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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME NOVEL 1,2,4-TRIAZOLES AND 1,3,4-THIADIAZOLES BEARING IMIDAZOLE NUCLUES

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Abstract - A series of N^1-[(4,5-di and 1,4,5-triphenylimidazol-2-yl)thioacetyl]-N^4-alkyl/aryl-thiosemicarbazides **3-7** were synthesized from (4,5-di and 3,4,5-triphenylimidazol-2-yl)thioacetic acid hydrazide **1**, **2**. The treatment of compounds **3-7** with NaOH gave 5-[(4,5-di and 1,4,5-triphenylimidazol-2-yl)-3-thiomethyl]-4-alkyl/aryl-2*H*-1,2,4-triazoles-3-thione **8-12**, while the acidic treatment of compounds **3-7** afforded 5-[(4,5-di- and 1,4,5-triphenylimidazol-2-yl)-3-thiomethyl]-2-alkyl/arylamino-1,3,4-thiadiazoles **13-17**. Moreover, potassium hydrazinecarbothionates **18, 19** were obtained from the reaction of acyl hydrazides **1, 2** with carbon disulfides in basic media and converted into 4-amino-1,2,4-triazole-3-thiones **20**, **21** and 1,3,4-thiadiazole-2-thiols **22**, **23** by the treatment with hydrazine hydrate and sulfuric acid, respectively. All newly synthesized compounds were screened for their antimicrobial activity.

INTRODUCTION

1,2,4-Triazole and its derivatives belong to a class of exceptionally active compounds possessing a wide spectrum of biological properties, including antibacterial,[1] antimicrobial,[2,3] antihypertensive,[4] analgesic,[5]

antiviral,[6] antioxidant,[7] anti-inflammatory,[8] antitumor,[9,10] anti-HIV,[11] pesticidal,[12] insecticidal,[13] herbicidal[14] and fungicidal activity.[15] Moreover, it was reported that compounds having triazole moieties, such as vorozole, letrozole and anastrazole appeared to be very effective aromatase inhibitors, which in turn prevented breast cancer.[16-18]

Furthermore, 1,3,4-thiadiazole nucleus takes part in the structure of several biologically active compounds, including antibacterial,[19-22] antifungal,[21,22] antitubercular,[23-25] analgesic,[26] antiinflammatory,[21,22,26] antidepressant,[27] leishmanicidal[28] activities.

Heterocycles containing an imidazole moiety constitute a class of compounds possessing a wide spectrum of biological activities such as anti-bacterial,[29] antiasthmatic[30] and antiulcerative[31] properties.

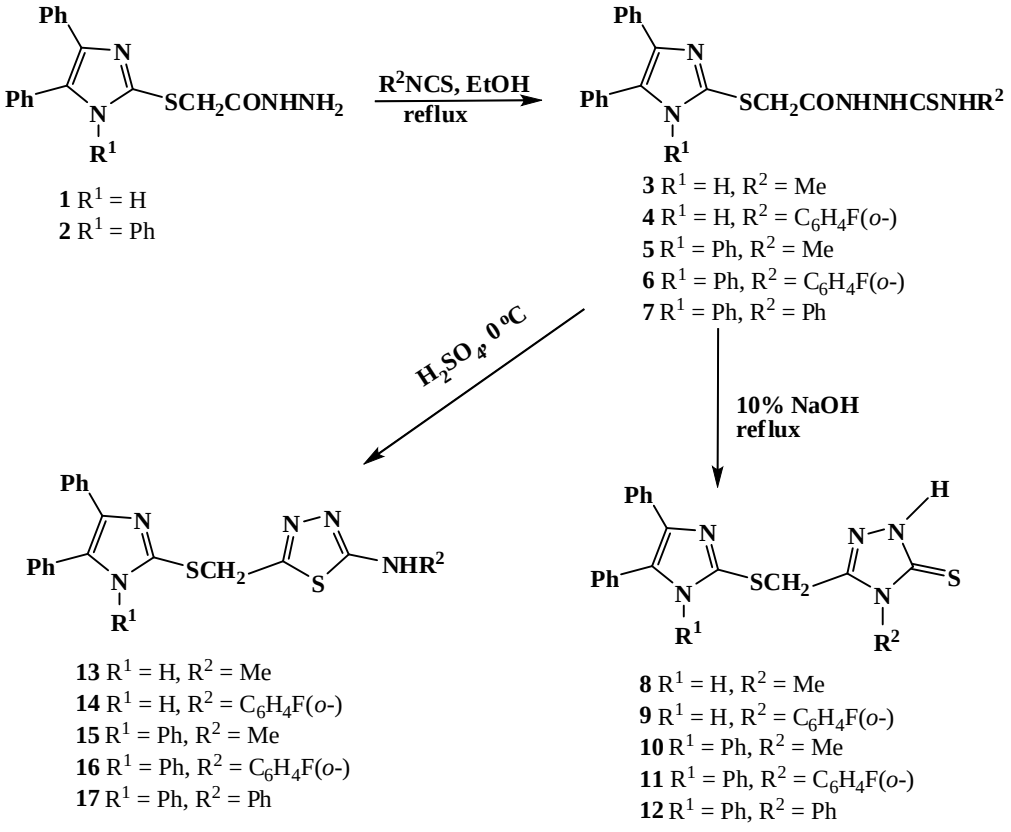
The binding of two or three heterocyclic rings having different sites or mode of action has commanded the world-wide attention of many research groups because of their high potential to exhibit antimicrobial activity.[32-34]

In view of these facts, the aim of the present study is to obtain 1,2,4-triazole and 1,3,4-thiadiazole derivatives carrying imidazole moiety as antimicrobial agents.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the 1,2,4-triazole and 1,3,4-thiadiazole derivatives is illustrated and outlined in Scheme 1.



Scheme 1. Synthetic pathway for the preparation of compounds **8**-**17**

The key intermediates **1** and **2** were prepared from ethyl(4,5-di- and 1,4,5-triphenylimidazol-2-yl)-thioacetate following the literature method.[35] Treatment of the acid hydrazides **1** and **2** with various alkyl/aryl isothiocyanates in ethanol gave corresponding N^1-[(4,5-di- and 1,4,5-triphenyl)thioacetyl]-N^4-alkyl/aryl-thiosemicarbazides **3-7** in good yield.

The structure of the compounds investigated **3-7** was confirmed by their IR spectra which displayed absorption peaks in the range of 3240-3365 cm^{-1} for NH, 1696-1703 cm^{-1} for C=O and 1296-1319 cm^{-1} corresponding to C=S stretching vibrations. Their ^1H NMR spectra showed a multiplet at δ_H 7.13-7.91 ppm for aromatic protons. The CONH and CSNH protons were observed as singlets at 8.05-8.56 and 10.08-10.36 ppm, respectively confirming the formation of acid thiosemicarbazides (controlled with D_2O). The carbon signals of these groups were recorded between 163 and 184.16 ppm.

Thiosemicarbazides undergo different cyclization reactions to give five member heterocycles. The product of cyclization depends on the reagent used. This cyclization leads to the formation of 1,3,4-oxadiazole ring through the oxidative cyclization of thiosemicarbazides using iodine and potassium iodide in ethanolic sodium hydroxide.[36,37] On the other hand, the 1,2,4-triazole and 1,3,4-thiadiazole derivatives were obtained by the treatment of thiosemicarbazides with sodium hydroxide and sulfuric acid, respectively.[32,36-38]

Therefore, the thiosemicarbazides **3-7** on heating with 10% aqueous NaOH underwent smooth cyclization through dehydration to afford 5-[(4,5-di- and 1,4,5-triphenylimidazol-2-yl)-3-thiomethyl]-4-alkyl/aryl-2*H*-1,2,4-triazoles-3-thione **8-12**.

These compounds displayed ^1H and ^{13}C NMR spectra and elemental analyses consistent with the assigned structures. The IR spectra of the triazoles **8-12** exhibited N-H bands in the region 3307-3341 cm^{-1}. The absorption bands at 1610-1630 cm^{-1} are due to the presence of C=N stretching of the triazole ring system. Absence of the C=O absorptions in **8-12** provided definitive proof for the formation of new products.

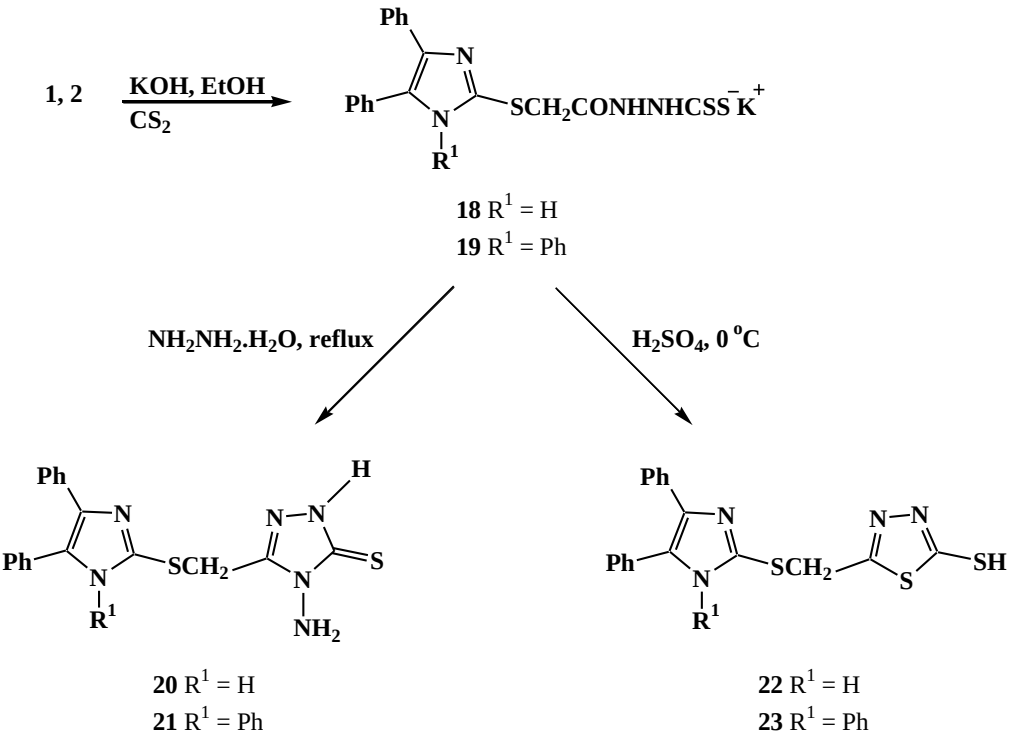
The exhibited chemical shifts obtained from ^1H NMR spectra were all supported the proposed structures of **8-12**. The ^1H NMR of **10-12** chosen as prototypes showed single NH triazole resonances in the 14.40-14.47 ppm regions (controlled with D_2O). The SCH_2 protons resonated at 4.62-4.79 ppm. Additional signals belonging to phenyl ring were observed in the aromatic region in the ^1H and ^{13}C NMR spectra of these compounds. Moreover, C=S group resonated at 181.80-183.64 ppm in the ^{13}C NMR spectra of compounds **8-12** confirming the presence of these compounds in their thione forms.

5-[(4,5-Di- and 1,4,5-triphenylimidazol-2-yl)-3-thiomethyl]-2-alkyl/arylamino-1,3,4-thiadiazoles **13-17** were obtained by cyclization of **3-7** by treating with cold concentrated sulfuric acid.

The IR spectra of the synthesized thiadiazoles **13-17** showed absorption peaks in the region 1613-1645 cm^{-1} due to C=N stretching vibrations. In the ^1H NMR spectra of **13-17**, the singlets of CONH and CSNH of thiosemicarbazides had disappeared, and the NH proton at 2-position of 1,3,4-thiadiazoles ring

appeared as singlet at 10.21-10.35 ppm (controlled with D_2O).

On the other hand, in ethanol solution of potassium hydroxide, carbohydrazides **1**, **2** interacted with carbon disulfide to potassium hydrazine carbodithionates **18** and **19**, which were used as precursors for the synthesis of 4-amino-1,2,4-triazole-3-thiones **20**, **21** and 1,3,4-thiadiazole-2-thiols **22**, **23** (scheme 2).



Scheme 2. Synthetic pathway for the preparation of compounds **18-23**

The refluxing of **18**, **19** and hydrazine hydrate led to the preparation of 4-amino-5-[(4,5-di and 1,4,5-triphenylimidazol-2-yl)-3-thiomethyl]-2*H*-1,2,4-triazole-3-thiones **20**, **21** in good yields. NMR spectral characteristic of the amino-1,2,4-triazoles **20**, **21**, revealed in their ^1H NMR spectra the two characteristic singlets of the NH_2 and NH (triazole) in the regions 7.90-8.03 ppm and 14.15-14.20 ppm, respectively (controlled with D_2O). In the NMR spectra of compounds **20** and **21**, the exocyclic C=S signals were observed at 182.30 and 183.76 ppm, respectively.

In sulfuric acid medium, the precursors **18**, **19** formed 5-[(4,5-di and 1,4,5-triphenylimidazol-2-yl)-3-thiomethyl]-1,3,4-thiadiazole-2-thiols **22**, **23**. In the ^{13}C NMR spectra of compounds **22**, **23**, the absence of the signals >180 ppm attributed to the thiocarbonyl carbons (C=S), confirmed the formation of the thiadiazoles in their thiol forms.

In addition, the chemical shifts of SH thiadiazole protons were detected as singlet at 13.92-13.98 ppm (controlled with D_2O), which confirmed the thiol form. The remaining protons and carbons were observed at the expected regions (see experimental part).

Antimicrobial activity

The antibacterial and antifungal activity, assay (Table 1) indicated that the acid hydrazides **1** and **2** show slight activity only against *E. coli* and *P. aeruginosa*. 1-[(4,5-Diphenylimidazol-2-yl)thioacyl]-4-methylthiosemicarbazide (**3**) displayed moderate to good activities against all tested microorganisms except *C. albicans* and *C. tropicalis*, whereas its 2-fluorophenyl analogue **4** displayed good activity against all bacterial and yeast strains. On the other hand, 1-[(1,4,5-triphenylimidazol-2-yl)thioacyl]-4-methylthiosemicarbazide (**5**) showed moderate antimicrobial activities towards *E. coli* and *P. aeruginosa*, while 1-[(1,4,5-triphenylimidazol-2-yl)thioacyl]-4-(2-fluorophenyl)thiosemicarbazide (**6**) showed good activity against all bacterial and yeast strains. 1-[(1,4,5-Triphenylimidazol-2-yl)thioacyl]-4-phenylthiosemicarbazide (**7**) indicated excellent activity against *E. coli*, *P. aeruginosa*, *C. albicans* and *C. tropicalis*. The antimicrobial activity of 4-alkyl/aryl-5-[(4,5-di- and 1,4,5-triphenylimidazole-2-yl)-thiomethyl]-2*H*-1,2,4-triazole-3-thiones **8-12** divulged that compound **9** showed good antimicrobial activity against tested bacterial and good antifungal activity against *C. albicans*.

Compounds **10** and **11** possessing three phenyl groups on imidazole ring exhibited excellent antibacterial activity against *S. aureus and B. subtilis* and good activity against *E. coli* and *P. aeruginosa*. The compound **12** having phenyl group on N-4 triazole ring showed excellent antifungal activity than antibacterial action. On other hand, the antimicrobial activity of the thiadiazoles **13-16** revealed that all the tested compounds possessed moderate to good inhibition, compounds **13** and **14** showed comparatively moderate activity against *E. coli* and *P. aeruginosa* and good inhibition towards *C. albicans and C. tropicalis*. Similarly, compounds **15** and **16** also showed significant activity against all tested microbial strains. In contradiction, compound **17** possessing phenyl substitution on thiadiazole ring displayed no activity.

The conversion of hydrazide structure in compound **1** to 1,2,4-triazole ring of 4-amino-5-[(4,5-diphenylimidazol-2-yl)thiomethyl]-2*H*-1,2,4-triazole-3-thione (**20**) caused excellent antimicrobial activities against all bacterial strains and *C. albicans*. 5-[(4,5-Diphenylimidazol-2-yl)thiomethyl]-1,3,4-thiadiazole-2-thiol (**21**), which is the thiadiazole derivative of compound **1**, demonstrated slight activity only towards *E. coli*. The conversion of compound **2** to compound **22** caused important antimicrobial activity. On the contrary to the activity of compound **22**, compound **23**, which was obtained from the reaction of compound **2** with H_2SO_4, indicated no activities towards all microbial strains.

Based on the activity data of all the synthesized compounds, it is concluded that imidazole bearing 1,2,4-triazole moiety exhibits better antibacterial activity than 1,3,4-thiadiazole moiety. Moreover, the presence of amino group at N-4 of triazole ring seems to have marginal effect on biological activity. In fact, upon *N*-amination the antibacterial activity marginally increases.

Interestingly, it was found that thiadiazole derivatives **13-16** are more active against fungal pathogens as compared to bacterial pathogens.

The antimicrobial activity of all the synthesized compounds could be attributed to the presence of triazole ring. This ring is incorporated into a wide variety of drugs used in medical therapy.

Table 1. Screening for antimicrobial activity of the synthesized compounds (MIC mcg/mL)

Comp. No.	E. coli	P. aur.	B. sub	S. au.	C. trop.	C. alb
1	9	10	-	-	-	-
2	11	11	-	-	-	-
3	20	18	15	22	-	-
4	22	21	20	22	20	22
5	17	20	-	-	-	-
6	20	22	22	20	21	21
7	32	34	-	-	21	22
8	8	9	7	8	-	-
9	24	23	21	22	12	20
10	21	19	34	31	-	-
11	22	21	35	34	-	-
12	20	18	16	22	25	25
13	21	20	8	11	20	20
14	20	21	10	11	20	19
15	24	22	20	18	19	20
16	22	23	20	19	20	20
17	-	-	-	-	-	-
20	31	30	32	35	9	21
21	9	-	-	-	-	-
22	33	30	30	35	18	19
23	-	-	-	-	-	-
DMF	-	-	-	-	-	-
Amp	10	18	15	35		
Flu					25	25

Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, Bs: *Bacillus. Subtilis*, Sa: *Staphylococcus aureus*, Ct: *Candida tropicalis*, Ca: *Candida albicans.* Amp: Ampicillin, Flu: Fluconazole, (-): no activity, solvent is DMF.

CONCLUSION

The research study reports the successful synthesis and antimicrobial activity of new 4-alkyl/aryl-2*H*-1,2,4-triazole-3-thiones, 4-amino-2*H*-1,2,4-triazole-3-thiones, 2-aminoalkyl/aryl-1,3,4-thiadiazoles and 1,3,4-thiadiazoles-3-thiols, carrying biologically active imidazole ring. Their antimicrobial activity study revealed that some of the compounds tested showed moderate to excellent antibacterial and antifungal activities against pathogenic strains.

EXPERIMENTAL

Chemistry

Melting points were determined on a Melt-temp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol 500 MHz spectrometer. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1430 series FTIR spectrometer. The microanalyses for C, H and N were performed on Perkin-Elmer elemental analyzer.

General method for the synthesis of compounds 3-7. A mixture of corresponding compound **1** or **2** (10 mmol) and the appropriate isothiocyanate derivatives (10 mmol) was refluxed in EtOH for 6 h. The solution was cooled and a white solid appeared. The obtained precipitate was filtered and recrystallized from ethanol to afford the desired product.

1-[(4,5-Diphenylimidazol-2-yl)thioacetyl]-4-methylthiosemicarbazide (3). Yield 88%, mp 158 °C; IR: 3355, 3270 (NH), 1700 (C=O), 1315 cm^{-1} (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 2.85 (3H, s, CH_3), 4.70 (2H, s, SCH_2), 7.18-7.63 (10H, m, arH), 8.05 (1H, s, NH), 8.55 (1H, s, NH), 10.10 (1H, s, NH), 12.54 (1H, s, NH imidazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_c 34.40 (CH_3), 40.97 (CH_2), (127.10, 127.94, 128.81, 129.21, 129.98, 131.66, 135.75, 137.89) arC, 163.00 (C=O), 181.70 (C=S). Anal. Calcd (%) for $C_{19}H_{19}N_5OS_2$: C, 57.41; H, 4.82; N, 17.62. Found: C, 57.30; H, 4.98; N, 17.52.

1-[(4,5-Diphenylimidazol-2-yl)thioacetyl]-4-(2-fluorophenyl)thiosemicarbazide (4). Yield 80%, mp 200-201 °C; IR: 3343, 3265 (NH), 1701 (C=O), 1296 cm^{-1} (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 4.61 (2H, s, SCH_2), 7.23-7.76 (14H, m, arH), 8.10 (1H, s, NH), 8.56 (1H, s, NH), 10.08 (1H, s, NH), 12.43 (1H, s, NH imidazole); ^{13}C NMR (500 MHz, DMSO-d_6) δ_C 41.75 (SCH_2), (125.33, 127.56, 128.07, 128.78, 129.15, 129.87, 130.45, 132.65, 131.80, 135.89, 138.24, 141.34) arC, 167.89 (C=O), 182.49 (C=S). Anal. Calcd (%) for $C_{24}H_{20}FN_5OS_2$: C, 60.36; H, 4.22; N, 14.66. Found: C, 60.12; H, 4.45; N, 14.46.

1-[(1,4,5-Triphenylimidazol-2-yl)thioacetyl]-4-methylthiosemicarbazide (5). Yield 84%, mp 167 °C; IR: 3345 and 3258 (NH), 1698 (C=O), 1319 cm^{-1} (C=S). ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 2.92 (3H, s, CH_3), 4.74 (2H, s, SCH_2), 7.15-7.78 (15H, m, arH), 8.20 (1H, s, NH), 8.36 (1H, s, NH), 10.24 (1H, s,

NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 35.61 (CH_3), 40.46 (CH_2), (126.87, 127.33, 127.27, 128.09, 128.74, 129.45, 129.81, 131.80, 136.09, 138.24) arC, 166.35 (C=O), 183.05 (C=S). Anal. Calcd (%) for $C_{25}H_{23}N_5OS_2$: C, 63.40; H, 4.89; N, 14.79. Found: C, 63.28; H, 4.80; N, 14.52.

1-[(1,4,5-Triphenylimidazol-2-yl)thioacetyl]-4-(2-fluorophenyl)thiosemicarbazide (6). Yield 80%, mp 216-217 °C; IR: 3346 and 3279 (NH), 1703 (C=O), 1310 cm^{-1} (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 4.61 (2H, s, SCH_2), 7.13-7.91 (19H, m, arH), 8.15 (1H, s, NH), 8.27 (1H, s, NH), 10.19 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 41.94 (SCH_2), (124.77, 126.45, 127.80, 127.98, 128.22, 128.51, 128.86, 129.05, 129.74, 132.11, 136.41, 138.40, 141.60) arC, 163.78 (C=O), 182.32 (C=S). Anal. Calcd (%) for $C_{30}H_{24}FN_5OS_2$: C, 65.08; H, 4.37; N, 12.65. Found: C, 64.89; H, 4.13; N, 12.51.

1-[(1,4,5-Triphenylimidazol-2-yl)thioacetyl]-4-phenylthiosemicarbazide (7). Yield 78%, mp 145 °C. IR: 3365 and 3240 (NH), 1696 (C=O), 1300 cm^{-1} (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 4.80 (2H, s, SCH_2), 7.20-7.75 (20H, m, arH), 8.10 (1H, s, NH), 8.25 (1H, s, NH), 10.34 (1H, s, NH),^{13}C NMR (125 MHz, DMSO-d_6) δ_C 40.07 (SCH_2), (127.88, 127.97, 128.14, 128.46, 129.80, 129.89, 130.24, 130.68, 131.80, 132,90, 136.65, 138.24) arC, 163.70 (C=O), 184.16 (C=S). Anal. Calcd (%) for $C_{30}H_{25}N_5OS_2$: C, 67.26; H, 4.70; N, 13.07. Found: C, 67.09; H, 4.55; N, 13.19.

General procedure for the synthesis of compounds 8-12. A solution of the corresponding thiosemicarbazide **3-7** (10 mmol) in 2 N NaOH was refluxed for 8 h. The resulting solution was cooled to room temperature and acidified with 37% HCl. The precipitate formed was filtered, washed with water and recrystallized from EtOH to afford the desired compounds.

4-Methyl-5-[(4,5-diphenylimidazol-2-yl)thiomethyl]-2*H*-1,2,4-triazole-3-thione (8). Yield 82%, mp 185-186 °C; IR: 3330 (NH), 1630 (C=N), 1306 cm^{-1} (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 3.42 (3H, s, CH_3), 4.72 (2H, s, SCH_2), 7.20-7.60 (10H, m, arH), 12.54 (1H, s, NH imidazole), 14.28 (1H, s, NH triazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 33.88 (CH_3), 38.43 (CH_2), (126.68, 127.80, 128.20, 129.56, 129.75, 130.98, 134.78, 137.20) arC, 155.36 (triazole C=N), 182.45 (C=S). Anal. Calcd (%) for $C_{19}H_{17}N_5S_2$: C, 60.13; H, 4.52; N, 18.45. Found: C, 60.29; H, 4.42; N, 18.61.

4-(2-Fluorophenyl)-5-[(4,5-diphenylimidazol-2-yl)thiomethyl]-2*H*-1,2,4-triazole-3-thione (9). Yield 77%, mp 230-231 °C; IR: 3341 (NH), 1624 (C=N), 1300 cm^{-1} (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 4.71 (2H, s, SCH_2), 7.18-7.73 (14H, m, arH), 12.45 (1H, s, NH imidazole), 14.37 (1H, s, NH triazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 40.76 (SCH_2), (125.33, 127.42, 127.80, 128.16, 128.51, 129.40, 130.70, 131.93, 134.38, 139.06, 141.56) arC, 154.52 (triazole C=N), 183.61 (C=S). Anal. Calcd (%) for $C_{24}H_{18}FN_5S_2$: C, 62.72; H, 3.95; N, 15.24. Found: C, 62.95; H, 4.10; N, 15.07.

4-Methyl-5-[(1,4,5-triphenylimidazol-2-yl)thiomethyl]-2*H*-1,2,4-triazole-3-thione (10). Yield 80%,

mp 209-211 °C; IR: 3307 (NH), 1615 (C=N), 1297 cm^{-1} (C=S); ^1H NMR (500 MHz, DMSO-d_6) δ_H 3.50 (3H, s, CH_3), 4.79 (2H, s, SCH_2), 7.24-7.82 (15H, m, arH), 14.45 (1H, s, NH triazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 33.54 (CH_3), 39.22 (CH_2), (126.60, 127.09, 127.41, 127.69, 128.40, 129.16, 130.00, 131.56, 135.78, 137.68) arC, 154.78 (triazole C=N), 181.80 (C=S). Anal. Calcd (%) for $C_{25}H_{21}N_5S_2$: C, 65.91; H, 4.65; N, 15.37. Found: C, 66.08; H, 4.51; N, 15.28.

4-(2-Fluorophenyl)-5-[(1,4,5-triphenylimidazol-2-yl)thiomethyl]-2*H*-1,2,4-triazole-3-thione (11). Yield 79%, mp 243-244 °C; IR: 3339 (NH), 1627 (C=N), 1313 cm^{-1} (C=S); ^1H NMR (500 MHz, DMSO-d_6) δ_H 4.62 (2H, s, SCH_2), 7.10-7.77 (19H, m, arH), 14.40 (1H, s, NH triazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 39.46 (SCH_2), (123.87, 126.45, 127.24, 128.34, 128.56, 129.24, 129.45, 130.50, 131.79, 131.78, 136.05, 138.24, 140.68) arC, 152.95 (triazole C=N), 183.00 (C=S). Anal. Calcd (%) for $C_{30}H_{22}FN_5S_2$: C, 67.27; H, 4.14; N, 13.07. Found: C, 67.43; H, 4.30; N, 12.90.

4-Phenyl-5-[(1,4,5-triphenylimidazol-2-yl)thiomethyl]-2*H*-1,2,4-triazole-3-thione (12). Yield 75%, mp 167-168 °C. IR: 3320 (NH), 1610 (C=N), 1308 cm^{-1} (C=S); ^1H NMR (500 MHz, DMSO-d_6) δ_H 4.74 (2H, s, SCH_2), 7.19-7.90 (20H, m, arH), 14.47 (1H, s, NH triazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 38.73 (SCH_2), (127.29, 127.70, 128.34, 128.98, 129.50, 130.05, 130.38, 130.72, 131.44, 132,67, 136.16, 139.78) arC, 152.84 (triazole C=N), 182.90 (C=S). Anal. Calcd (%) for $C_{30}H_{23}N_5S_2$: C, 69.60; H, 4.48; N, 13.53. Found: C, 69.74; H, 4.66; N, 13.59.

General method for the synthesis of compounds 13-17. A mixture of the corresponding thiosemicarbazide **3-7** (10 mmol) in cold concentrated sulfuric acid (30 mL) was stirred for 30 min. Then, the mixture was allowed reach to cool to room temperature. After stirring for an additional 3 h, the resulting solution was poured into ice-cold water and made alkaline to pH 8 with ammonia. The precipitated product was filtered and recrystallized from EtOH to afford the desired product.

5-[(4,5-Diphenylimidazol-2-yl)thiomethyl]-2-(*N*-methylamino)-1,3,4-thiadiazole (13). Yield 73%, mp 249-250 °C; IR: 3230 (NH), 1645 and 1624 cm^{-1} (C=N); ^1H NMR (500 MHz, DMSO-d_6) δ_H 3.34 (3H, s, CH_3), 4.72 (2H, s, SCH_2), 7.20-7.67 (10H, m, arH), 10.22 (1H, s, NH), 12.40 (1H, s, NH imidazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 32.65 (CH_2), 35.12 (CH_3), (125.90, 127.45, 127.77, 129.09, 129.57, 131.60, 134.89, 136.73) arC, 154.45 and 157.05 (thiadiazole C=N). Anal. Calcd (%) for $C_{19}H_{17}N_5S_2$: C, 60.13; H, 4.52; N, 18.45. Found: C, 60.30; H, 4.39; N, 18.36.

5-[(4,5-Diphenylimidazol-2-yl)thiomethyl]-2-[*N*-(2-fluorophenyl)]amino-1,3,4-thiadiazole (14). Yield 72%, mp 269-270 °C; IR: 3246 (NH), 1629 and 1613 cm^{-1} (C=N); ^1H NMR (500 MHz, DMSO-d_6) δ_H 4.66 (2H, s, SCH_2), 7.14-7.82 (14H, m, arH), 10.33 (1H, s, NH), 12.42 (1H, s, NH imidazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 39.05 (SCH_2), (123.88, 126.73, 127.23, 127.80, 128.34, 128.70, 129.57,

131.88, 134.50, 137.19, 140.80) arC, 153.57 and 157.30 (thiadiazole C=N). Anal. Calcd (%) for $C_{24}H_{18}FN_5S_2$: C, 62.72; H, 3.95; N, 15.24. Found: C, 62.86; H, 3.76; N, 15.41.

5-[(1,4,5-Triphenylimidazol-2-yl)thiomethyl]-2-(*N*-methylamino)-1,3,4-thiadiazole (15). Yield 71%, mp 260-261 °C; IR: 3245 (NH), 1639 and 1616 cm^{-1} (C=N); ^1H NMR (500 MHz, DMSO-d_6) δ_H 3.18 (3H, s, CH_3), 4.76 (2H, s, SCH_2), 7.21-7.70 (15H, m, arH), 10.26 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 32.97 (CH_2), 36.00 (CH_3), (126.21, 126.78, 127.64, 127.80, 128.33, 128.96, 130.15, 130.67, 135.90, 137.46) arC, 153.94 and 159.14 (thiadiazole C=N). Anal. Calcd (%) for $C_{25}H_{21}N_5S_2$: C, 65.91; H, 4.65; N, 15.37. Found: C, 65.78; H, 4.81; N, 15.50.

5-[(1,4,5-Triphenylimidazol-2-yl)thiomethyl]-2-[*N*-(2-fluorophenyl)]amino-1,3,4-thiadiazole (16). Yield 74%, mp 279-280 °C; IR: 3278 (NH), 1638 and 1613 cm^{-1} (C=N); ^1H NMR (500 MHz, DMSO-d_6) δ_H 4.63 (2H, s, SCH_2), 7.13-7.80 (19H, m, arH), 10.21 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 40.33 (SCH_2), (123.87, 126.23, 127.67, 127.89, 128.75, 128.90, 129.34, 130.41, 131.51, 135.70, 137.66, 140.22) arC, 153.11 and 157.50 (thiadiazole C=N). Anal. Calcd (%) for $C_{30}H_{22}FN_5S_2$: C, 67.27; H, 4.14; N, 13.07. Found: C, 67.01; H, 3.97; N, 13.32.

5-[(1,4,5-Triphenylimidazol-2-yl)thiomethyl]-2-(*N*-phenylamino)-1,3,4-thiadiazole (17). Yield 67%, mp 223-224 °C; IR: 3217 (NH), 1640 and 1617 cm^{-1} (C=N); ^1H NMR (500 MHz, DMSO-d_6) δ_H 4.83 (2H, s, SCH_2), 7.10-7.78 (20H, m, arH), 10.35 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 33.82 (SCH_2), (126.55, 127.86, 128.35, 129.14, 130.21, 130.42, 131.50, 131.67, 132,51, 135.83, 138.46) arC, 154.67 and 157.08 (thiadiazole C=N). Anal. Calcd (%) for $C_{30}H_{23}N_5S_2$: C, 69.60; H, 4.48; N, 13.53. Found: C, 69.47; H, 4.60; N, 13.40.

General method for the synthesis of compounds 18 and 19. Carbon disulfide (15 mmol) was added dropwise to a solution of **1** or **2** (10 mmol) in absolute EtOH (30 mL) containing potassium hydroxide (15 mmol) at 0 ºC. The reaction was stirred at room temperature for 16 h, and then cooled and diluted with Et_2O. The precipitate was filtered, washed with Et_2O and dried. The potassium dithiocarbazinates **18** and **19** were obtained in nearly quantitative yield and used without further purification as it were moisture sensitive.

General method for the synthesis of compounds 20 and 21. Hydrazine hydrate (95%, 20 mmol) was added to a suspension of the potassium salt **18** or **19** (10 mL) and the mixture was refluxed with stirring for 4 h. After cooling, it was diluted with water then acidified with aqueous hydrochloric acid. The precipitate was filtered, washed with water and recrystallized from EtOH to give yellow needles.

4-Amino-5-[(4,5-diphenylimidazol-2-yl)thiomethyl]-2*H*-1,2,4-triazole-3-thione (20). Yield 77%,

mp 190 °C; IR: 3210-3320 (NH, NH_2), 1632 (C=N), 1308 cm^{-1} (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 4.59 (2H, s, SCH_2), 7.26-7.56 (10H, m, arH), 7.90 (2H, s, NH_2), 12.31 (1H, s, NH imidazole), 14.15 (1H, s, NH triazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 36.16 (SCH_2), (125.70, 126.38, 127.28, 128.44, 129.35, 130.92, 134.71, 136.08) arC, 153.08 (triazole C=N), 182.30 (C=S). Anal. Calcd (%) for $C_{18}H_{16}N_6S_2$: C, 56.82; H, 4.24; N, 22.09. Found: C, 57.02; H, 4.31; N, 21.93.

4-Amino-5-[(1,4,5-triphenylimidazol-2-yl)thiomethyl]-2*H*-1,2,4-triazole-3-thione (21). Yield 76%, mp 229 °C; IR: 3224-3330 (NH, NH_2), 1620 (C=N), 1296 cm^{-1} (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 4.66 (2H, s, SCH_2), 7.12-7.60 (15H, m, arH), 8.03 (2H, s, NH_2), 14.20 (1H, s, NH triazole); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 37.20 (SCH_2), (126.63, 126.86, 127.08, 127.67, 128.11, 128.84, 129.20, 130.48, 134.63, 136.52) arC, 152.14 (triazole C=N), 183.76 (C=S). Anal. Calcd (%) for $C_{24}H_{20}N_6S_2$: C, 63.13; H, 4.42; N, 18.41. Found: C, 63.29; H, 4.60; N, 18.59.

General method for the synthesis of compounds 22 and 23. A mixture of the corresponding dithiocarbazate **18** or **19** (10 mmol) in cold concentrated sulfuric acid (30 mL) was stirred for 30 min. Then, the mixture was allowed reach to cool to room temperature. After stirring for an additional 5 h, the resulting solution was poured into ice-cold water and made alkaline to pH 8 with ammonia. The precipitated product was filtered and recrystallized from EtOH to afford the desired product.

5-[(4,5-Diphenylimidazol-2-yl)thiomethyl]-1,3,4-thiadiazole-2-thiol (22). Yield 68%, mp 260-261 °C; IR: 2615 (SH), 1635, 1617 cm^{-1} (C=N); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 4.65 (2H, s, SCH_2), 7.18-7.60 (10H, m, arH), 12.40 (1H, s, NH imidazole), 13.92 (1H, s, SH); ^{13}C NMR (500 MHz, DMSO-d_6) δ_C 34.50 (CH_2), (126.24, 126.66, 127.50, 127.92, 128.71, 129.87, 134.56, 137.34) arC, 153.12 and 157.94 (thiadiazole C=N). Anal. Calcd (%) for $C_{18}H_{14}N_4S_3$: C, 56.52; H, 3.69; N, 14.65. Found: C, 56.38; H, 3.86; N, 14.52.

5-[(1,4,5-Triphenylimidazol-2-yl)thiomethyl]-1,3,4-thiadiazole-2-thiol (23). Yield 66%, mp 239-240 °C; IR: 2600 (SH), 1640, 1621 cm^{-1} (C=N); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 4.72 (2H, s, SCH_2), 7.20-7.73 (15H, m, arH), 13.98 (1H, s, SH); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 35.38 (CH_2), (126.00, 126.51, 127.13, 127.63, 129.81, 130.69, 135.38, 137.93) arC, 154.26 and 159.40 (thiadiazole C=N). Anal. Calcd (%) for $C_{24}H_{18}N_4S_3$: C, 62.85; H, 3.96; N, 12.22. Found: C, 63.03; H, 3.89; N, 12.37.

Antimicrobial activity

All bacterial and yeast strains were obtained from the microbiology Laboratory-Faculty of medicine, University of Djillali Liabes (Sidi-Bel-Abbes-Algeria) and were as follows: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa, Candida tropicalis and Candida albicans*. All the newly synthesized 1,2,4-triazoles and 1,3,4-thiadiazoles were screened for their antimicrobial and

antifungal activity. Thus, they were dissolved in dimethylformamide (DMF) to prepare chemical stock solution of 10 mg/1 mL.

Agar-well diffusion method was the simple screening method used for this study.[39,40] Thus, each microorganism was suspended in Mueller Hinton (MH) broth and diluted approximately to 10^6 colony forming unit (cfu)/mL. They were "flood-inoculated" onto the surface of MH agar and sabouraud Dextrose Agar and then dried. For *Candida tropicalis and Candida albicans*, SDA were used. Five millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µL of the chemical substances was delivered into the wells. The plates were incubated for 16-18 h at 37 ºC. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 µg) and Fluconazole (100 µg) were standard drugs. Dimethylformamide was used as solvent controls. The antimicrobial activity results are summarized in Table 1.

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