–29 h. After completion of the reaction (monitoring by TLC plate), the mixture was cooled and neutralized with a solution of 10 % NaOH. The precipitate was filtered and washed thoroughly with water (5 ml), dried, and recrystallized from EtOH/H_2O to give the pure products **6a**–**d**.

*1-(4-Nitrophenyl)-5-(pyridin-4-yl)-1H-imidazole-2(3H)-thione (**6a**)*

White needles (EtOH/H_2O); this compound was prepared by refluxing for 22 h in 76 % yield; mp 177–180 °C; FT-IR (KBr) ν_{max} 3288 (N–H), 3075 (C–H), 1602, 1553 (C=N, C=C), 1505, 1335 (NO_2), 1200 (C=S), 1103, 1033, 839 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 6.98$ (d, 2H, $J = 8.85$ Hz, H–Ar), 7.46–7.55 (m, 2H, H–Ar), 8.18–8.24 (m, 4H, H–Ar), 8.29 (s, 1H, H–Ar), 11.62 (br, 1H, NH), ppm; ^{13}C-NMR (125 MHz, DMSO–d_6): $\delta = 119.4$ (CH, C-3), 121.5 (CH, C-8,10), 125.1 (C, C-4), 128.3 (CH, C-15,17), 129.1 (CH, C-14,18), 138.5 (C, C-13), 143.3 (C, C-16), 144.4 (C, C-9), 150.4 (CH, C-7,11), 167.9 (C=S) ppm; Anal. Calcd. for $C_{14}H_{10}N_4O_2S$: C, 56.37; H, 3.38; N,

18.78; S, 10.75: found: C, 56.77; H, 3.41; N, 18.62; S, 10.61 %.

*1-(4-Chlorophenyl)-5-(pyridin-4-yl)-1H-imidazole-2(3H)-thione (**6b**)*

White needles (EtOH/H_2O); this compound was prepared by refluxing for 24 h in 73 % yield; mp 102–103 °C; FT-IR (KBr) ν_{max} 3238 (N–H), 3050 (C–H), 1599, 1544, 1484, 1429, 1388 (C=N, C=C), 1322 (C=S), 1035, 815 (C–Cl), 718 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 7.23–7.27$ (m, 5H, H–Ar), 7.30 (d, 2H, $J = 7.50$ Hz, H–Ar), 8.62 (d, 2H, $J = 8.50$ Hz, H-Pyr), 11.62 (s, 1H, NH or SH) ppm; the NH or SH proton disappeared on D_2O addition; ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 119.4$ (CH, C-3), 122.7 (CH, C-8,10), 129.0 (C, C-4), 130.5 (CH, C-15,17), 134.7 (C, C-13), 135.6 (C, C-16), 138.0 (CH, C-14,18), 142.6 (C, C-9), 150.5 (CH, C-7,11), 162.8 (C=S) ppm; Anal. Calcd. for $C_{14}H_{10}ClN_3S$: C, 58.43; H, 3.50; Cl, 12.32; N, 14.60; S, 11.14: found: C, 58.93; H, 3.55; N, 14.70; S, 11.04 %.

*5-(Pyridin-4-yl)-1-(p-tolyl)-1H-imidazole-2(3H)-thione (**6c**)*

White needles (EtOH/H_2O); this compound was prepared by refluxing for 27 h in 55 % yield; mp 86–88 °C; FT-IR (KBr) ν_{max} 3224 (N–H), 3039, 2987 (C–H), 1598, 1540, 1408 (C=N, C=C), 1323 (C=S), 1211, 1035 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 2.34$ (s, 3H, CH_3), 7.14–7.22 (m, 5H, H–Ar), 7.42 (br s, 2H, H-Pyr), 8.37 (d, 2H, $J = 7.50$ Hz, H-Pyr), 10.85 (br, 1H, NH or SH) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 20.9$ (CH_3), 117.1 (CH, C-3), 119.9 (CH, C-8,10), 121.5 (C, C-4), 123.8 (CH, C-15,17), 129.5 (C, C-13), 135.1 (CH, C-14,18), 138.5 (C, C-16), 148.5 (C, C-9), 148.9 (CH, C-7,11), 166.3 (C=S) ppm; Anal. Calcd. for $C_{15}H_{13}N_3S$: C, 67.39; H, 4.90; N, 15.72; S, 11.99: found: C, 67.09; H, 4.93; N, 15.52; S, 11.80 %.

*1-Phenyl-5-(pyridin-4-yl)-1H-imidazole-2(3H)-thione (**6d**)*

White needles (EtOH/H_2O); this compound was prepared by refluxing for 29 h in 50 % yield; mp 67–70 °C; FT-IR (KBr) ν_{max} 3215 (N–H), 3044 (C–H), 1598, 1544, 1408 (C=N, C=C), 1327 (C=S), 1198, 1028, 739 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 7.19$ (br s, 3H, H–Ar), 7.55 (m, 5H, H–Ar), 8.41 (d, 2H, $J = 8.50$ Hz, H-Pyr), 11.37 (s, 1H, NH) ppm; ^{13}C-NMR (500 MHz, $CDCl_3$): $\delta = 117.1$ (CH, C-3), 119.4 (CH, C-8,10), 121.4 (C, C-4), 125.2 (C, C-16), 129.0 (CH, C-15,17), 135.7 (CH, C-14,18), 137.6 (C, C-13), 148.2 (C, C-9), 149.7 (CH, C-7,11), 166.6 (C=S) ppm; Anal. Calcd. for $C_{14}H_{11}N_3S$: C, 66.38; H, 4.38; N, 16.59; S, 12.66: found: C, 66.67; H, 4.39; N, 16.63; S, 12.73 %.

Biological screening

The antibacterial activity of synthesized compounds were screened at a concentration of 10 mg/ml against five reference strains of bacteria including *Bacillus cereus* ATCC 11778 (Gram-positive), *S. aureus* ATCC 25923 (Gram-positive), *Escherichia coli* ATCC 25922 (Gram-negative), *Pseudomonas aeruginosa* ATCC 27853 (Gram-negative), and *Enterococcus faecalis* ATCC 29212 (Gram-positive). Tested compounds were dissolved in dimethyl sulfoxide (DMSO) for the preparation of stock solution. The solvent control was included, although no antibacterial activity has been noted. All samples were tested in triplicate and the average results were recorded. Microbial susceptibility testing of all compounds was carried out by diffusion agar and minimal inhibitory concentration (MIC) methods according to Clinical and Laboratory Standards Institute (CLSI) guideline (Wayne, 2011; Chand *et al.*, 1994). The suspension of bacteria was adjusted to 0.5 MacFarland Standard (10^6 c.f.u/ml) and spread over Muller-Hinton agar. The tested compounds are placed in random well position on the plate, after overnight incubation at 37 °C the zone of inhibition determinate. The results of the bioassay are given in Tables 1 and 2.

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