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New Imidazolidineiminothione, Imidazolidin-2-one and Imidazoquinoxaline Derivatives: Synthesis and Evaluation of Antibacterial and Antifungal Activities

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Abstract: A series of new 5-imino-4-thioxo-2-imidazolidinone derivatives **3** with various halogenated and alkylated aromatic substituents at N^1 and N^3 was synthesized. Imidazolidineiminothione derivatives **3** were prepared from the reaction of *N*-arylcyanothioformamide derivatives with aryl isocyanates. These compounds were used as key synthons for the preparation of wide variety of new substituted imidazole compounds. Imine hydrolysis of **3** with ethanolic HCl produced the corresponding 4-thioxo-2,5-imidazolidindiones **4.** Condensation of **3** with benzophenonhydrazone furnished the corresponding 4-azine derivatives **5**. Monohydrazono and dihydrazono derivatives **6** and **8** were obtained upon treatment of imidazolidinone derivatives **3** with hydrazine hydrate. Finally, imidazolidinones **3** were reacted with *o*-phenylenediamines or pyrazol-5(4*H*)-ones and afforded the corresponding imidazoquinoxaline and imidazolidin-4-ylidenepyrazolone-5(4*H*)-one derivatives **11** and **12,** respectively. Evaluation of the antibacterial and antifungal activities for the synthesized compounds was carried out to probe their activities. Most of the tested compounds showed significant activities. The best antimicrobial activity was observed for 1-(3-ethoxyphenyl)-6-methyl-1-phenyl-1*H*-imidazo[4,5-b]quinoxalin-2(3*H*)-ones **(11c)** followed by 5-imino-3-(3-methoxy- phenyl)-1-phenyl-4-thioxoimidazolidin-2-one **(3f)**.

Keywords: *N*-arylcyanothioformanilides, imidazolidineiminothiones, imidazolidinone, imidazoquinoxaline, antibacterial and antifungal activities.

INTRODUCTION

Infectious diseases caused by bacteria and fungi remain a major worldwide health problem due to rapid development of resistance to the existing antimicrobial drugs. The increasing use and misuse of the existing antimicrobial drugs have resulted in the development of resistant pathogens. The medical community faces a serious problem when treating infections caused by pathogenic microbes and needs an effective therapy and search for novel antimicrobial agents [1-6]. In addition, systemic and dermal fungal infections have significantly increased, specifically in individuals with suppressed immune systems such as those receiving cancer chemotherapy and AIDS patients. Although there are different antifungal drugs used in the treatment of fungal infections, some of them have undesirable side effects because of the biochemical similarity between human cell and fungi forms. The search for new and effective antimicrobial agents, resistant to the mechanisms of defense of these bacteria, is of paramount importance [1, 2]. Imidazoles and their fused derivatives are keys in many bioactive compounds of both natural and synthetic origins [7] such as histidine, purines, biotin and hydantoin. Nitroimidazoles are a well-known family of antibacterial and antiprotozoal drugs [8, 9], including antitrypanosomal drugs or compounds with known anti-trypanosomal activity [10, 11]. Metronidazole, the first drug to be introduced for this purpose and probably also the best known drug in this class, has been in use for more than 50 years [12]. 4-Oxoimidazolidine-2-thiones and their 2-alkylthio-3,5-dihydro-4*H*-imidazol-4-one derivatives are important compounds in the fields of drugs, pharmaceutical intermediates. As an example isatinylidene derivatives exhibit immunosuppressive activity [13]. 2-Thiohydantoin derivatives have not only been used in medicinal chemistry, but have also been developed as fungicides e.g. fenamidone [14, 15].

In light of these facts, and in continuation of our ongoing study on antimicrobial agents [16-19], the present study was designed to synthesize new imidazolinone derivatives and evaluate their antimicrobial activity. Various functional groups were introduced into the target compounds in order to investigate their preliminary structure activity relationships.

RESULTS AND DISCUSSION

Chemistry

The starting compounds, *N*-(4-fluoro, 3-chloro, 4-methyl)-cyanothioformanilides **2a-c** were prepared according to the previously reported procedure [20]. The new compounds, *N*-(3-methoxy, 3-ethoxy)-cyanothioformanilides **2d,e** were prepared from the reaction of *N*-aryl isothiocyanates with potassium cyanide. The elemental analysis and spectroscopic data of **2d,e** are consistent with the assigned structure. IR measurement of **2e** for example displayed absorption bands at: 3264 and 2227 cm^{-1} for NH and C≡N groups. ^1H NMR spectrum revealed triplet and quartet signals at: δ = 1.23 and 4.14 ppm corresponding to CH_3 and CH_2, respectively and the presence of a D_2O exchangeable broad singlet at: δ = 13.36 ppm attributable to the NH proton. In addition, ^{13}C NMR spectrum of **2e** showed two signals at 14.1 (CH_3), 63.3 (CH_2), also it showed a farthermost downfield signal for the thione group (C=S) at: 161.1

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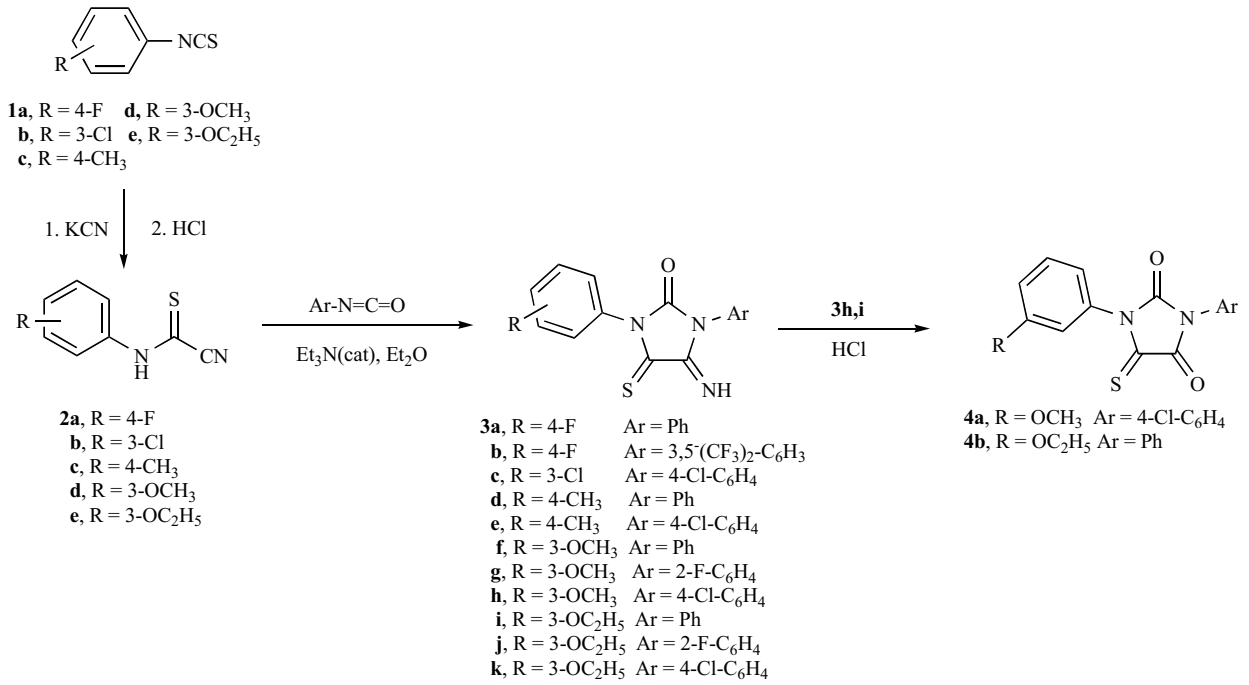
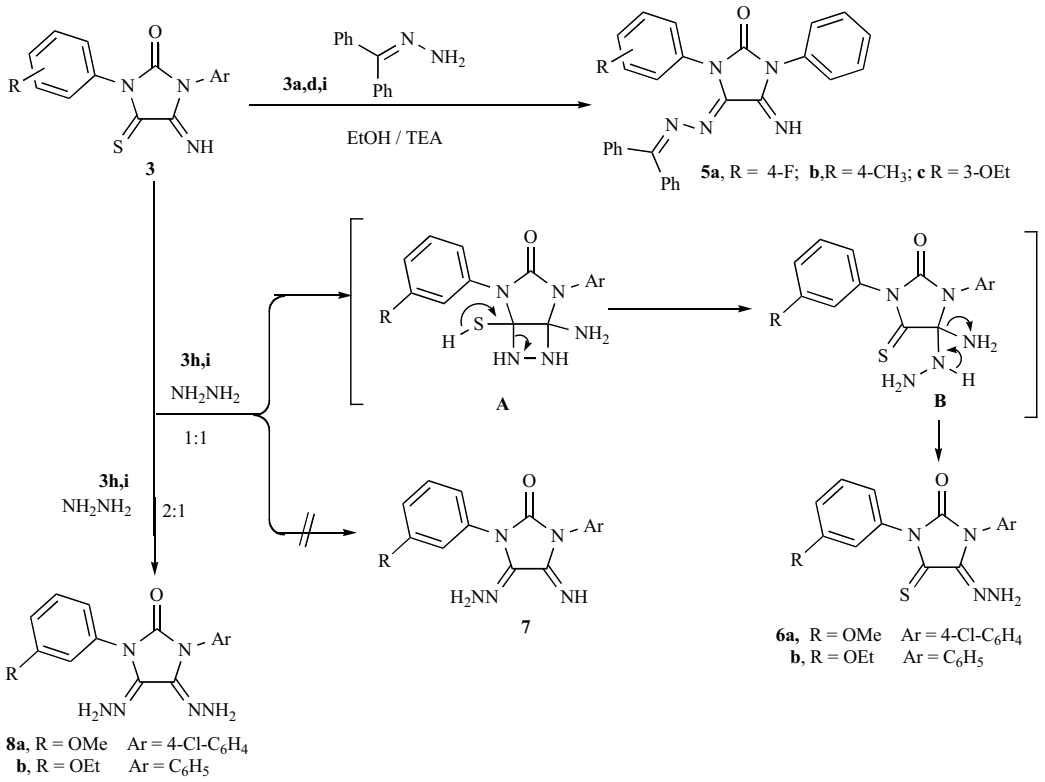


Scheme 1. Synthesis of *N*-arylcyanothioformanilide, imidazolidin-2-one and imidazolidineiminothione derivatives.

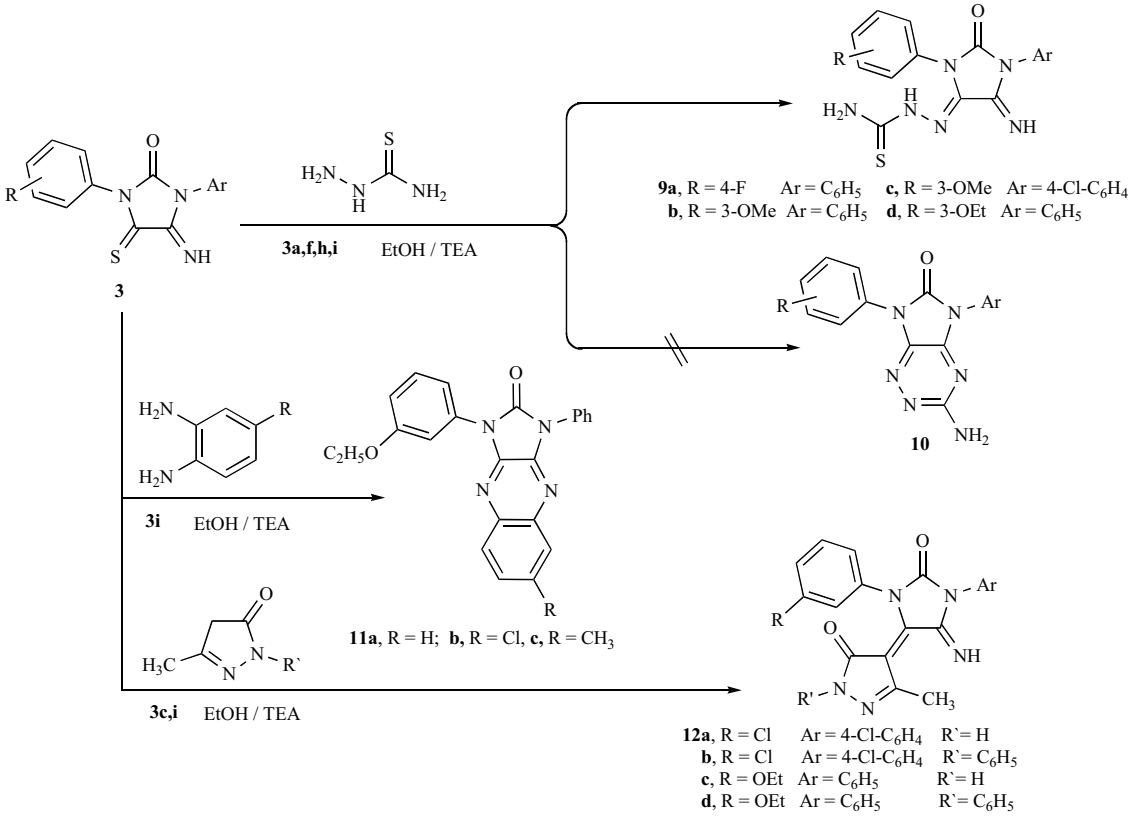
due to the tautomeric thione and thiole mixture and an upfield signal resulting from the nitrile group (107.9). The behavior of the cyanothioformanilides towards isocyanate derivatives was investigated. Thus, reaction of cyanothioformanilides with aryl isocyanates in ether and in the presence of triethylamine caused cyclization to furnish the corresponding 5-imino-4-thioxo-2-imidazolidinones **3a-k**. The structure of **3** was assigned on the basis of analytical and spectral data. IR spectrum of **3f**, as representative example, displayed absorption bands at: 3261 cm^{-1} due to NH and at 1777 cm^{-1} due to C=O functional group. ^1H NMR spectrum exhibited two singlets at: δ = 3.80 and 9.66 ppm indicative of methoxy protons and NH proton, respectively. ^{13}C NMR spectrum of **3f** displayed four signals at: 183.3, 160.1, 154.5 and 65.3 for C=S, C=O, C=N and OCH_3, respectively, whereas the aromatic signals ranged from 114.1 to 154.1 corresponding for 12C. Mass spectrum showed a molecular ion peak at *m/z* = 311, corresponding to a molecular formula $C_{16}H_{13}N_3O_2S$. Hydrolysis of **3h,i** with dilute HCl in boiling ethanol afforded the corresponding dione derivatives **4a,b** (Scheme **1**). IR spectrum has no absorption band characteristic to an NH group.

The imidazolidine derivatives contain adjacent imino and thione functional groups in the 5-and 4-positions appear promising for further chemical transformations. Therefore, it was interesting to study the reaction of imidazolidineiminothiones **3** with some amino compounds as nitrogen nucleophiles. Condensation of **3a,d,i** with benzophenonehydrazone in boiling ethanol using triethylamine as a basic catalyst furnished the corresponding 4-azine derivatives **5a-c**. The analytical and spectral data are in agreement with the proposed structure. Thus IR spectrum of **5a** showed the presence of three signals at 3265, 1758 and 1624 cm^{-1} due to the presence of three groups NH, C=O and C=N, respectively. ^1H NMR spectrum showed singlet signal at: 10.11 ppm corresponding NH proton. While, ^{13}C NMR showed the complete disappearance of the signal in the range around 180 ppm due to C=S and the presence of four signals at 160.4, 160.7, 162.4 and 167.5 corresponding 3C=N and C=O groups. In addition, the reactivity of iminothione derivatives **3** towards binucliophiles was also investigated. Thus equimolecular amounts of **3h,i** and hydrazine hydrate furnished the monohydrazono derivatives for which structure **6** or **7** seemed possible. The positive element test for sulfur and spectral data favored the 5-hydrazono derivatives **6a,b**. Elemental analysis, IR and NMR are in agreement with the proposed structure. The formation of **6** may be rationalized *via* the nucleophilic addition of nitrogen atom of hydrazine to the thio group followed by nucleophilic addition of the other nitrogen to the imino group followed by elimination of ammonia as shown in Scheme **2.** Dihydrazono derivatives **8a,b** were achieved as a sole product by treating the iminothione **3h,i** with two moles of hydrazine hydrate in ethanol (Scheme **2**). The product of this reaction was identified on the basis of both elemental analyses and spectral data. ^1H NMR spectrum of **8b** displayed triplet and quartet signals at: δ =1.32, 4.01 due to the ethoxy group and broad singlet signals at: δ = 5.65 assignable the $2NH_2$. ^{13}C NMR showed complete disappearance of the signal in the range around 180 ppm due to the functional group change from C=S to C=N and the presence of signals at: 152.6, 159.0 corresponding C=N and C=O groups.

Furthermore, upon reaction of the iminothiones **3a,f,h,i** with thiosemicarbazide, the nucleophilic addition occurred at the thio group and the corresponding 4-thiosemicarbazone derivatives **9a-d** were obtained in good yield. Compounds **9** were not converted into the triazine derivatives **10** under these conditions. The chemical structure of **9a-d** was elucidated on the basis of elemental analyses and spectral data. ^1HNMR spectrum of **9d** as a representative example revealed triplet and quartet signals at: δ = 1.30 and 4.03 ppm, respectively, characteristic for ethoxy protons, in addition to three broad singlet signals at: 8.46, 9.47, 12.79 due to NH and NH_2 protons. Now, we have extended our synthetic program to the synthesis of otherwise inaccessible heterocyclic ring system utilizing imidazolidineiminothiones **3** as the key starting material. Thus condensation of the imidazolidineiminothione **3i** with *o*-phenylenediamine derivatives as 1,4-binucleophile in ethanol under reflux afforded yellow products which were identified as imidazo[4,5-*b*]quinoxalines **11a-c**. The spectral data of the isolated product were in complete agreement with structure **11**. IR spectrum revealed lack of an absorption band corresponding to a C=NH functional group. Mass spectrum of compound **11c** showed molecular ion peak at m/z 396 (base peak) corresponding to molecular formula $C_{24}H_{20}N_6O_2$. Finally, it was interesting to study the behavior of imidazolidi-



Scheme 2. Reactions of imidazolidineiminothiones with hydrazone derivatives and hydrazine hydrate.



Scheme 3. Reactions of imidazolidineiminothione derivatives with different reagents.

neiminothiones toward carbon nucleophiles. Thus, condensation of the imidazolidineiminothiones **3c,i** with 3-methyl-1*H*-pyrazol-5(4*H*)-one derivatives in boiling ethanol using triethylamine as a basic catalyst afforded a product which was identified as imidazolidin-4-ylidenpyrazolones **12a-d** (Scheme **3**). IR spectrum of **12d** as a representative example revealed absorption bands at: 3280, 1717 and 1603 characteristic to NH, C=O and C=N functional groups, respectively. The ^{1}H NMR spectrum exhibited triplet, quar-

Table 1. Antimicrobial activity of the synthesized compounds against the pathological organisms expressed as inhibition diameter zones in millimeters (mm) based on well diffusion assay.

Compd. No.	Gram Positive Bacteria			Gram Negative Bacteria			Fungi		
	S. aureus	*S. epidermidis*	*B. subtilits*	*N. gonorrhoeae*	*E. coli*	*K. pneumoniae*	*A. fumigatus*	*A. clavatus*	*G. candidum*
3b	0	14.6 ± 0.43	16.2 ± 0.53	10.2 ± 0.72	13.7 ± 0.63	15.6 ± 0.25	12.6 ± 0.58	13.6 ± 0.25	15.2 ± 0.38
3f	20.3 ± 0.63	20.7 ± 0.22	22.4 ± .036	16.2 ± .058	17.6 ± 0.58	18.3 ± 1.2	20.5 ± 0.22	18.3 ± 0.26	22.1 ± 0.15
3g	14.3 ± 0.58	15.9 ± 0.44	17.2 ± 0.58	16.3 ± 0.63	18.9 ± 0.63	20.3 ± 0.63	16.3 ± 0.44	18.4 ± 0.58	19.1 ± 0.37
3h	13.6 ± 0.58	15.2 ± 0.44	16.1 ± 0.67	10.2 ± 0.46	13.0 ± 0.46	14.5 ± 0.46	10.6 ± 0.25	11.7 ± 0.34	16.5 ± 0.58
3i	18.9 ± 0.14	16.2 ± 0.15	19.8 ± 0.42	12.3 ± 0.53	15.3 ± 0.53	16.2 ± 0.53	15.7 ± 0.33	15.9 ± 0.25	16.8 ± 0.34
3j	18.9 ± 0.58	20.3 ± 0.43	21.4 ± 0.53	14.3 ± 0.25	16.9 ± 0.25	19.2 ± 0.25	17.6 ± 0.58	18.2 ± 0.25	20.3 ± 0.38
3k	15.8 ± 0.44	17.8 ± 0.17	19.8 ± 0.22	0	0	0	18.7 ± 0.11	19.3 ± 0.23	20.3 ± 0.27
4a	20.3 ± 0.58	17.3 ± 0.63	20.2 ± 0.44	13.6 ± 0.37	15.9 ± 0.37	16.8 ± 0.37	16.3 ± 0.44	18.6 ± 0.58	19.8 ± 0.25
4b	13.6 ± 0.25	20.6 ± 0.63	22.4 ± 0.44	14.3 ± 0.63	16.9 ± 0.58	17.8 ± 1.2	17.8 ± 0.63	18.9 ± 0.44	20.3 ± 0.25
6a	12.8 ± 0.58	14.3 ± 0.63	15.2 ± 0.72	0	0	0	13.3 ± 0.44	16.2 ± 0.58	17.2 ± 0.37
6b	17.3 ± 0.58	18.4 ± 0.17	20.3 ± 0.22	0	0	0	16.3 ± 0.39	18.2 ± 0.16	20.4 ± 0.58
8a	10.3 ± 0.58	11.6 ± 0.58	13.3 ± 0.72	0	0	0	12.3 ± 0.25	13.2 ± 0.25	15.2 ± 0.58
8b	9.6 ± 1.2	10.6 ± 0.58	12.7 ± 0.72	0	0	0	8.6 ± 0.36	9.3 ± 0.44	11.4 ± 0.58
9a	11.6 ± 0.58	12.4 ± 0.63	14.3 ± 0.63	0	0	0	16.3 ± 0.25	15.2 ± 0.58	17.3 ± 0.17
9b	16.3 ± 0.37	17.2 ± 0.20	19.4 ± 0.29	0	11.2 ± 0.33	15.9 ± 0.77	15.6 ± 0.44	16.2 ± 0.58	17.9 ± 0.37
9c	12.3 ± 0.58	14.2 ± 0.17	16.4 ± 0.22	0	0	0	10.3 ± 0.25	11.6 ± 0.34	13.4 ± 0.58
11a	16.6 ± 0.20	19.8 ± 0.12	21.4 ± 0.17	11.4 ± 0.42	18.3 ± 0.58	16.3 ± 0.81	20.6 ± 0.63	21.1 ± 0.27	21.9 ± 0.35
11b	15.6 ± 0.44	18.6 ± 0.44	21.1 ± 0.58	14.3 ± 0.20	16.3 ± 0.33	17.2 ± 0.34	18.3 ± 0.34	21.1 ± 0.25	21.3 ± 0.38
11c	21.6 ± 0.25	22.3 ± 0.25	25.8 ± 0.25	20.6 ± 0.25	21.6 ± 0.19	23.2 ± 0.42	20.9 ± 0.25	22.3 ± 0.25	24.8 ± 0.58
12a	17.2 ± 0.24	19.2 ± 0.15	19.8 ± 0.42	10.4 ± 0.25	13.4 ± 0.63	14.5 ± 0.51	15.7 ± 0.33	17.2 ± 0.25	19.8 ± 0.34
12b	19.2 ± 0.25	20.6 ± 0.25	20.9 ± 0.63	11.6 ± 0.43	15.7 ± 0.56	15.9 ± 0.77	16.8 ± 025	18.3 ± 0.44	20.4 ± 0.63
St. (A)	28.9 ± 0.14	25.4 ± 0.18	29.8 ± 0.35	-------	-------	-------	-------	-------	-------
St. (B)	-------	-------	-------	22.3 ± 0.58	23.4 ± 0.3	26.3 ± 0.15	-------	-------	-------
St. (C)	-------	-------	-------	-------	-------	-------	23.7 ± 0.10	21.9 ± 0.12	25.4 ± 0.16

St. (A): Ampicillin, St. (B): gentamycin and St. (C): Amphotericin b

tet and singlet at: 1.14, 4.19 and 2.41 ppm due to ethoxy and methyl protons, singlet signal at 12.83 specific for NH. Moreover, its ^{13}C NMR spectrum revealed signals at: 14.8, 15.1, 63.3 assigned ethoxy and methyl carbons signals 105.0, 144.7 corresponding for C=C and signals at 159.3, 159.4, 162.3 and 166.6 assigned to 2 imino and 2 carbonyl carbons.

Antibacterial and Antifungal Activities

The synthesized compounds were tested *in vitro* for antibacterial and antifungal activities against the following strains: three Gram-positive bacteria, *Staphylococcus aureus* RCMB 010027, *Staphylococcus epidermidis* RCMB 010024 and *Bacillis subtilis* RCMB 010063; three Gram-negative bacteria, *Neisseria gonorrhoeae* RCMB 010079, *Esherichia coli* RCMB 010052 and *Klebsiella pneumoniae* RCMB 010093, and three Fungi, *Aspergillus fumigatus* RCMB 02564, *Aspergillus clavatus* RCMB 02593 and *Geotricum Candidum* RCMB 05096, and the results were summarized in Table **1**. The synthesized compounds were tested for antimicrobial activity by the agar diffusion method [21] using a 1 cm microplate well diameter and a 100 µL of each concentration. The antifungal agents were evaluated against clinical isolates of standard strains of fungi by the broth dilution methods Broth dilution method according to NCCLs [22, 23]. Tested compound solution prepared by dissolving 5 mg of the chemical compound in 1 mL of dimethyl sulfoxide (DMSO). The inoculated plates were then incubated for 24 h at 37 °C. Ampicillin, Gentamycin and Amphotericin B (1 mg/mL) were used as standard references for Gram positive bacteria, Gram negative bacteria and antifungal activity, respectively. After incubation time, antimicrobial activity was evaluated by measuring the inhibition zone diameters against the test organisms and compared with standard zone size ranges that determine susceptibility, intermediate susceptibility, or resistance to the screened compounds. Visual bacterial growth is observed only in areas in which the drug concentrations are below those required for growth inhibition. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

The mean values of the inhibition zone diameter obtained for these compounds suggest that all of the imidazole derivatives evaluated possess significant antibacterial activity against most of the test organisms used in these assays. A moderate difference in antibacterial activity is noted between the tested compounds, this indicate that the main effect related to the presence of the imidazolidinone moiety. Using the general structure provided in (Fig. **1**), certain aspects of the structure activity relationships for these compounds can be more clearly highlighted.

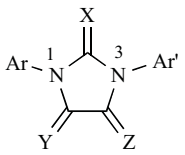


Fig. (1). General formula of the synthesized compounds.

A series of imidazolidineiminothione derivatives **3** which contain 4-fluorophenyl, 3-methoxyphenyl, 3-ethoxyphenyl moieties (Ar) at N^1 and phenyl, 2-fluorophenyl, 4-chlorophenyl, 3,5-di(trifluoromethyl)-phenyl moieties (Ar') at N^3, X = O, Y = S and Z = NH was synthesized and their biological activates were measured and studied. Regarding the effect of Ar and Ar' groups. The type of the substitutions on the benzene ring of aryl moiety is important. It was noticed that the presence of 3-methoxyphenyl and phenyl in **3f** and 3-ethoxyphenyl and 4-chlorophenyl in **3j** showed the maximum activities against most of the test organisms which showed near the reference drug. On the other hand, compounds **3b,g,h,i** showed moderate to good activity against most of the tested organisms. Compound **3k** with 4-fluorophenyl and 3,5-di(trifluoromethyl)-phenyl moieties has moderate activity the Gram +ve bacteria and no activity toward the Gram -ve bacteria. Regarding the effect of changing the substituents on N^1 and N^3, the results of screening demonstrated the presence of good correlation between the antifungal and antibacterial activities. Changing the substituent on C4 from imino to oxo (**3h,i → 4a,b**) was carried out to show the deference between imino and oxo on the effect of the antimicrobial activity. Compound **4a** showed higher activity more than its imino analogues **3h** while compound **4b** has less activity than its imino analogues compound **3i**. Y and Z substituents in imidazolidineiminothione derivatives **3** were changed to monohydrazono, dihydrazono and thiosemicarbazono moieties to do the same study. Compounds **6, 8, 9** with mono hydrazono, dihydrazono and thiosemicarbazono moieties have weak activity against Gram +ve bacteria and no activity against Gram -ve activity. Changing the substituent at C4 from imino to hydrazono (**3h,i → 6a,b**) showed lower activity than their imino analogues **3h,i.** Also, changing the substituents on C4 and C5 from thioxo and imino to hydrazono (**3h,i → 8a,b**) showed lower activity than its analogues **3h,i**. Moreover, changing the substituent on C4 from thioxo to thiosemicarbazono (**3a,f,h → 9a,b,c**) showed lower activity than their analogues. The authors also studied the effect of changing of the substituent at C4 and C5 to cyclic substituents at imidazolidine ring on the biological activities. Compounds **11a-c** with quinoxaline moiety showed strong activity against all of the tested organisms, where compound **11c** was equipotent to the reference drug. Finally, the vary substitution on C5 from thioxo to substituted pyrazolone moiety (**3i → 12c,d**) had slight effect on antibacterial activity. Compounds **12c,d** with moiety showed moderate activity with all the tested Gram positive and negative bacteria. The comparison between the antimicrobial activity of our potent synthesized compounds and standard reference drug against the used Gram positive, Gram negative bacteria and fungi is represented graphically in Figure **2**.

MIC of the Most Active Compounds

Minimum inhibitory concentration (MIC) of the most active synthesized compounds **3f, 3k, 4a, 11c, 12a,** and **12b** was evaluated *in vitro* using the Broth dilution method according to NCCLs [22, 23]. The results of minimum inhibitory concentration are depicted in Table **2**. Regarding the effect of each substituent at C2, C4, C5 and those at N^1 and N^3 against bacterial and fungal strains, results of antimicrobial activity in this study revealed that the presence of quinoxaline moiety in compound **11c** resulted in highest antimicrobial activity among all the investigated compounds. The latter compound showed better results when compared with standard drugs as revealed from its MIC values (0.12 - 0.98 µg/mL). Compound **11c** was equipotent to Ampicillin in inhibiting the growth of *B. subtilis* (MIC 0.15 µg/mL). This compound was equipotent to Gentamycin in inhibiting the growth of *K. pneumonia* (MIC 0.12 µg/mL). Also, this compound was equipotent to Amphotericin B in inhibiting the growth of *A. clavatus* (MIC 0.49 µg/mL) and *G. candidum* (MIC 0.12 µg/mL). In certain cases, such values are low enough to render such agents as potential candidates for further studies.

CONCLUSION

The aim of the present investigation is to synthesize different series of imidazolidin-2-one bearing various substituents at N^1 and others at N^3 beside various substituents at C1, C3 and C4. The authors measured the antibacterial and antifungal activities of these derivatives. They studied the effect of each substituent on these

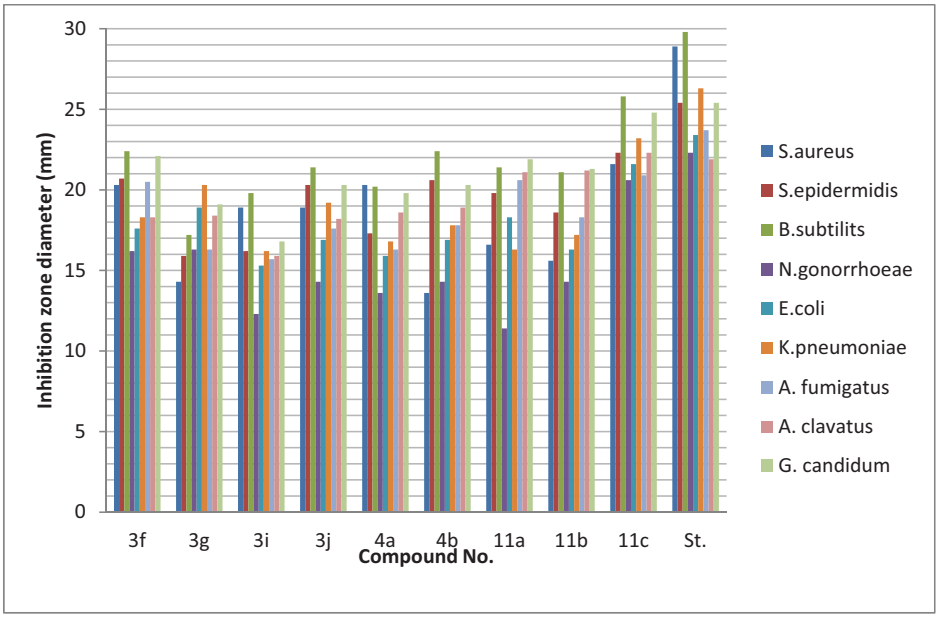


Fig. (2). The comparison between the antimicrobial activity of our potent synthesized compounds and standard drug against the used Gram positive, Gram negative bacteria and fungi.

Table 2. Minimum inhibitory concentration (µg/mL) of the more potent synthesized compounds against the pathological organisms.

Compd. No.	Gram +ve			Gram -ve			Fungi		
	S. aureus	*S.epidermidis*	*B. subtilis*	*N.gonorrhoeae*	*E. coli*	*K.pneumoniae*	*A. fumigatus*	*A. clavatus*	*G. candidum*
3f	1.95	0.98	0.49	31.25	7.81	7.81	0.98	7.81	0.49
3k	3.9	1.95	0.98	125	15.63	3.9	7.81	7.81	1.95
4a	1.95	15.63	1.95	125	31.25	15.63	31.25	3.9	1.95
11c	0.49	0.49	0.12	0.98	0.49	0.12	0.98	0.49	0.12
12a	15.63	3.9	1.95	500	125	125	62.5	15.6	1.95
12b	3.9	0.98	0.98	500	31.25	31.25	15.63	7.8	1.95
St. (A)	0.03	0.12	0.15	-----	-----	-----	-----	-----	-----
St. (B)	-----	-----	-----	0.49	0.24	0.12	-----	-----	-----
St. (C)	-----	-----	-----	-----	-----	-----	0.12	0.49	0.12

St. (A): Ampicillin, St. (B): gentamycin and St. (C): Amphotericin b

activities and make a comparative study between them to deduce a structure activity relationship. Most compounds displayed antibacterial and antifungal activities. A moderate difference in antimicrobial activity is noted between the tested compounds, this indicates the main effect related to the presence of the imidazolidinone moiety. Our results clearly revealed that the best antimicrobial activity was observed for 1-(3-ethoxyphenyl)-6-methyl-1-phenyl-1*H*-imidazo[4,5-b]quinoxalin-2(3*H*)-ones **(11c)** followed by 5-imino-3-(3-methoxy- phenyl)-1-phenyl-4-thioxoimidazolidin-2-one **(3f)**.

EXPERIMENTAL SECTION

All melting points are recorded on digital Gallen Kamp MFB-595 instrument and are uncorrected. The IR spectra (KBr) (cm^{-1}) were measured on a Shimadzu 440 spectrophotometer. NMR spectra (δ, ppm) were obtained in deuterated dimethyl sulfoxide on a Varian Gemini 500 (500 MHz) spectrometer, using TMS as an internal standard; chemical shifts are reported as δ ppm units. Mass spectra (m/z, %) were obtained on GC MS-QP 100 Exmass spectrometer at 70 eV. Elemental analyses were carried out at Microanalytical Unit, Cairo University, Cairo, Egypt.

General Procedure for the Synthesis of Arylcarbamothioyl Cyanide Derivatives 2d,e

To a solution of isothiocyanate derivative (3-methoxyphenyl isothiocyanate or 3-ethoxyphenyl isothiocyanate) (0.01 mol) in ethanol (50 ml), a solution of KCN (0.01 mol) in 5 ml water was added slowly while stirring. After complete addition, stirring of the reaction mixture was continued for additional 1 h. The reaction mixture was poured into acidified crushed ice. The resulting precipitate was filtrated off, dried and crystallized from ethanol to give **2d, e**.

(3-Methoxyphenyl)carbamothioyl cyanide (2d): as yellow crystals; yield 65 %; mp 75-77 °C; IR: ν/cm^{-1} = 3251 (NH), 3041 (CH-Ar), 2996 (CH-aliph.), 2221 (C≡N); ^{1}H NMR: δ/ppm = 3.83 (s, 3H, CH_3), 6.96 -7.68 (m, 4H, Ar-H), 9.55 (br, 1H, NH; cancelled with D_2O); ^{13}C NMR: 55.6 (OCH_3), 113.7, 114.4, 124.2, 129.9, 130.2, 137.4, 159.0, 161.0; Anal. Calcd for $C_9H_8N_2OS$ (192.24): C, 56.23; H, 4.19; N, 14.57; Found: C, 56.44; H, 4.35; N, 14.81%.

(3-Ethoxyphenyl)carbamothioyl cyanide (2e): as yellow crystals; yield 73%; mp 83-85 °C; IR: ν/cm^{-1} = 3264 (NH), 3066 (CH-Ar), 2980 (CH-aliph.), 2227 (C≡N); ^{1}H NMR: δ/ppm = 1.23 (t, 3H, *J* = 6.9 Hz, CH_3), 4.14 (q, 2H, *J* = 7.0 Hz, CH_2), 6.83-7.68 (m, 4H, Ar-H), 13.36 (br, 1H, NH; cancelled with D_2O); ^{13}CNMR: 14.1 (CH_3), 63.3 (CH_2), 107.9, 113.7, 114.5, 130.3, 138.7, 140.3, 158.8, 161.1; Anal. Calcd. for $C_{10}H_{10}N_2OS$ (206.26): C, 58.23; H, 4.89; N, 13.58; Found: C, 58.42; H, 4.76; N, 13.84%.

General Procedure for the Synthesis of 5-imino-4-thioxoimidazolidin-2-one Derivatives 3

To a solution of **2** (0.0l mo1) in ether (20 ml), isocyanates (0.0l mo1) and triethylamine (0.5 ml) were added; the reaction mixture was stirred at room temperature for 1 hr. The obtained products were crystallized from the chloroform / *n*-hexane to afford **3.**

5-Imino-4-thioxoimidazolidin-2-ones (3a,c-e) were prepared according to the reported method [20]. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-fluorophenyl)-5-imino-4-thioxoimidazolidin-2-one (3b): as orange crystals; yield 83 %; mp 105-107 °C; IR: ν/cm^{-1} = 3215 (NH), 1774 (C=O), 1664 (C=N); ^{1}H NMR: δ/ppm = 7.12-8.35 (m, 7H, Ar-H), 10.44 (br, 1H, NH; cancelled with D_2O); ^{13}C NMR: 116.8, 117.0, 120.1, 122.2, 124.4, 126.6, 127.9, 129.7, 130.5, 131.3, 131.5, 134.9, 153.5, 153.8, (12C-Ar + 2CF_3), 161.6 (C=N), 163.6 (C=O), 182.9 (C=S); Anal. Calcd. for $C_{17}H_8F_7N_3OS$ (435.32): C, 46.90; H, 1.85; N, 9.65; Found: C, 46.55;H, 1.87; N, 9.76%.

4-Imino-1-(3-methoxyphenyl)-3-phenyl-5-thioxoimidazolidin-2-one (3f): as orange crystals; yield 78 %; mp 118-120 °C; IR: ν/cm^{-1} = 3261 (NH), 2988, 2933 (CH-aliph), 1777 (C=O), 1600 (C=N); ^{1}H NMR: δ/ppm = 3.80 (s, 3H, OCH_3), 7.10-7.50 (m, 9H, Ar-H), 9.66 (br, 1H, NH; cancelled with D_2O); ^{13}C NMR: 65.3 (OCH_3), 115.3, 118.5, 120.3, 122.3, 128.5, 129.3, 130.3, 133.1, 134.9, 140.1, 153.0, 154.1 (12C-Ar), 154.5 (C=N), 160.1 (C=O), 183.3 (C=S); MS, m/z (%): 311 (M^+, 1.30), 261 (13.67), 205 (2.4), 180 (13.25), 166 (14.11), 165 (81.01), 119 (20.49), 104 (13.67), 91 (46.48), 77 (100); Anal. Calcd. for $C_{16}H_{13}N_3O_2S$ (311.36): C, 61.72; H, 4.21; N, 13.50; Found: C, 61.53; H, 4.36; N, 13.62%.

1-(2-Fluorophenyl)-5-imino-3-(3-methoxyphenyl)-4-thioxoimidazolidin-2-one (3g): as orange crystals, yield 62 %; mp 143-145 °C; IR: ν/cm^{-1} = 3264 (NH), 3064 (CH-Ar), 2986, 2936 (CH-aliph), 1778 (C=O), 1602 (C=N); ^{1}H NMR: δ/ppm = 3.80 (s, 3H, OCH_3), 7.10-7.69 (m, 8H, Ar-H), 9.82 (br, 1H, NH; cancelled with D_2O), ^{13}CNMR 65.3 (OCH_3), 115.7, 116.9, 117.1, 120.2, 125.1, 125.5, 130.3, 131.8, 134.3, 153.5, 153.5, 153.5 (12C-Ar), 154.0 (C=N), 160.1 (C=O), 183.1 (C=S); Anal. Calcd. for $C_{16}H_{12}FN_3O_2S$ (329.35): C, 58.35; H, 3.67; N, 12.76; Found: C, 58.53; H, 3.84; N, 12.54%.

1-(4-Chlorophenyl)-5-imino-3-(3-methoxyphenyl)-4-thioxoimidazolidin-2-one (3h): as yellow crystals; yield 68 %; mp 127-129 °C, IR: ν/cm^{-1} = 3231 (NH), 2934 (CH-aliph.), 1772 (C=O), 1665 (C=N); ^{1}H NMR: δ/ppm = 3.79 (s, 3H, OCH_3), 7.11-7.66 (m, 8H, Ar-H), 9.74 (s, 1H, NH; cancelled with D_2O), ^{13}C NMR: 55.9 (OCH_3), 114.0, 115.5, 120.2, 122.3, 129.1, 129.3, 129.4, 130.5, 131.9, 133.0, 134.8, 153.9 (12C-Ar), 154.3 (C=N), 160.1 (C=O), 183.2 (C=S); MS, m/z (%): 345 (M^+, 100), 347 (M+2; 39.0), 314 (2.0), 192 (22.1), 191 (20.4), 165 (44.1), 161 (10.8), 135 (10.2), 125 (4.3), (3.8), 77 (15.9); Anal. Calcd. for $C_{16}H_{12}ClN_3O_2S$ (345.80): C, 55.57; H, 3.50; N, 12.15; Found: C, 55.76; H, 3.37; N, 12.23%.

1-(3-Ethoxyphenyl)-4-imino-3-phenyl-5-thioxoimidazolidin-2-one (3i): as orange crystals; yield 82 %; mp 100-102 °C; IR: ν/cm^{-1} = 3217 (NH), 2974, 2879 (CH-aliph), 1769 (C=O), 1599 (C=N); ^{1}H NMR: δ/ppm = 1.34 (t, 3H, *J* = 6.9 Hz, CH_3), 4.05 (q, 2H, *J* = 6.9 Hz, CH_2), 7.08-7.59 (m, 9H, Ar-H), 9.67 (br, 1H, NH; cancelled with D_2O); ^{13}C NMR: 15.0 (CH_3), 63.9 (CH_2), 114.4, 116.0, 120.1, 127.6, 128.6, 129.3, 130.4, 130.5, 131.9, 133.0, 134.9, 154.1 (12C-Ar), 154.6 (C=N), 159.3 (C=O), 183.3 (C=S); Anal. Calcd. for $C_{17}H_{15}N_3O_2S$ (325.38): C, 62.75; H, 4.65; N, 12.91; Found: C, 62.84; H, 4.42; N, 12.75%.

1-(3-Ethoxyphenyl)-3-(2-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-one (3j): as orange crystals; yield 71%, mp 105-107 °C; IR: ν/cm^{-1} = 3262 (NH), 2969, 2886 (CH-aliph.), 1775 (C=O), 1597 (C=N); ^{1}H NMR: δ/ppm = 1.34 (t, 3H, *J* = 6.8 Hz, CH_3), 4.04 (q, 2H, *J* = 6.9 Hz, CH_2), 7.08-7.68 (m, 8H, Ar-H), 9.81 (br, 1H, NH; cancelled with D_2O); ^{13}C NMR: 14.3 (CH_3), 63.9 (CH_2), 114.3, 116.1, 116.9, 117.1, 120.3, 125.5, 130.5, 133.5, 130.9, 131.9, 134.7, 153.5 (12C-Ar), 157.0 (C=N), 159.4 (C=O), 183.1 (C=S); MS, m/z (%): 343 (M^+, 100), 344 (M+1; 20.6), 324 (12.9), 205 (7.6), 179 (16.0), 161 (8.2), 151 (28.8), 93 (6.4), 65 (9.3); Anal. Calcd. for $C_{17}H_{14}FN_3O_2S$ (343.38): C, 59.46; H, 4.11; N, 12.24; Found: C, 59.65; H, 4.32; N, 12.42%.

1-(4-Chlorophenyl)-3-(3-ethoxyphenyl)-5-imino-4-thioxoimidazolidin-2-one (3k): as golden crystals; yield 78 %; mp 142-144 °C; IR: ν/cm^{-1} = 3242 (NH), 2980, 2921 (CH-aliph), 1770 (C=O), 1604 (C=N); ^{1}H NMR: δ/ppm = 1.36 (t, 3H, *J* = 7.0 Hz, CH_3), 4.09 (q, 2H, *J* = 6.9 Hz, CH_2), 7.07-7.63 (m, 8H, Ar-H), 9.72 (s, 1H, NH; cancelled with D_2O); ^{13}C NMR: 14.9 (CH_3), 63.9 (CH_2), 114.4, 116.0, 120.1, 120.3, 125.9, 129.0, 129.4, 130.5, 133.0, 134.1, 139.1, 152.4 (12C-Ar), 153.3 (C=N), 154.3 (C=O), 183.3 (C=S); Anal. Calcd. for $C_{17}H_{14}ClN_3O_2S$ (359.83): C, 56.74; H, 3.92; N, 11.68; Found: C, 56.91; H, 3.75; N, 11.76%.

Synthesis of Imidazolidin-2,5-diones 4a,b

The imidazolidineiminothione derivative **3h** or **3i** (5 mmol) was dissolved in boiling ethanol (20 mL) and treated with dil. HCl (1:1 molar ratio). The obtained products were filtered off, washed with cold water, air-dried, and recrystallized from chloroform/n-hexane to give the corresponding diones **4a,b,** respectively.

3-(4-Chlorophenyl)-1-(3-methoxyphenyl)-5-thioxoimidazolidine-2,4-dione (4a): as orange crystals; yield 62 %; mp 100-102 °C; IR: ν/cm^{-1} = 2918, 2826 (CH-aliph), 1749, 1797 (C=O); ^{1}H NMR: δ/ppm = 3.82 (s, 3H, OCH_3), 7.06-7.68 (m, 8H, Ar-H); Anal. Calcd. for $C_{16}H_{11}ClN_2O_3S$ (346.79): C, 55.41; H, 3.20; N, 8.08; Found: C, 55.64; H, 3.42; N, 8.26%.

1-(3-Ethoxyphenyl)-3-phenyl-5-thioxoimidazolidine-2,4-dione (4b): as orange crystals; mp 157-159 °C; yield 76 %; IR: ν/cm^{-1} = 2982, 2895 (CH-aliph), 1745, 1802 (C=O); ^{1}H NMR: δ/ppm = 1.34 (t, 3H, *J* = 6.8 Hz, CH_3), 4.11 (q, 2H, *J* = 6.9 Hz, CH_2), 7.03-7.65 (m, 8H, Ar-H); Anal. Calcd. for $C_{17}H_{13}ClN_2O_3S$ (360.81): C, 56.59; H, 3.63; N, 7.76; Found: C, 56.75; H, 3.84; N, 7.45%.

Synthesis of Azine Derivatives 5a-c

To a solution of **3a, 3d, 3i (**0.0l mo1) in ethanol (20 ml), benzophenonehydrazone (0.01mol) was added and the reaction mixture was heated under reflux for 3hr. The obtained product was filtered, washed with ethanol and crystallized from ethanol/dioxane to afford **5a-c.**

4-((Diphenylmethylene)hydrazono)-3-(4-fluorophenyl)-5-imino-1-phenylimidazolidin-2-one (5a): as yellow crystals; yield 62 %, mp 170-172 °C, IR: ν/cm^{-1} = 3265 (NH), 1758 (C=O), 1624 (C=N); ^{1}H NMR: δ/ppm = 7.34-7.62 (m, 19H, Ar-H), 10.11 (br, 1H, NH; cancelled with D_2O); ^{13}C NMR: 115.6, 126.5, 127.3, 128.1, 128.3, 128.4, 128.5, 128.8, 129.2 (3C), 129.4 (2C), 130.0, 130.1, 130.2, 133.7, 134.4, 134.8, 137.4, 142.4, 150.7, 152.1, 152.8 (24 C-Ar), 160.4, 160.7, 162.4 (3C=N), 167.5 (C=O); Anal. Calcd. For $C_{28}H_{20}FN_5O$ (461.49): C, 72.87; H, 4.37; N, 15.18; Found: C, 72.77; H, 4.27; N, 15.29%.

4-((Diphenylmethylene)hydrazono)-5-imino-1-phenyl-3-p-tolylimidazolidin-2-one 5b: as yellow crystals; mp 162-164 °C; yield 72 %; IR: ν/cm^{-1} = 3249 (NH), 1757 (C=O), 1612 (C=N); ^{1}H NMR: δ/ppm = 2.27 (s, 3H, CH_3), 7.13-7.61 (m, 19H, Ar-H), 10.04 (br, 1H, NH; cancelled with D_2O); ^{13}C NMR: 21.1 (CH_3), 115.4, 126.5, 127.3, 128.1, 128.3, 128.4, 128.5, 128.8, 129.1 (2C), 129.2, 129.3, 129.9, 130.0, 130.1, 131.3, 132.8, 134.3, 134.4, 137.7, 137.5, 142.2, 145.3, 148.1 (24 C-Ar), 150.8, 153.7, 153.0 (3C=N), 167.3 C=O); Anal. Calcd. for $C_{29}H_{23}N_5O$ (457.53): C, 76.13; H, 5.07; N, 15.31; Found: C, 76.28; H, 5.19; N, 15.43%.

4-((Diphenylmethylene)hydrazono)-3-(3-ethoxyphenyl)-5-imino-1-phenylimidazolidin-2-one (5c): as yellow crystals; yield 67 %; mp 165-167 °C; IR: ν/cm^{-1} = 3240 (NH), 1751 (C=O), 1609 (C=N); ^{1}H NMR: δ/ppm = 1.23 (t, 3H, *J* = 6.8 Hz, CH_3), 3.86 (q, 2H, *J* = 6.8 Hz, CH_2), 6.86-7.61 (m, 19H, Ar-H), 10.07 (br, 1H, NH; cancelled with D_2O); ^{13}C NMR: 14.9 (CH_3), 63.6 (CH_2), 114.5, 120.0, 125.9, 127.6, 127.7, 128.1, 128.2, 128.3, 128.5, 128.7, 128.9, 129.0, 129.1, 129.2, 129.4, 129.8, 130.0, 130.7, 131.3, 132.8, 133.5, 134.2, 137.6, 142.3 (24 C-Ar), 150.7, 152.9, 158.8 (3C=N), 167.4 (C=O); MS, m/z (%): 487 (M^+, 3.0), 488 (M+1, 1.6), 459 (41.2), 458 (100), 430 (14.5), 410 (30.9), 368 (15.9), 323 (51.6), 291 (18.6), 135 (11.0), 119 (14.8), 77 (44.3); Anal. Calcd. for $C_{30}H_{25}N_5O_2$ (487.55): C, 73.90; H, 5.17; N, 14.36; Found: C, 73.76; H, 5.29; N, 14.44%.

Synthesis of Hydrazone Derivatives 6a,b

A solution of **3h, 3i** (0.01 mol**)** in ethanol (30 ml) was treated with hydrazine hydrate (0.012 mol), and the reaction mixture was stirred at room temperature for 15 min. The product was collected and recrystallized from ethanol/dioxane to give **6a,b**.

1-(4-Chlorophenyl)-5-hydrazono-3-(3-methoxyphenyl)-4-thioxoimidazolidin-2-one (6a): as yellow crystals; yield 61 %; mp 213-215 °C; IR: ν/cm^{-1} = 3372, 3119 (NH_2), 2965, 2886 (CH-aliph), 1725 (C=O), 1598 (C=N); ^{1}H NMR: δ/ppm = 1.22 (s, 3H, CH_3), 5.78 (br, 2H, NH_2), 7.02-7.87 (m, 8H, Ar-H); MS, m/z (%): 360 (M^+, 100), 361 (M+1**,** 22.7), 362 (M+2**,** 36.4), 334 (17.1), 332 (40.8), 166 (11.0), 164 (9.4), 148 (9.1), 134 (60.2), 111 (11.7), 107 (18.4), 77 (14.3); Anal. Calcd. for $C_{16}H_{13}ClN_4O_2S$ (360.82): C, 53.26; H, 3.63; N, 15.53; Found: C, 53.42; H, 3.76; N, 15.48%.

1-(3-Ethoxyphenyl)-4-hydrazono-3-phenyl-5-thioxoimidazolidin-2-one (6b): as yellow crystals**;** yield 65 %; mp 158-160 °C; IR: ν/cm^{-1} = 3327, 3169 (NH_2), 3109 (CH-Ar), 2984, 2884 (CH-aliph.), 1722 (C=O), 1598 (C=N); ^{1}H NMR: δ/ppm = 1.42 (t, 3H, *J* = 7.1 Hz, CH_3), 4.05 (q, 2H, *J* = 7.0 Hz, CH_2), 5.56 (br, 2H, NH_2), 7.01-7.62 (m, 9H, Ar-H); ^{13}C NMR: 14.7 (CH_3), 63.6 (CH_2), 113.7, 114.0, 116.1, 120.7, 126.1, 127.8, 128.9, 130.1, 132.1, 133.1, 135.2, 150.1, 152.6, 159.0, 183.6 (C=S); MS, m/z (%): 340 (M^+, 100), 341 (M+1**,** 25.1), 313 (17.2), 312 (82.1), 295 (19.7), 164 (11.4), 149 (14.2), 148 (36.7), 136 (20.4), 135 (17.3),

121 (20.1), 104 (16.8), 93 (11.6), 77 (38.8); Anal. Calcd. for $C_{17}H_{16}N_4O_2S$ (340.40): C, 59.98; H, 4.74; N, 16.46; Found: C, 59.75; H, 4.63; N, 16.73%.

Synthesis of Dihydrazone Derivatives 8a,b

A mixture of **3h, 3i** (0.01 mol) and hydrazine hydrate (0.03 mol) in ethanol (30 ml) was stirred for 30 min. at room temperature. The product was recrystallized from ethanol/dioxane to give **8a, b**.

1-(4-Chlorophenyl)-4,5-dihydrazono-3-(3-methoxyphenyl)imidazolidin-2-one (8a): as yellow crystals; yield 63 %; mp 193-195 °C; IR: ν/cm^{-1} = 3373, 3193 (NH_2), 2978 (CH-aliph), 1724 (C=O), 1603 (C=N); ^{1}H NMR: δ/ppm = 1.21 (s, 3H, CH_3), 5.65 (br, 4H, $2NH_2$), 6.91-7.58 (m, 8H, Ar-H); MS, m/z (%): 358 (M^+, 1.76), 343 (32.7), 177 (12.8), 173 (15.6), 161 (16.7), 151 (100), 136 (18.7), 135 (37.4), 122 (33.6), 107 (41.8), 80 (13.3); Anal. Calcd. for $C_{16}H_{15}ClN_6O_2$ (358.78): C, 53.56; H, 4.21; N, 23.42; Found: C, 53.78; H, 4.45; N, 23.56%.

1-(3-Ethoxyphenyl)-4,5-dihydrazono-3-phenylimidazolidin-2-one (8b): as yellow crystals; yield 77%; mp 150-152 °C, IR: ν/cm^{-1} = 3372, 3210 (NH_2), 3067 (CH-Ar), 2925, 2833 (CH-aliph), 1724 (C=O), 1600 (C=N); ^{1}H NMR: δ/ppm = 1.32 (t, 3H, *J* = 7.0 Hz, CH_3), 4.01 (q, 2H, *J* = 6.9 Hz, CH_2), 5.65 (br, 4H, $2NH_2$), 6.91-7.58 (m, 9H, Ar-H); ^{13}C NMR: 15.0 (CH_3), 63.6 (CH_2), 113.8, 114.5, 116.0, 120.2, 127.4, 128.4, 129.0, 129.7, 131.2, 133.0, 134.7, 150.1 (12C-Ar), 152.6 (2C=N), 159.0 (C=O); MS, m/z (%): 338 (M^+, 100), 339 (M+1; 22.5), 307 (34.0), 175 (11.9), 148 (53.3), 135 (13.4), 119 (14.2), 77 (32.4); Anal. Calcd. for $C_{17}H_{18}N_6O_2$ (338.36): C, 60.34; H, 5.36; N, 24.84; Found: C, 59.95; H, 5.53; N, 24.73%.

Synthesis of Thiosemicarbazone Derivatives 9a-d

To a solution of **3a, 3f, 3h, 3i (**0.0l mo1) in ethanol (20 ml), thiosemicarbazide (0.01mol) was added and the reaction mixture was heated under reflux for 3hr. The obtained product was filtered, washed with ethanol and crystallized from ethanol/dioxane to afford **9a-d**.

2-(3-(4-Fluorophenyl)-5-imino-2-oxo-1-phenylimidazolidin-4- ylidene)hydrazinecarbothioamide (9a): as orange crystals; yield 62%; mp 215-217 °C; IR: ν/cm^{-1} = 3426, 3260, 3148 (NH_2, NH), 1758 (C=O), 1611 (C=N); ^{1}H NMR: δ/ppm = 6.87-7.74 (m, 9H, Ar-H), 8.43, 9.72, 11.63 (3br, 4H, NH_2+2NH); Anal. Calcd. For $C_{16}H_{13}FN_6OS$ (356.38): C, 53.92; H, 3.68; N, 23.58; Found: C, 53.69; H, 3.88; N, 23.76%.

2-(5-Imino-3-(3-methoxyphenyl)-2-oxo-1-phenylimidazolidin-4-ylidene)hydrazinecarbothioamide (9b): as yellow crystals; yield 61%; mp 218-220 °C; IR: ν/cm^{-1} = 3442, 3251, 3203 (NH_2, NH), 2964, 2930 (CH-aliph), 1765 (C=O), 1645 (C=N); ^{1}H NMR: δ/ppm = 1.23 (s, 3H, CH_3), 6.91-7.63 (m, 9H, Ar-H), 8.52, 9.33, 11.87 (3br, 4H, NH_2+2NH); Anal. Calcd. for $C_{17}H_{16}N_6O_2S$ (368.41): C, 55.42; H, 4.38; N, 22.81; Found: C, 55.67; H, 4.69; N, 22.75%.

2-(1-(4-Chlorophenyl)-5-imino-3-(3-methoxyphenyl)-2-oxoimidazolidin-4-ylidene)hydrazinecarbothioamide (9c): as yellow crystals; mp 222-224 °C; yield 59%; IR: ν/cm^{-1} = 3421, 3238, 3139 (NH_2, NH), 2934 (CH-aliph), 1757 (C=O), 1640 (C=N); ^{1}H NMR: δ/ppm = 1.21 (s, 3H, CH_3), 6.95-7.87 (m, 8H, Ar-H), 8.53, 9.34, 11.92 (3br, 4H, NH_2+2NH); MS, m/z (%): 402 (M^+, 9.2), 346 (21.3), 255 (37.0), 253 (100), 252 (50.6), 249 (32.0), 180 (11.9), 166 (13.7), 155 (31.6), 153 (99.0), 119 (13.5), 77 (49.4); Anal. Calcd. For $C_{17}H_{15}ClN_6O_2S$ (402.86): C, 50.68; H, 3.75; N, 20.86; Found: C, 50.39; H, 3.86; N, 20.79%.

2-(3-(3-Ethoxyphenyl)-5-imino-2-oxo-1-phenylimidazolidin-4-ylidene)hydrazinecarbothioamide (9d): as yellow crystals yield 75%, mp 188-190 °C, IR: ν/cm^{-1} = 3382, 3254, 3162 (NH_2, NH), 2978 (CH-aliph), 1775 (C=O), 1607 (C=N); ^{1}H NMR: δ/ppm = 1.30 (t, 3H, *J* = 7.0 Hz, CH_3), 4.03 (q, 2H, *J* = 7.0 Hz, CH_2), 6.91-7.63 (m, 9H, Ar-H), 8.46, 9.47, 12.79 (3s, 4H, NH_2+2NH); MS, m/z (%): 382 (M^+, 16.5), 383 (M+1, 8.1), 365 (38.9), 348 (15.8), 323 (1.5), 263 (17.3), 219 (66.4), 218 (50.2), 180 (28.7), 163 (27.9), 135 (47.5), 119 (62.0), 93 (100), 77 (45.0); Anal. Calcd. for $C_{18}H_{18}N_6O_2S$ (382.44): C, 56.53; H, 4.74; N, 21.97; Found: C, 56.34; H, 4.84; N, 21.76%.

Synthesis of imidazo[4,5-b]quinoxalin-2(3H)-one derivatives 11a-c

A solution of **(3i,** 0.0l mo1) in ethanol (20 ml) was treated with *o*-phenylenediamine derivatives (0.0l mo1) and triethylamine (0.5 ml). The reaction mixture was heated under reflux for 3hr and the solid that obtained after filtration was crystallized from ethanol/dioxane to give **11a-c**.

1-(3-Ethoxyphenyl)-3-phenyl-1H-imidazo[4,5-b]quinoxalin-2(3H)-one (11a): as white crystals; yield 62%; mp 160-162 °C; IR: ν/cm^{-1} = 3055 (CH-Ar), 2975 (CH-aliph.), 1744 (C=O), 1597 (C=N); ^{1}H NMR: δ/ppm = 1.37 (t, 3H, *J* = 7.0 Hz, CH_3), 4.10 (q, 2H, *J* = 7.0 Hz, CH_2), 7.06-7.98 (m, 13H, Ar-H); ^{13}C NMR: 15.0 (CH_3), 63.8 (CH_2), 113.6, 114.6, 119.1, 119.2, 127.2, 127.5, 128.6, 129.3, 129.6, 130.3 (2C), 132.9, 133.9, 136.9, 137.0, 137.4, 138.8, 140.6 (18C-Ar), 152.6 (2C=N), 159.2 (C=O); Anal. Calcd. for $C_{23}H_{18}N_4O_2$ (382.41): C, 72.24; H, 4.74; N, 14.65; Found: C, 72.36; H, 4.44; N, 14.76%.

6-Chloro-1-(3-ethoxyphenyl)-3-phenyl-1H-imidazo[4,5-b]quinoxalin-2(3H)-one (11b): as gray crystals; yield 63%; mp 130-132 °C; IR: ν/cm^{-1} = 3064 (CH-Ar), 2960 (CH-aliph.), 1748 (C=O), 1600 (C=N); ^{1}H NMR: δ/ppm = 1.37 (t, 3H, *J* = 6.8 Hz, CH_3), 4.09 (q, 2H, *J* = 7.0 Hz, CH_2), 7.09-7.98 (m, 12H, Ar-H); ^{13}C NMR: 16.0 (CH_3), 53.9 (CH_2), 113.6, 115.4, 118.5, 119.3, 126.2, 126.8, 127.3 (3C), 127.8, 128.9, 129.5, 130.9, 131.5, 132.3, 132.5, 138.5, 152.6, 159.3 (C=N), 159.4 (C=N), 160.0 (C=O); MS, m/z (%): 416 (M^+, 100), 417 (M+1, 54.2), 418 (M+2, 30.3), 402 (11.2), 387 (71.4), 320 (46.1), 253 (20.3), 163 (10.0), 176 (14.5), 163 (17.1), 135 (12.5), 91 (19.5), 77 (58.5); Anal. Calcd. for $C_{23}H_{17}ClN_4O_2$ (416.86): C, 66.27; H, 4.11; N, 13.44; Found: C, 66.13; H, 4.24; N, 13.21%.

1-(3-Ethoxyphenyl)-6-methyl-3-phenyl-1H-imidazo[4,5-b]quinoxalin-2(3H)-one (11c): as brown crystals; yield 67%; mp 118-120 °C; IR: ν/cm^{-1} = 3054 (CH-Ar.), 2975, 2922 (CH-aliph.), 1744 (C=O), 1597 (C=N); ^{1}H NMR: δ/ppm = 1.37 (t, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.09 (q, 2H, CH_2), 7.09-7.92 (m, 12H, Ar-H); ^{13}C NMR: 15.0 (CH_3), 21.4 (CH_3), 63.8 (CH_2), 113.6, 114.7, 119.3, 119.4, 127.3, 127.6, 127.8, 127.9, 128.7, 129.6, 130.4, 132.8, 133.8, 138.9, 139.0, 140.6, 140.7, 150.5 (18C-Ar), 152.6 (2C=N), 159.3 (C=O); MS, m/z (%): 396 (M^+, 100), 397 (M+1, 31.4), 381 (27.6), 368 (50.7), 320 (46.1), 77 (8.5); Anal. Calcd. for $C_{24}H_{20}N_4O_2$ (396.44): C, 72.71; H, 5.08; N, 14.13; Found: C, 72.86; H, 5.21; N, 14.24.

Synthesis of Imidazolidin-4-Ylidene Derivatives 12a-d

To a solution of iminothione **3c,i** (0.0l mo1) in ethanol (20 ml) pyrazolone derivatives (0.01 mol) and triethylamine (0.5 ml) was added. The reaction mixture was heated under reflux for 10 hr. left to cool, the solid that obtained after filtration was crystallized from ethanol/dioxane to give **12a-d.**

4-(3-(3-Chlorophenyl)-1-(4-chlorophenyl)-5-imino-2-oxoimidazolidin-4-ylidene)-3-methyl-1H-pyrazol-5(4H)-one (12a): as red crystals; yield 72%; mp 118-120 °C; IR: ν/cm^{-1} = 3335 (NH), 3047 (CH-Ar), 2980, 2927 (CH-aliph.), 1726 (C=O), 1594 (C=N); ^{1}H NMR: δ/ppm: 2.31 (s, 3H, CH_3), 7.08-8.41 (m, 8H, Ar-H), 8.08 (br, 1H, NH; cancelled with D_2O); 12.72 (br, 1H, NH; cancelled with D_2O); Anal. Calcd. for $C_{19}H_{13}Cl_2N_5O_2$ (414.24): C, 55.09; H, 3.16; N, 16.91; Found: C, 55.29; H, 3.34; N, 16.71%.

4-(3-(3-Chlorophenyl)-1-(4-chlorophenyl)-5-imino-2-oxoimidazolidin-4-ylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (12b): as violet crystals; yield 60%; mp 93-95 °C, IR: ν/cm^{-1} = 3345 (NH), 3063 (CH-Ar), 2922 (CH-aliph.), 1718 (C=O), 1621 (C=N); ^{1}H NMR: δ/ppm: 2.41 (s, 3H, CH_3), 7.11-8.79 (m, 13H, Ar-H), 12.64 (br, 1H, NH; cancelled with D_2O); MS, m/z (%): 490 (M^+, 1.0), 478 (2.4), 417 (5.1), 405 (3.6), 379 (4.4), 358 (3.1), 355 (5.3), 333 (30.9), 315 (4.3), 304 (6.3), 278 (41.6), 255 (21.2), 208 (38.9), 179 (49.0), 159 (16.73), 122 (60.4), 106 (100), 68 (8.47); Anal. Calcd. for $C_{25}H_{17}Cl_2N_5O_2$ (490.34): C, 61.24; H, 3.49; N, 14.28; Found: C, 61.39; H, 3.54; N, 14.59%.

4-(3-(3-Ethoxyphenyl)-5-imino-2-oxo-1-phenylimidazolidin-4-ylidene)-3-methyl-1Hpyrazol-5(4H)-one (12c): as red crystals; yield 65%; mp 175-177 °C; IR: ν/cm^{-1} = 3291 (NH), 3050 (CH-Ar), 2978 (CH-aliph.), 1713 (C=O), 1610 (C=N); ^{1}H NMR: δ/ppm: 1.12 (t, 3H, *J* = 6.9 Hz, CH_3), 2.32 (s, 3H, CH_3), 4.18 (q, 2H, *J* = 6.8 Hz, CH_2), 7.11-8.79 (m, 9H, Ar-H), 8.11 (br, 1H, NH; cancelled with D_2O); 12.71 (br, 1H, NH; cancelled with D_2O); MS, m/z (%): 389 (M^+, 3.1), 390 (M+1, 3.1), 309 (12.2), 304 (100), 295 (12.6), 287 (13.6), 275 (13.9), 244 (24.9), 200 (23.2), 163 (13.6), 135 (30.3), 124 (44.1), 119 (18.0), 90 (2.3), 77 (26.3); Anal. Calcd. for $C_{21}H_{19}N_5O3$ (389.41): C, 64.77; H, 4.92; N, 17.98; Found: C, 64.86; H, 4.72; N, 17.76%.

4-(3-(3-Ethoxyphenyl)-5-imino-2-oxo-1-phenylimidazolidin-4-ylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (12d): as brown crystals; yield 68%; mp 70-72 °C; IR: ν/cm^{-1} = 3280 (NH), 3055 (CH-Ar), 2978, 2914 (CH-aliph.), 1717 (C=O), 1603 (C=N); ^{1}H NMR: δ/ppm: 1.14 (t, 3H, *J* = 6.8 Hz, CH_3), 2.41 (s, 3H, CH_3), 4.19 (q, 2H, *J* = 6.9 Hz, CH_2), 7.13- 8.01 (m, 14H, Ar-H), 12.83 (br, 1H, NH; cancelled with D_2O); ^{13}CNMR 14.8 (CH_3), 15.1 (CH_3), 63.3 (CH_2), 105.0, 110.7, 112.8, 115.4, 119.2, 120.7, 122.7, 123.7, 124.2, 127.3, 127.9, 128.8, 129.4, 130.3, 137.3, 139.8, 140.5, 144.7, 147.8, 153.9, 159.34 (C=N), 159.4 (C=N), 162.3 (C=O), 166.6 (C=O); Anal. Calcd. for $C_{27}H_{23}N_5O_3$ (465.50): C, 69.66; H, 4.98; N, 15.04; Found: C, 69.49; H, 4.76; N, 15.21%.

Antimicrobial Activity

Chemical compounds were individually tested against a panel of gram positive and gram negative bacterial pathogens and fungi. Antimicrobial tests were carried out by the agar well diffusion method using 100 µL of suspension containing 1 x10^8 CFU/mL of pathological tested bacteria and 1 x10^6 CFU/ml of fungi spread on nutrient agar and Sabouraud dextrose agar media, respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested compound solution prepared by dissolving 5 mg of the chemical compound in one mL of dimethyl sulfoxide (DMSO). The inoculated plates were then incubated for 24 h at 37 °C. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ampicillin, Gentamycin and Amphotericin B (1 mg/mL) were used as standard for antibacterial and antifungal activity, respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table **1**. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Minimal Inhibitory Concentration (MIC) Measurement

Screening was evaluated *in vitro* using the Broth dilution method according to NCCLs [22, 23]. All the bacteria were incubated and activated at 30 °C for 24 h inoculation into nutrient broth and the fungi were incubated in malt extract broth for 48 h. The compounds were dissolved in DMSO and then diluted using cautiously adjusted Mueller-Hinton broth. Two-fold serial concentrations of the compounds were employed to determine the (MIC). In each case triplicate tests were performed and the average was taken as the final reading. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37 °C for 24 hours for tested microorganisms (1×10^8 CFU/mL for bacteria and 1 x10^6 CFU/mL of fungi), each 5 ml received 0.1 mL of the above inoculum and incubated at 37 °C for 24 hours. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Abbas, S.Y.; El-Sharief, A.M.; Basyouni, W.M.; Fakhr, I.M.I.; El-Gammal, E.W. Thiourea derivatives incorporating a hippuric acid moiety: Synthesis and evaluation of antibacterial and antifungal activities. *Eur. J. Med. Chem.* **2013**, *64*, 111-120.

[2] El-Sharief, A.M.; Abbas, S.Y.; El-Bayouki, K.A.; El-Gammal, E.W. Synthesis of thiosemicarbazones derived from N-(4-hippuric acid)thiosemicarbazide and different carbonyl compounds as antimicrobial agents. *Eur. J. Med. Chem*. **2013**, *67*, 263-268.

[3] Aneja, D.K.; Lohan, P.; Arora, S.; Sharma, C.; Aneja, K.R.; Prakash, O. Synthesis of new pyrazolyl-2, 4-thiazolidinediones as antibacterial and antifungal agents. *Bioorg. Med.Chem. Lett*. **2011**, 1-15.

[4] Berber, I.; Cokmus, C.; Atalan, E. Characterization of *Staphylococcus* species by SDS–page of whole-cell and extracellular proteins. *Microbiology* **2003**, *72*, 42-47.

[5] Bildirici, I.; Sener A.; Tozlu, I. Further derivatives of 4-benzoyl-1, 5-diphenyl-1H pyrazole-3-carboxylic acid and their antibacterial activities. *Med. Chem. Rev*. **2007**, *16*, 418-426.

[6] Appelbaum, P.C. The emergence of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. *Clin. Microbiol. Infect.* **2006**, *12*, 16.

[7] Graziak, M.P.; Ding, H. Halogenated (acylamino)imidazoles and benzimidazoles for directed halogen-metal exchange-based functionalization. *Acta Chim. Solv.* **2000**, *47*, 1.

[8] Trunz, B.B.; Jedrysiak, R.; Tweats, D.; Brun, R.; Kaiser, M.; Suwinski, J.; Torreele, E. 1-Aryl-4-nitro-1H-imidazoles, a new promising series for the treatment of human African trypanosomiasis. *Eur. J. Med. Chem*. **2011**, *46*, 1524-1535.

[9] Raether, W.; Hänel, H. Nitroheterocyclic drugs with broad spectrum activity. *Parasitol. Res*. **2003**, *90*, 519-539.

[10] Winkelmann, E.; Raether, W.; Gebert, U.; Sinharay, A. Chemotherapeutically active nitro compounds. 4. 5-Nitroimidazoles (Part I-IV). *Arzneimittelforschung* **1977**, 27-28

[11] Marie-Daragon, A.; Rouillard, M.C.; Bouteille, B.; Bisser, S.; de Albuquerque, C.; Chauvière, G.; Périé, J.; Dumas, M. An efficacy trial on Trypanosoma brucei brucei of molecules permeating the blood-brain barrier and of megazol. *Bull. Soc. Pathol. Exot.* **1994**, *87*, 347-352.

[12] Freeman, C.D.; Klutman, N.E.; Lamp, K.C. Metronidazole. A therapeutic review and update. *Drugs***1997**, *54*, 679-708.

[13] Chazeau, V.; Cussac M.; Boucherle, A. Synthesis and immunomodulating activity of 1-amino-2- thiohydantoin derivatives. *Eur. J. Med. Chem*. **1992**, *27*, 839-843.

[14] Bascou, J.P.; Lacroix, G.; Gadras, A.; Perez, J. Dérivés optiquement actifs de 2-imidazoline-5-ones et 2-imidazoline-5-thiones fongicides. EP Patent 0 629616 A2, 1994.

[15] Ding, M.W.; Fu, B.Q.; Yuan, J.-Z. Facile synthesis of 2-alkylthio-3-amino-4 H-imidazol-4-ones and 2H-imidazo[2,1-b]-1,3,4-thiadiazin-6(7H)-ones via N-vinylic iminophosphorane. *Heteroatom Chem*. **2005**, *16*, 76-80.

[16] Aly, M.M.; Mohamed, Y.A.; El-Bayouki, Kh.A.M.; Basyouni, W.M.; Abbas, S.Y. Synthesis of some new 4 (3H)-quinazolinone-2-carboxaldehyde thiosemicarbazones and their metal complexes and a study on their anticonvulsant, analgesic, cytotoxic and antimicrobial activities–Part-1. *Eur. J. Med. Chem.*, **2010**, *45*, 3365-3373.

[17] Helal, M.H.; Abbas, S.Y.; Salem, M.A.; Farag, A.A.; Ammar, Y.A. Synthesis and characterization of new types of 2-(6-methoxy-2-naphthyl) propi-

onamide derivatives as potential antibacterial and antifungal agents. *Med. Chem. Res*. **2013**, *22*, 5598-5609.

[18] Abbas, S.Y.; Farag, A.A.; Ammar, Y.A.; Atrees, A.A.; Mohamed, A.F.; El-Henawy, A.A. Synthesis, characterization, and antiviral activity of novel fluorinated isatin derivatives. *Monatsh Chem*. **2013**, *144*, 1725-1733.

[19] Bondock, S.; Naser, T.; Ammar, Y.A. Synthesis of some new 2-(3-pyridyl)-4,5-disubstituted thiazoles as potent antimicrobial agents. *Eur. J. Med. Chem.* **2013**, *62*, 270-279

[20] El-Sharief, A.M.; Moussa, Z. Synthesis, characterization and derivatization of some novel types of mono- and bis-imidazolidineiminothiones and imidazolidineiminodithiones with antitumor, antiviral, antibacterial and antifungal activities – part I. *Eur. J. Med. Chem*. **2009**, *44*, 4315

[21] Cooper, R.E. Analytical Microbiology, Ed. Kavangeh, F.W. Vol. *1&2*, Academic press, New York and London, **1972**.

[22] Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards NCCLS); Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. NCCLS M7 A4, 4th Edition, Approved Standard, NCCLS: Pennsylvania, **1997**.

[23] Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards NCCLS); Method M27-A2, 2nd Edition.; Wayne, Ed.; NCCLS: Pennsylvania, **2002**, *22*(15), 1-29.

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