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Synthesis and characterization of new imidazolidineiminothione and *bis*-imidazolidineiminothione derivatives as potential antimicrobial agents

Yousry Ahmed Ammar [1], Samir Youssef Abbas [2], Marwa Ahmed Mohamed Shehab El-Sharief [3], Mohamed Abd El-Rashid Salem [1,4,*] and Ahmed Ragab Mohamed [1]

[1] *Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, 11284, Egypt*
[2] *Organometallic and Organometalloid Chemistry Department, National Research Centre, Cairo, 12622, Egypt*
[3] *Applied Organic, Chemistry Department, National Research Centre, Cairo, 11284, Egypt*
[4] *Chemistry Department, Faculty of Science and Arts, King Khalid University, Mohail Assir, 900, Kingdom of Saudi Arabia*

** Corresponding author at: Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, 11284, Egypt. Tel.: +966.536.563810. Fax: +966.536.563810. E-mail address: m_eltayyeb@hotmail.com (M.A. Salem).*

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ABSTRACT

A series of new imidazolidineiminothiones (2-4) and *bis*-imidazolidineiminothiones (5) were synthesized through the cycloaddition reaction of *N*-arylcyanothioformamides (1) with some electrophilic reagents. Structure of imidazolidineiminothione derivatives were established based on spectroscopic IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analyses data. These compounds were screened for their antibacterial and antifungal activities. Among the synthesized compounds, imidazolidineiminothione derivative 3a showed about 25% less potent effect than Ampicillin against *S. epidermidis* and *B. subtilis* (MIC, 0.49 µg/mL) and about 50 % less potent effect than Amphotericin B against *A. clavatus* and *G. Candidum*. *Bis*-imidazolidineiminothione derivative 5a was equipotent to the Gentamycin in inhibiting the growth of *N. gonorrhoeae* (MIC, 0.49 µg/mL), and displayed 50% less active than Amphotericin B against *A. clavatus*.

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1. Introduction

Infectious diseases caused by bacteria and fungi remain a major worldwide health problem due to rapid development of resistance to the existing antibacterial and antifungal drugs. So, the medical community faces a serious problem against infections caused by the pathogenic microbes and needs effective therapies and search for novel antimicrobial agents. The quest for new and effective antimicrobial agents, resistant to the mechanisms of defense of these bacteria, is of paramount importance [1-3].

Imidazoles and their fused derivatives are key components of great many bioactive compounds of both natural and synthetic origin [4] such as histidine, purines and biotin. Antimicrobial activity was stated among derivatives possessing aromatic substituents at the imidazole nitrogen e.g. *N*-acyl and 5-arylidene derivatives of hydantoin and 4-thiohydantoin [5,6]. Moreover, previous reports demonstrated that synthetic imidazoles act either as inhibitors of α-adrenoceptor mediated events in platelets [7] or inducers of platelets activation [8]. Imidazolidineiminothiones and the various heterocycles derived from them were shown to exhibit an interesting and a wide range of pharmacological effects including antitumor, antiviral, antimicrobial and antifungal strains [9-15]. *N*-Substituted cyanothioformamides were used as a key intermediate for a number of interesting heterocyclic ring closures such as imidazole [16], oxazole [17], and thiazole [18,19]. In particular, their reactions with isocyanates [16] and isothiocyanates [17] give 1,3-disubstituted imidazolidine systems.

In view of the above-mentioned findings, and as a continuation of our effort to identify new candidates [9-15], which are crucial in designing new, potent, selective, and less toxic antimicrobial agents, its report herein the synthesis and antimicrobial evaluation of some novel imidazolidineimino thione. The target compounds were rationalized so as to comprise some pharmacophores that are believed to be responsible for the biological activity of some relevant chemotherapeutic such as the carbonyl, thiocarbonyl, imino and cyano groups functionalities. The substitution pattern of the imidazolidineiminothione ring was carefully selected in order to confer different electronic environment to the molecules.

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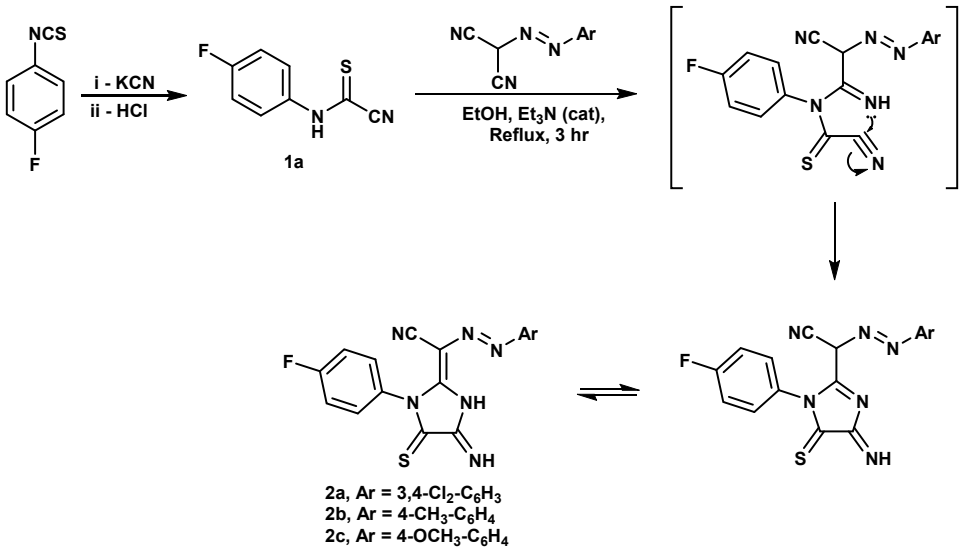


Scheme 1

2. Experimental

All the melting points are uncorrected. IR (KBr) spectra were measured on Shimadzu 440 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained in DMSO-d_6 on a Varian Gemini 300 MHz spectrometer using TMS as internal standard. The chemical shifts are reported as δ ppm units at the Micro-analytical Center in National Research Center, Egypt. Mass spectra were obtained on GCMS/QP 1000 Ex mass spectrometer at 70 eV at National Research Center, Egypt. Elemental analyses were carried out at the Department of Chemistry, Faculty of Science, Cairo University, Egypt. Microbiology screening was carried out in the Regional Center for Microbiology and Biotechnology (RCMB), Antimicrobial unit test organisms, Al-Azhr University, Cairo, Egypt.

2.1. Chemistry

2.1.1. Synthesis of 2-(aryldiazenyl)-2-(1-(4-fluorophenyl)-4-imino-5-thioxoimidazolidin-2-ylidene)acetonitriles (2a-c)

A mixture of cyanothioformamide (**1a**) (0.01 mol), arylhydrazonomalononitriles (0.01 mol) and of triethylamine (3 drops) in absolute ethanol was heated under reflux for 4 h. left to cool. The solid product was collected and recrystallized from dioxane to give compounds **2a-c** (Scheme 1).

2-((3,4-Dichlorophenyl)diazenyl)-2-(1-(4-fluorophenyl)-4-imino-5-thioxoimid-azolidin-2-ylidene)acetonitrile (**2a**): Color: Gray. Yield: 73 %. M.p.: 258-260 °C. IR (KBr, ν, cm^{-1}): 3410 (NH), 2201 (C≡N), 1671 (C=N), 1574 (N=N), 1186 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.43-7.64 (m, 7H, Ar-H), 8.87 (br, 1H, NH; exchangeable with D_2O), 9.81 (br, 1H, NH, exchangeable with D_2O). MS (EI, *m/z* (%)): 418 (M$^+$; 24.7), 419 (M+1; 28.2), 245 (12.6), 174 (18.4), 159 (19.2), 145 (100), 109 (56.8), 95 (67.5), 75 (36.4). Anal. calcd. for $C_{17}H_9Cl_2FN_6S$: C, 48.70; H, 2.16; N, 20.05. Found: C, 48.48; H, 2.02; N, 20.18%.

2-(1-(4-Fluorophenyl)-4-imino-5-thioxoimidazolidin-2-ylidene)-2-(p-tolyldiazenyl)acetonitrile (**2b**): Color: Gray. Yield: 72 %. M.p.: 190-191 °C. IR (KBr, ν, cm^{-1}): 3401 (NH), 2213 (C≡N), 1671 (C=N), 1584 (N=N), 1182 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.26 (s, 3H, CH_3), 6.73-7.52 (m, 8H, Ar-H), 8.63 (br, 1H, NH; exchangeable with D_2O), 9.56 (br, 1H, NH, exchangeable with D_2O). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 21.4 (CH_3), 105.1, 113.3, 116.7, 116.9, 122.5 (2C), 129.9 (2C), 130.7, 130.8, 135.4, 141.5, 150.8, 160.5, 161.9, 164.6, 188.2 (C=S). MS (EI, *m/z* (%)): 364 (M$^+$; 54.2 %), 365 (M+1; 19.2), 264 (22.4), 119 (21.2), 9 (100), 65 (65.8). Anal. calcd. for $C_{18}H_{13}FN_6S$: C, 59.33; H, 3.60; N, 23.06. Found: C, 59.46; H, 3.81; N, 23.42%.

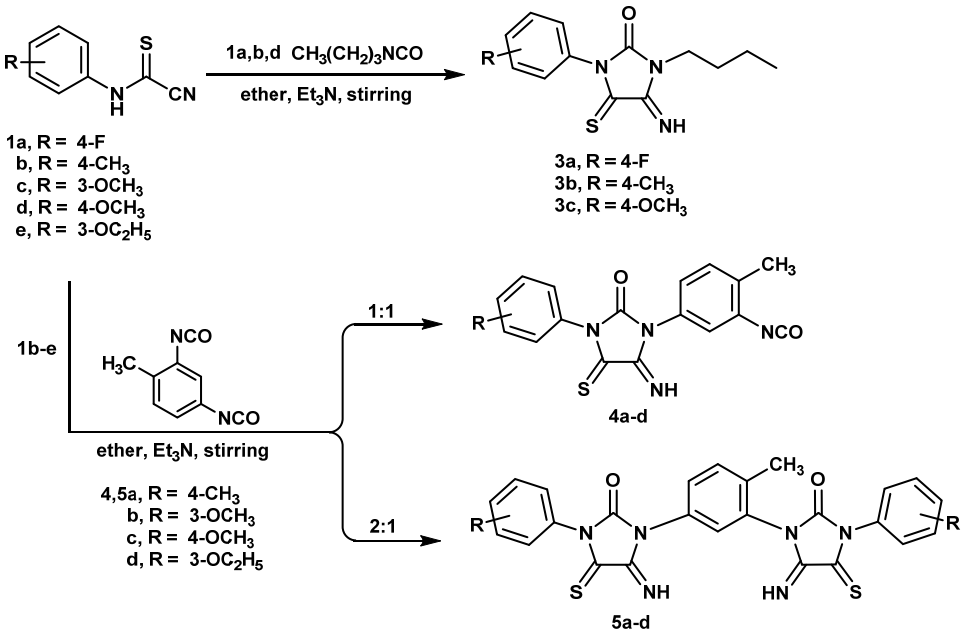
2-(1-(4-Fluorophenyl)-4-imino-5-thioxoimidazolidin-2-ylidene)-2-(4-methoxy-phenyldiazenyl)acetonitrile (**2c**): Color: White. Yield: 71%. M.p.: 260-262 °C. IR (KBr, ν, cm^{-1}): 3401 (NH), 2214 (CN), 1666 (C=N), 1585 (N=N), 1137 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.86 (s, 3H, OCH_3), 6.81-7.53 (m, 8H, Ar-H), 8.52 (br, 1H, NH, exchangeable with D_2O), 9.45 (br, 1H, NH, exchangeable with D_2O). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 56.1 (OCH_3), 105.2, 114.6, 116.7, 116.9 (2C), 122.5 (2C), 124.5 (2C), 130.3 (2C), 130.7, 147.2, 160.4, 161.9, 163.6, 187.8 (C=S). MS (EI, *m/z* (%)): 380 (M$^+$; 77.2 %), 380 (M+1; 22.2), 235 (22.4), 107 (100), 93 (44.8) 77 (15.2). Anal. calcd. for $C_{18}H_{13}FN_6OS$: C, 56.83; H, 3.44; N, 22.09. Found: C, 57.11; H, 3.28; N, 21.97 %.

2.1.2. Synthesis of 1-butyl-5-imino-3-(aryl)-4-thioxo imidazolidin-2-ones (3a-c)

To a solution of compound **1a**,**b** and **d** (0.01 mol) in ether (20 mL), *n*-butylisocyanate (0.01 mol) and triethylamine (0.5 mL) were added; the reaction mixture was stirred at room temperature for 2 hr. The obtained products were crystallized from absolute ethanol to form compounds **3a-c** (Scheme 2).

1-Butyl-3-(4-fluorophenyl)-5-imino-4-thioxoimidazolidin-2-one (**3a**): Color: White. Yield: 83 %. M.p.: 80-82 °C. IR (KBr, ν, cm^{-1}): 3250 (NH), 2960 (CH-aliph), 1766 (C=O), 1660 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 0.91 (t, 3H, *J* = 11.5 Hz, CH_3), 1.33 (h, 2H, *J* = 12.0 Hz, CH_2), 1.67 (p, 2H, *J* = 12.0 Hz, CH_2), 3.68 (t, 2H, *J* = 11.5 Hz, CH_2), 7.35-7.42 (m, 2H, Ar-H), 7.54-7.58 (m, 2H, Ar-H), 9.47 (br, 1H, NH, exchangeable with D_2O). Anal. calcd. for $C_{13}H_{14}FN_3OS$: C, 55.90; H, 5.05; N, 15.04. Found: C, 56.14; H, 4.89; N, 15.11%.

1-Butyl-5-imino-4-thioxo-3-p-tolylimidazolidin-2-one (**3b**): Color: Yellow. Yield: 79 %. M.p.: 95-97 °C. IR (KBr, ν, cm^{-1}): 3250 (NH), 2951 (CH-aliph), 1765 (C=O), 1660 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 0.95 (t, 3H, *J* = 11.5 Hz, CH_3), 1.32 (h, 2H, *J* = 11.5 Hz, CH_2), 1.67 (p, 2H, *J* = 12.0 Hz, CH_2), 2.36 (s, 3H, CH_3), 3.67 (t, 2H, *J* = 12.0 Hz, CH_2), 7.32 (s, 4H, Ar-H), 9.44 (br, 1H, NH; exchangeable with D_2O).



Scheme 2

^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 13.5 (CH_3), 19.3 (CH_2), 20.7 (CH_3), 20.8 (CH_2), 29.0 (CH_2), 127.2, 127.3, 129.4, 129.6, 130.6, 138.9, 153.8 (C=N), 154.6 (C=O), 183.1 (C=S). Anal. calcd. for $C_{14}H_{17}N_3OS$: C, 61.06; H, 6.22; N, 15.26. Found: C, 61.32; H, 6.42; N, 15.52%.

1-Butyl-5-imino-3-(4-methoxyphenyl)-4-thioxoimidazolidin-2-one (**3c**): Color: Gray. Yield: 80 %. M.p.: 110-112 °C. IR (KBr, ν, cm^{-1}): 3242 (NH), 2951 (CH-aliph), 1763 (C=O), 1656 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 0.91 (t, 3H, *J* = 11.5 Hz, CH_3), 1.3 (h, 2H, *J* = 11.5 Hz, CH_2), 1.69 (p, 2H, *J* = 11.5 Hz, CH_2), 3.69 (t, 2H, *J* = 12.0 Hz, CH_2), 3.81 (s, 3H, OCH_3), 7.05 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.36 (d, 2H, *J* = 8.5 Hz, Ar-H), 9.42 (br, 1H, NH; exchangeable with D_2O). Anal. calcd. for $C_{14}H_{17}N_3O_2S$: C, 57.71; H, 5.88; N, 14.42. Found: C, 57.62; H, 5.74; N, 14.23%.

2.1.3. Synthesis of 1-(aryl)-4-imino-3-(3-isocyanato-4-methylphenyl)-5-thioxoimid-azolidin-2-ones (4a-d)

A mixture of cyanothioformamides **1b**-**e** (0.01 mol) in ether (20 mL), toluenediisocyanate (0.01 mol) and triethylamine (0.5 mL) were added, the reaction mixture was stirred at room temperature for 0.5 hr. The obtained products were crystallized from proper solvent to afford 5-imino-4-thioxo-2-imidazolidinones **4a-d** (Scheme 2).

4-Imino-3-(3-isocyanato-4-methylphenyl)-5-thioxo-1-p-tolyl imidazolidin-2-one (**4a**): Color: Gray. Yield: 77 %. M.p.: 217-219 °C. IR (KBr, ν, cm^{-1}): 3238 (NH), 2971 (CH-aliph), 2272 (NCO), 1775 (C=O), 1667 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.82 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 7.05-7.53 (m, 7H, Ar-H), 9.52 (s, 1H, NH, exchangeable with D_2O). MS (EI, *m/z* (%)): 350 (M^+, 14.2), 351 (M+1, 3.5), 149 (35.9), 148 (100), 122 (13.2), 107 (12.7), 92 (10.8), 77 (27.4). Anal. calcd. for $C_{18}H_{14}N_4O_2S$: C, 61.70; H, 4.03; N, 15.99. Found: C, 61.42; H, 4.12; N, 15.77%.

4-Imino-3-(3-isocyanato-4-methylphenyl)-1-(3-methoxy phenyl)-5-thioxoimidazolidin-2-one (**4b**): Color: Beige. Yield: 81 %. M.p.: 233-235 °C. IR (KBr, ν, cm^{-1}): 3343 (NH), 2968 (CH-aliph), 2270 (NCO), 1779 (C=O), 1665 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.35 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 7.07-7.97 (m, 7H, Ar-H), 9.60 (s, 1H, NH, D_2O exchanagable). Anal. calcd. for $C_{18}H_{14}N_4O_3S$: C, 59.01; H, 3.85; N, 15.29. Found: C, 59.21; H, 3.77; N, 15.42%.

4-Imino-3-(3-isocyanato-4-methylphenyl)-1-(4-methoxy phenyl)-5-thioxoimidazolidin-2-one (**4c**): Color: Gray. Yield: 78 %. M.p.: 248-250 °C. IR (KBr, ν, cm^{-1}): 3352 (NH), 2924 (CH-aliph), 2267 (NCO), 1778 (C=O), 1666 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.34 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.08-7.52 (m, 7H, Ar-H), 9.70 (s, 1H, NH, exchangeable with D_2O). Anal. calcd. for $C_{18}H_{14}N_4O_3S$: C, 59.01; H, 3.85; N, 15.29. Found: C, 58.85; H, 3.72; N, 15.33%.

1-(3-Ethoxyphenyl)-4-imino-3-(3-isocyanato-4-methylphen yl)-5-thioxoimidazolidin-2-one (**4d**): Color: Beige. Yield: 79 %. M.p.: 216-218 °C. IR (KBr, ν, cm^{-1}): 3346 (NH), 2978 (CH-aliph), 2271 (NCO), 1779 (C=O), 1665 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.34 (t, 3H, *J* = 6.9 Hz, CH_3), 2.35 (s, 3H, CH_3), 4.05 (q, 2H, *J* = 6.9 Hz, CH_2), 7.07-7.97 (m, 7H, Ar-H), 9.60 (s, 1H, NH, exchangeable with D_2O). Anal. calcd. for $C_{19}H_{16}N_4O_3S$: C, 59.99; H, 4.24; N, 14.73. Found: C, 60.15; H, 4.13; N, 14.59%.

2.1.4. Synthesis of 3,3'-(4-methyl-1,3-phenylene)bis(1-(aryl)-5-imino-4-thioxoimid-azolidin-2-ones (5a-d)

A mixture of cyanothioformamides **1b**-**e** (0.02 mol) in ether (20 mL), toluenediisocyanate (0.01 mol) and triethylamine (0.5 mL) were added, the reaction mixture was stirred at room temperature for 1 hr. The obtained products were crystallized from ethanol to afford *bis*-imidazolidineimino thiones **5a-d** (Scheme 2).

3,3'-(4-Methyl-1,3-phenylene)bis(4-imino-5-thioxo-1-p-tolyl imidazolidin-2-one) (**5a**): Color: Beige. Yield: 83 %. M.p.: 165-167 °C. IR (KBr, ν, cm^{-1}): 3304 (br, 2NH), 2922 (CH-aliph), 1779 (br, 2C=O), 1665 (br, 2C=NH), 1219 (br, 2C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.28 (s, 6H, $2CH_3$), 2.39 (s, 3H, CH_3), 7.08-8.47 (m, 11H, Ar-H), 9.58 (s, 1H, NH, exchangeable with D_2O), 9.59 (s, 1H, NH, D_2O exchangeable). Anal. calcd. for $C_{27}H_{22}N_6O_2S_2$: C, 61.58; H, 4.21; N, 15.96. Found: C, 61.44; H, 4.15; N, 15.77%.

3,3'-(4-Methyl-1,3-phenylene)bis(4-imino-1-(3-methoxy phenyl)-5-thioxoimidazolidin-2-one) (**5b**): Color: Gray. Yield: 85 %. M.p.: 139-141°C. IR (KBr, ν, cm^{-1}): 3340 (NH), 2923 (CH-

aliph), 1779 (br, C=O), 1664 (br, C=NH), 1223 (br, C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.36 (s, 3H, CH_3), 3.80 (s, 6H, $2OCH_3$), 7.09-7.49 (m, 11H, Ar-H), 9.61 (s, 1H, NH, exchangeable with D_2O), 9.71 (s, 1H, NH, exchangeable with D_2O). Anal. calcd. for $C_{27}H_{22}N_6O_4S_2$: C, 58.05; H, 3.97; N, 15.04. Found: C, 58.14; H, 3.88; N, 15.17%.

3,3'-(4-Methyl-1,3-phenylene)bis(4-imino-1-(4-methoxyphenyl)-5-thioxoimidazolidin-2-one) (**5c**): Color: Gray. Yield: 84 %. M.p.: 248-250 °C. IR (KBr, ν, cm^{-1}): 3232 (br, 2NH), 2927 (CH-aliph), 1778 (br, 2C=O), 1666 (br, 2C=NH), 1167 (br, 2C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.34 (s, 3H, CH_3), 3.83 (s, 6H, $2OCH_3$), 7.11-7.78 (m, 11H, Ar-H), 9.66 (s, 1H, NH, exchangeable with D_2O), 9.70 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 17.9 (CH_3), 55.9 ($2OCH_3$), 114.8 (3C), 126.4, 128.0, 129.4 (3C), 129.5 (3C), 131.3, 131.5, 132.1, 137.2, 154.1 (2C), 154.3, 154.5, 160.1 (3C), 183.5 (C=S), 183.7 (C=S). Anal. calcd. for $C_{27}H_{22}N_6O_4S_2$: C, 58.05; H, 3.97; N, 15.04. Found: C, 57.89; H, 4.04; N, 14.89%.

3,3'-(4-Methyl-1,3-phenylene)bis(1-(3-ethoxyphenyl)-4-imino-5-thioxoimidazolidin-2-one) (**5d**): Color: Gray. Yield: 80 %. M.p.: 148-150 °C. IR (KBr, ν, cm^{-1}): 3343 (br, 2NH), 2977 (CH-aliph), 1779 (br, 2C=O), 1665 (br, 2C=NH). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.36 (t, 6H, *J* = 6.9 Hz, CH_3), 2.27 (s, 3H, CH_3), 4.04-4.06 (q, 4H, *J* = 7.1 Hz, CH_2), 7.07-7.79 (m, 11H, Ar-H), 9.70 (s, 1H, NH; exchangeable with D_2O), 9.74 (s, 1H, NH; exchangeable with D_2O). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 15.0 ($2CH_3$), 17.9 (CH_3), 65.4 ($2CH_2$), 114.5 (3C), 115.9, 120.2 (3C), 126.4, 128.0, 130.4 (3C), 131.5, 132.1, 134.9, 154.0, 154.1 (2C), 154.3, 159.3 (3C), 183.3 (2C=S). Anal. calcd. for $C_{29}H_{26}N_6O_4S_2$: C, 59.37; H, 4.47; N, 14.32. Found: C, 59.23; H, 4.31; N, 14.19%.

2.2. 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 1×10^8 CFU/mL of pathological tested bacteria and 1×10^6 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 for bacteria, and 72 h at 28 °C for fungi. 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. 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.

2.2.1. Minimal inhibitory concentration (MIC) measurement

The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, ampicillin, gentamycin, amphotericin B and sulfisoxazole were prepared in DMF at concentrations 1000 µg/mL. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500-0.007 µg/mL. 10 mL of the broth containing about 10^6 CFU/mL of test bacteria or 10^4 CFU/mL of the test fungus was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37 °C for 24 h for antibacterial activity and at 25 °C for 48 h for antifungal activity in a humid chamber. At the end of the incubation period, the minimal inhibitory concentrations (MIC) values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions.

3. Results and discussion

3.1. Chemistry

N-arylcyanothioformamide derivative **1a-d** were prepared by treating arylisothiocyanate with potassium cyanide according to literature procedures [10,14]. Reaction of *N*-(4-florophenyl)cyanothioformamide derivative (**1a**) with aryl hydrazonomalononitrile derivatives in ethanol in the presence of triethylamine as a catalyst under reflux condition afforded imidazolidineiminothione derivatives (**2a-c**) containing arylazo moiety in good yields (Scheme 1). The structure of the synthesized compounds was corroborated on the basis of spectral and analytical results. Infrared spectra of these compounds revealed absorption bands corresponding to NH, C≡N, C=N and C=S functional groups. The ^{1}H NMR spectra of compounds **2a-c** exhibited two singlet signals around δ 8.60 (C=NH) and 9.60 ppm (NH imidazole ring) along with aromatic protons. ^{13}C NMR spectra of compounds **2b**,**c** displayed signals at δ 187.8-188.2 ppm due to C=S. The reaction mechanism was assumed to proceed through a nucleophilic attack of the nitrogen atom of the cyanothioformamide moiety (**1a**) to the cyano group in arylhydrazonomalononitrile to form intermediate that followed by intramolecular cyclization to yield the cyclized products **2** as illustrated in Scheme 1.

Treating an ethereal solution of various *N*-arylcyano thioformamides (**1a**,**b**,**d**) with an equimolar amount of butyl isocyanate, followed by addition of a few drops of triethylamine as catalyst afforded imidazolidineiminothione derivatives (**3a-c**) (Scheme 2). The structure of compound **3** has been assigned as a reaction product on the basis of analytical and spectral data. The IR spectra of compounds **3a-c** displayed absorption bands around 3250 cm^{-1} due to NH and 1765 cm^{-1} due to C=O function. The ^{1}H NMR spectra of compounds **3a-c** exhibited triplet, sextet, quintet and triplet signals around δ 0.91, 1.33, 1.67 and 3.68 ppm, specific for butyl protons, respectively. Also, ^{1}H NMR spectra showed another specific broad signal around δ 9.42 ppm for NH proton. ^{13}C NMR spectrum of compound **3b** displayed four signals at δ 13.5, 19.3, 20.8 and 29.0 ppm for butyl carbons, three signals at δ 153.8, 154.6 and 183.1 ppm for C=N, C=O and C=S, respectively. 5-Imino-5-thioxo-2-imidazolidinone derivatives (**3a-c**) were furnished as the sole products, indicating that the ring closing reaction proceeds *via* a single path, which involves attack via the nitrogen atom. The behavior of the cyanothioformanilides towards 2,4-diisocyana totoluene was discussed. Thus, equimolar reaction between cyanothioformanilides (**1b-e**) and 2,4-diisocyanato-toluene in ether and in the presence of triethylamine caused cyclo-addition reaction to furnish 5-thioxoimidazo-lidin-2-ones (**4a-d)**. On the other hand, *bis*-imidazolidineiminothiones (**5a-d**) were obtained via the reaction of cyanothioformanilide (**1**) with 2,4-diisocyanato-toluene (2:1 molar ratio) under the same reaction condition (Scheme 2). The structures of compounds **4** and **5** have been assigned as a reaction product on the basis of analytical and spectral data. IR spectra of compound **4** showed signal around 2270 cm^{-1} due to the presence of isocyanate group while IR spectra of compound **5** showed the complete disappearance of this signal. Moreover IR spectra of compounds **4** and **5** showed the presence of three signals around 3340, 1779 and 1665 cm^{-1} due to the presence of three groups NH, C=O and C=N, respectively.

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.

Compound	Gram positive bacteria			Gram negative bacteria			Fungi		
	S. aureus	*S. epidermidis*	*B. subtilis*	*N. gonorrhoeae*	*E. coli*	*K. pneumoniae*	*A. fumigatus*	*A. clavatus*	*G. candidum*
2a	17.8±0.48	14.9±0.23	17.2±0.48	16.9±0.41	17.5±0.43	14.6±0.53	17.6±0.35	12.7±0.43	11.7±0.43
2b	0	0	0	0	0	0	0	0	0
2c	0	0	0	0	0	0	0	0	0
3a	28.2±0.54	25.4±0.47	28.6±0.51	19.8±0.63	20.1±0.48	24.5±0.58	19.0±0.12	17.3±0.26	19.8±0.26
3b	11.5±0.43	10.3±0.23	16.5±.042	12.3±0.43	10.6±0.26	9.5±0.34	23.3±0.54	22.6±0.57	26.9±0.38
4a	20.9±0.44	19.7±0.59	21.8±0.47	17.3±0.32	18.8±0.54	16.47±0.54	16.6±0.34	15.6±0.43	13.9±0.32
4b	15.9±0.45	14.8±0.24	17.2±0.64	13.2±0.36	15.3±0.35	17.4±0.38	15.7±0.36	14.6±0.43	12.8±0.38
4c	10.2±0.42	9.3±0.50	12.2±0.63	0	0	0	9.6±0.23	9.8±0.46	11.5±0.27
5a	22.7±0.65	20.8±0.45	22.7±0.56	21.4±0.41	22.5± 0.36	21.4±0.36	18.7±0.28	20.93±0.38	24.8±0.54
5b	0	0	12.23±0.2	0	0	0	0	10.9±0.34	9.8±0.27
5c	19.9±0.64	20.8±0.32	21.7±0.37	20.9±0.45	20.9±0.36	22.1±0.53	19.7±0.28	21.8±0.27	22.7±0.39
Ampicillin	28.9±0.14	25.4±0.18	29.8±0.35	-	-	-	-	-	-
Gentamycin	-	-	-	22.3±0.58	23.4±0.3	26.3±0.15	-	-	-
Amphotericin B	-	-	-	-	-	-	23.7±0.10	21.9±0.12	25.4±0.16

The ^{1}H NMR spectra of compounds **4** and **5** displayed characteristic signals due aliphatic protons and broad signals assignable the NH. The ^{13}C NMR spectra of compounds **4** and **5** displayed characteristic signals for the carbon of CH_3, OCH_3, C=NH, C=O and C=S groups.

3.2. Antimicrobial activity

The search for newer antibacterial and antifungal agents is still in continuation; due to the rapid development of the resistance among bacteria and fungi. Imidazolidineimino thione derivatives may comprise a new class of antimicrobial agents with diminished resistance. Therefore, the aim of the present investigation is to synthesize different series of imidazolidineiminothione derivatives which bearing various substituent at *C*2, *N*1 and *N*3. Accordingly, these compounds were synthesized and tested for their expected effect(s) against selected Gram-positive, Gram-negative bacteria and fungi species.

3.2.1. 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 *Bacillus subtilis* (RCMB 010063); three Gram-negative bacteria, *Neisseria gonorrhoeae* (RCMB 010079), *Escherichia coli* (RCMB 010052) and *Klebsiella pneumoniae* (RCMB 010093) and three Fungi, *Aspergillus fumigatus* (RCMB 02564), *Aspergillus clavatus* (RCMB 02593) and *Geotrichum candidum* (RCMB 05096), and the results were summarized in Table 1. The synthesized compounds were tested for anti-microbial activity by the agar diffusion method [20] using a 1 cm microplate well diameter and a 100 µL of each concentration. Antimicrobial tests were carried out using 100 µL of tested compound solution prepared by dissolving 5 mg of the chemical compound in 1 mL of dimethyl sulfoxide (DMSO). Ampicillin, gentamycin and amphotericin B (1 mg/mL) were used as standard references for Gram positive bacteria, Gram negative bacteria and antifungal activity, respectively. Certain aspects of the structure activity relation-ships of the prepared compounds were clearly highlighted.

The results of the antimicrobial screening demonstrated the following assumptions about the structural activity relationship (SAR). Incorporating arylazo moiety as in structure **2** had a detrimental effect on antimicrobial activity. Structure **2** has at *C*2 side chain ending with arylazo moiety (Aryl: **2a**; 3,4-dichlorophenyl, **2b**; 4-methylphenyl, **2c**; 4-methoxyphenyl). The presence of 3,4-dichlorophenyl moiety (**2a**) resulted in the highest antimicrobial activity among all the compounds investigated in this structure. The presence of 3,4-dichlorophenyl moiety exhibited moderate activity against most of the tested organisms. 4-Methylphenyl (**2b**) and 4-methoxyphenyl (**2c**) showed no activity against all tested organisms. Imidazolidineiminothione derivatives (**3a-c**) contain 4-fluorophenyl (**3a**), 4-methylphenyl (**3b**), 4-methoxy phenyl (**3c**) moieties at *N*3 and butyl moiety at *N*1: Regarding the effect of the aryl substitution at *N3*, the type of the substitutions on the benzene ring of aryl moiety is important. It was noticed that the presence of 4-fluorophenyl in compound **3a** showed the maximum activities against most the test bacterial strains which showed near the reference drug. On the other hand, compound **3a** showed moderate to good activity against the tested fungi strains. Unlike compound **3a**, 4-methylphenyl (**3b**) and 4-methoxyphenyl (**3c**) moieties showed moderate activities against most the test bacterial strains and showed high activity against the tested fungi near the reference drug. Imidazolidineiminothione derivatives (**4a-d**) contains 4-methylphenyl (**4a**), 3-methoxylphenyl (**4b**), 4-methoxyphenyl (**4c**), 3-ethoxylphenyl (**4d**) moieties at *N*1 and 3-isocyanato-4-methylphenyl moiety at *N*3. Regarding the effect of the aryl substitution at *N1*, it was noticed that the presence of methylphenyl moiety (**4a**) resulted in the highest antimicrobial activity among all the compounds investigated in this structure. The presence of methylphenyl moiety exhibited moderate to good activity against most of the tested organisms while 4-methoxyphenyl (**4c**) showed weak to moderate activity against all tested organisms. *Bis*-imidazolidineimino thione derivatives (**5a-d**) contains 4-methyl-phenyl (**5a**), 3-methoxyphenyl (**5b**), 4-methoxyphenyl (**5c**), 3-ethoxylphenyl (**5b**), moieties at *N*1 and methylphenylene linker at *N*3. Regarding the effect of the aryl substitution at *N*1, It was noticed that the presence of *para* substitution at phenyl moiety resulted in the highest antimicrobial activity among all the compounds investigated in this structure. *Bis*-compounds **5a**,**c** with two imidazolidineiminothione moiety showed activity in some cases equipotent to the reference drug; especially against fungi.

3.2.1.1. MIC of the most active compounds

Minimum inhibitory concentration (MIC) of the most active synthesized compounds **3a**, **5a** and **5c** was evaluated *in vitro* using the related reference [21]. The results of minimum inhibitory concentration were depicted in Table 2. Regarding the effect of each substituent at *C*2 and those at *N*1 and *N*3 against bacterial and fungal strains, results of antimicrobial activity in this study revealed that: Compound **3a** showed about 25% less potent effect than Ampicillin against *S. epidermidis* and *B. subtilis* (MIC, 0.49 µg/mL) and showed about 50% less potent effect than Amphotericin B against *A. clavatus* and *G. Candidum.*

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

Compound	Gram positive bacteria			Gram negative bacteria			Fungi		
	S. aureus	*S. epidermidis*	*B. subtilis*	*N. gonorrhoeae*	*E. coli*	*K. pneumoniae*	*A. fumigatus*	*A. clavatus*	*G. candidum*
2a	0.98	0.49	0.49	15.63	7.81	15.63	1.95	0.98	0.24
5a	0.49	1.95	1.95	0.49	3.9	15.63	0.98	0.98	1.95
5c	1.95	1.95	0,98	1.95	0.49	1.95	0.98	7.81	1.95
Ampicillin	0.03	0.12	0.15	-	-	-	-	-	-
Gentamycin	-	-	-	0.49	0.24	0.12	-	-	-
Amphotericin B	-	-	-	-	-	-	0.12	0.49	0.12

Compound **5a** was equipotent to the Gentamycin in inhibiting the growth of *N. gonorrhoeae* (MIC, 0.49 μg/mL), and displayed 50% less activity compared to Amphotericin B against *A. clavatus.* Compound **5c** showed about 50% less potent effect than Gentamycin against *E. coli* (MIC, 0.49 μg/mL).

4. Conclusion

The objective of the present study was to synthesize and investigate the antimicrobial activities of some new imidazolidineiminothione which bearing various substituent at *N*1 and others at *N*3 beside various substituent at *C*2 with the hope of discovering new structure leads serving as potent antimicrobial agents. Our aim has been verified by the synthesis of imidazolidineiminothione, imidazolidineimino thione containing arylazo moiety and *bis*-imidazolidine-iminothione derivatives. It is worth mentioning that, imidazole dineiminothione derivative **3a** contain 4-fluorophenyl at *N*3 and butyl moiety at *N*1 showed the maximum activities against most the test bacterial strains which showed near the reference drug. *Bis*-imidazolidineiminothione **5a** was equipotent to the Gentamycin in inhibiting the growth of *N. gonorrhoeae* (MIC, 0.49 μg/mL), and displayed 50% less activity compared to Amphotericin B against *A. clavatus.* While, *bis*-imidazolidine iminothione **5c** showed about 50% less potent effect than Gentamycin against *E. coli* (MIC, 0.49 μg/mL).

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