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Sythesis Of Novel 2-Thioxo-4-Imidazolidinone Derivatives And Evaluate Their Antibacterial, And Antifungal Activities

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Abstract

The infectious diseases caused by antimicrobials pathogens are difficult, harmful, and sometimes impossible to treat. Therefore, design new drugs to treat antimicrobial infections is the biggest challenge for modern medicine. 2-Thioxo-4-imidazolidinone is used for the synthesis of a wide variety of new substituted imidazolidinone derivatives. In this study, we designing eight novel compounds derived for the 2-thioxo-4-imidazolidinones (5a-h). The preparation was in two steps via Micheal addition of phenyl hydrazide (2a), 4-methyl phenyl hydrazide 2b on N-substituted maleimides 1a-d in ethanol**,** and the second step by reaction of maleimide derivatives with cyclohexyl isothiocyanate 4 in acetonitrile. The chemical structures of the compounds were identified using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectra, as well as the melting point. The antibacterial and antifungal evaluation was carried out to target their activities. Compound N-(5-(2-((4-chlorophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl) benzamide (5b) exhibited antibacterial activity toward *Staphylococcus aureus* and *Pseudomonas aeruginosa* with equal minimum inhibitory concentration (MIC) values of 25 mg/mL. Compounds N-(3-cyclohexyl-4-oxo-5-(2-oxo-2-(phenylamino)ethyl)-2-thioxoimidazolidin-1-yl) benzamide (5a), N-(5-(2-((4-chlorophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl) benzamide (5b), N-(5-(2-((4-bromophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxo imidazolidin-1-yl) benzamide (5c), and N-(3-cyclohexyl-4-oxo-5-(2-oxo-2-(phenylamino)ethyl)-2-thioxoimidazolidin-1-yl)-4-methyl benzamide (5e) exhibited antifungal activity toward *Candida albicans*, while all compounds exhibited antifungal activity toward *Aspergillus niger* except for compound 5h, with various MIC values. In conclusion, the results demonstrate that the new compounds have to promise as antifungal agents. Moreover, compound 5b could develop as an antibacterial agent.

Keywords:Antifungal; Diffusion method; Gram positive bacteria; Gram negative bacteria; Maleimide; Thioimidazolidine.

Introduction

The risk of infectious diseases caused by bacterial and fungal is increased with the increase of some factors such as environmental pollution, patient demographics, neutropenia, transplantation modality, product type, and era of transplantation [1,2]. The type of disease depends on bacterial and fungal species, with various levels of danger for human, animals, and plants [3]. There are about 30,000 bacteria species, based on pure culture and the investigated physiology, some of them are antibiotic-resistant bacteria. The structure of bacteria cell wall is plays a role in bacteria-resistance and classification, such as gram positive has no outer lipid membrane and gram negative bacteria has outer lipid membrane [4,5]. The diversity of bacteria species causes numerous diseases, gram negative bacteria have been developing dangerous resistance and classified by the centers for diseases control and prevention (CDC) as a more serious threat, while gram positive bacteria causes tremendous and many eradication efforts [6]. For example, *staphylococcus aureus* species is gram negative bacteria that causes skin infections, pneumonia, endocarditis, osteomyelitis, and toxic reactions [7]. *Pseudomonas aeruginosa* species is gram-positive bacteria that

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causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bone and joint infections [8]. *Candida albicans* species causes skin infections such as oral thrush, nail fungus, and diaper rash [9]. *Aspergillus niger* species causes health problems such as allergic reactions and lung infections [10]. On the other hand, many types of antibiotics with different mechanisms are used to treat bacterial and fungal diseases such as amoxicillin, erythromycin, fluconazole, and caspofungin [11,12]. The antibiotic resistance increased health problems like mortality, hospital stays, and higher medical costs [13]. So, it is important to use medicinal chemistry for designing new bioactive compounds to treat various diseases with fewer side effects and high efficiency [14]. The biological activities of heterocyclic compounds are increased by a variety of functional groups alone or combined in different positions such as the para or ortho positions in a phenyl ring [15,16]. Among these bioactive compounds is thiohydantoin, which is an important class of biologically active compounds [17,18]. Thiohydantoins are sulfur analogs of hydantoins with one or two carbonyl groups that have been substituted by thiocarbonyl groups [19]. 2-Thioxoimidazolidin-4-ones are very useful synthetic intermediates and have found a myriad of applications in the area of therapeutics [20,21]. Hence, 2-thiohydantoins were used as reference standards for the development of C-terminal protein sequencing [22], reagents for dying development [23], the complexation of metal cations in textile printing, and polymerization catalysis [24,25].

The limited treatment option of pathogens leads to a variety of the drugs are efficacy levels in preventing the risk of infection diseases. Hence, some of novel antimicrobial drugs under the evaluation of clinical development to assess their ability in the current resistance problems. So, we need to develop new approaches using medicinal chemistry to design and synthesis new models of effective heterocyclic compounds [26]. 2-Thioxo-4-imidazolidinone is a well-known compound that exhibits various biological activities. In this study, we synthesized eight new compounds derived from 2-thioxo-4-imidazolidinone which involved various functional groups. The antibacterial and antifungal activities of these new compounds were examined for the first time.

Experimental

Chemistry

The melting point was determined in an open capillary tube using a Gallenkamp melting point apparatus. FT-IR spectra were recorded using Perkin-Elmer equipment. The ^{1}H and ^{13}C-NMR spectra were recorded in DMSO-d6 on a BRUCKER-500 spectrometer operating at 500 MHz and 125 MHz, respectively. All chemical shifts are reported in ppm using TMS as an internal standard. All the compounds were checked for purity by thin-layer chromatography (TLC) using a silica gel plate, and ethyl acetate : hexane (3:2) as a mobile phase. TLC spots were made visible in UV and I_2. Mass spectra were obtained on a Probe Agilent (HP) MSD5973 spectrometer operating at 70 eV.

Synthesis of compounds (5a-h): [27,28]

1. **Step** 1: A mixture of variously substituted maleimides 1a-d (0.01 mol) and phenylhydrazide 2a or 4-methylphenylhydrazide 2b (0.01 mol) in 30 ml of 98% ethanol was refluxed under magnetic stirring for 6-12 hours. The white precipitate produced was filtered and re-crystallized in ethanol to give maleimide derivatives 3a-h.

2. **Step** 2: Cyclohexyl isothiocyanate 4 (0.011 mol) with three drops of glacial acetic acid was added to a solution of compounds 3a-h (0.01 mol) in 30 ml of acetonitrile. The resulting mixture was under reflux for 16 to 70 hours. The white solid obtained after cooling was filtered and recrystallized from acetonitrile to give compounds 5a-h. After re-crystallization, the purity of the compounds were confirmed by measuring the melting point and TLC.

3. Structure analysis data of the new compounds:

N-(3-cyclohexyl-4-oxo-5-(2-oxo-2-(phenylamino) ethyl)-2-thioxoimidazolidin-1-yl) benzamide (5a):

White solid powder; yield 50 %, mp.= 200-202 $^{\circ}C$; IR (KBr, cm^{-1}): 3329 (NH *amide*), 3267 (Ph-NH), 3055 (CH-*arom.*), 2927, 2858 (CH-*aliph.*),1747 (C=O *asym.*),1666 (C=O *sym.*),1600 (C=O *amide*),1552, 1500 (C=C *arom.*), 1444 (C=S), 1406 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.36 (s, 1H, H_d), 10.08 (s, 1H, H_e), 7.92 (d, *J* = 8.4 Hz, 2H, H-*Ar*), 7.62 – 7.49 (m, 5H, H-*Ar*), 7.29 (t, *J* = 7.9 Hz, 2H, Ar-H), 7.03 (t, *J* = 7.4 Hz, 1H, H-*Ar*), 4.64 (t, *J* = 4.1 Hz, 1H, H_c), 4.50 (t, *J* = 12.2 Hz, 1H, H_f), 3.03-2.97 (m, 2H, H_a, H_b), 2.22 (dq, *J* = 27.1, 14.5, 14.1 Hz, 2H, H-*cyclohexyl*), 1.84–1.63 (m, 5H, H-*cyclohexyl*), 1.29–1.14 (m, 3H, H-*cyclohexyl*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 184.66 (C=S), 172.05 (PhNHC=O), 166.41 (C=O *thioimidazole*), 165.57 (NNHC=O), 138.95, 132.41, 131.53, 128.67, 128.51, 127.70, 123.19, 119.01 (C-*Ar*), 59.13 (CH_c), 55.62 (CH_f), 34.79 (CH_aH_b), 28.60, 27.87, 25.54, 24.93 (C-*cyclohexyl*); MS *m/z* (%): 450 (M^+, 45), 330 (15), 188 (20), 105 (100), 77 (42), 41 (12).

N-(5-(2-((4-chlorophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl) benzamide (5b):

White solid powder; yield 89 %, mp.=264-265 °C; IR (KBr, cm^{-1}): 3315 (N-H *amide*), 3203 (PhNH), 3050 (CH *arom.*), 2939, 2856 (CH-*aliph.*), 1749 (C=O *asym.*) ,1660 (C=O *sym.*), 1610 (C=O *amide*),1552, 1480 (C=C *arom.*), 1429 (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (s, 1H, H_d), 10.21 (s, 1H, H_e), 7.89-7.87 (m, 2H, H-*Ar*), 7.62–7.48 (m, 5H, H-*Ar*), 7.35–7.33 (m, 2H, H-*Ar*), 4.61 (t, *J* = 4.2 Hz, 1H, H_c), 4.54–4.44 (m, 1H, H_f), 3.01–2.98 (m, 2H, H_a, H_b), 2.19 (dq, *J* = 25.2, 12.6 Hz, 2H, H-*cyclohexyl*), 1.84–1.63 (m, 5H, H-*cyclohexyl*), 1.28–1.13 (m, 3H, H-*cyclohexyl*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 185.09 (C=S), 172.44 (PhNHC=O), 167.07 (C=O *thioimidazole*), 166.01 (NNHC=O), 138.34, 132.89, 131.96, 129.08, 129.00, 128.15, 127.21, 121.01 (C-*Ar*), 59.53 (CH_c), 56.09 (CH_f), 35.24 (CH_aH_b), 29.05, 28.32, 26.05, 25.91, 25.38 (C-*cyclohexyl*); MS *m/z* (%): 485 (M^+, 6), 368 (40), 236 (30), 111 (25), 105 (100), 83 (52), 43 (62).

N-(5-(2-((4-bromophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl) benzamide (5c):

White solid powder; yield 53 %, mp.= 279-280 °C; IR (KBr, cm^{-1}): 3315 (N-H *amide*), 3257 (NH), 3035 (CH-*arom.*), 2937, 2856 (CH-*aliph.*), 1749 (C=O *asym.*), 1662 (C=O (*sym.*),1608 (C=O *amide*), 1546, 1487 (C=C *arom.*),1429 (C=S), 1359 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.32 (s, 1H), 10.21 (s, 1H), 7.89 (d, *J* = 7.7 Hz, 2H, H-*Ar*), 7.62 – 7.46 (m, 7H, H-*Ar*), 4.62 (t, *J* = 4.3 Hz, 1H, Hc), 4.48 (ddt, *J* = 12.7, 9.1, 4.1 Hz, 1H, H_f), 3.06-2.98 (m, 2H, H_a, H_b), 2.26 – 2.14 (m, 2H, H-*cyclohexyl*), 1.84 – 1.63 (m, 5H, H-*cyclohexyl*), 1.32 – 1.13(m, 3H, H-*cyclohexyl*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 185.10 (C=S), 172.43 (PhNHC=O), 167.10 (C=O *thioimidazole*), 165.99 (NNHC=O), 138.75, 132.89, 131.99, 131.96, 129.00, 128.15, 121.40, 115.23 (C-*Ar*), 59.52 (CH_c), 56.09 (CH_f), 35.26 (CH_aH_b) , 29.05, 28.32, 26.05, 25.91, 25.38 (C-*cyclohexyl*); MS *m/z* (%): 530 (M^+, 15%, Br^{81}), 408 (6), 210 (13), 171 (10), 105 (100), 77 (60), 41 (21).

N-(3-cyclohexyl-4-oxo-5-(2-oxo-2-(p-Tolylamino) ethyl)-2-thioxoimidazolidin-1-yl)benzamide (5d):

White solid powder; yield 84 %, mp.= 238-239 °C; IR (KBr, cm^{-1}): 3329 (NH amide), 3277 (NH), 3050 (CH *arom.*), 2939, 2858 (CH-*aliph.*), 1743 (*asym.* C=O), 1660 (*sym.* C=O), 1608 (C=O *amide*),1546, 1512 (C=C *arom.*), 1404 (C=S), 1357 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 1H, H_d), 9.98 (s, 1H, H_e), 7.93–7.09 (m, 9H, H-*Ar*), 4.61 (t, *J* = 4.1 Hz, 1H, H_c), 4.53–4.45 (m, 1H, H_f), 3.07 – 2.91 (m, 2H, H_a, H_b), 2.23 (s, 3H, CH_3), 2.19-2.14 (m, 2H, H-*cyclohexyl*), 1.86 – 1.64 (m, 5H, H-*cyclohexyl*), 1.34 – 1.11 (m, 3H, H-*cyclohexyl*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 185.13 (C=S), 172.53 (PhNHC=O), 166.59 (C=O *thioimidazole*), 166.00 (NNHC=O), 136.91, 132.88, 132.53, 131.99, 129.51, 128.99, 128.16, 119.49 (C-*Ar*), 59.59 (CH_c), 56.07 (CH_f), 35.19 (CH_aH_b), 29.06, 28.33, 26.05, 25.92, 25.39 (C-*cyclohexyl*), 20.90 (CH_3); MS (m/z): MS *mlz* (%): 464 (M^+, 18), 344 (10), 236 (8), 210 (8), 129 (9), 105 (100), 91 (10), 77 (75), 55 (23), 41 (14).

N-(3-cyclohexyl-4-oxo-5-(2-oxo-2-(phenylamino) ethyl)-2-thioxoimidazolidin-1-yl)-4-methyl benzamide (5e):

White solid powder; yield 52 %, mp.= 193-195 °C; IR (KBr, cm^{-1}): 3304, 3140 (NH), 3041 (CH *arom.*), 2933, 2856 (CH-*aliph.*),1751(C=O *asym.*), 1712 (C=O *sym.*),1676 (C=O *amide*),1600, 1517, 1498 (C=C),1444 (C=S),1359 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.25 (s, 1H, H_d), 10.06 (s, 1H, H_e), 7.80 (d, *J* = 8.0 Hz, 2H, H-*Ar*), 7.53 (d, *J* = 8.4 Hz, 2H, H-*Ar*), 7.31–7.01 (m, 5H, H-Ar), 4.60 (t, *J* = 4.1 Hz, 1H, H_c), 4.48 (m, 1H, H_f), 3.00 (d, *J* = 3.9 Hz, 2H, H_a, H_b), 2.37 (s, 3H, CH_3), 2.26-2.18 (m, 2H, H-*cyclohexyl*), 1.84 –1.63 (m, 5H, H-*cyclohexyl*), 1.3–1.13 (m, 3H, H-*cyclohexyl*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 185.14 (C=S), 172.54 (PhNHC=O), 166.85 (C=O *thioimidazole*), 165.93 (NNHC=O), 143.04 , 139.39, 129.50, 129.15, 128.18, 123.66, 119.46 (C-*Ar*), 59.59 (CH_c), 56.06 (CH_f), 35.21 (CH_aH_b), 29.04, 28.31, 26.05, 25.90, 25.38 (C-*cyclohexyl*), 21.53 (CH_3); MS *m/z* (%): 465 ([M + H]$^+$, 25), 330 (7), 236 (5), 210 (10), 119 (100), 91 (20), 77 (4), 41 (5).

N-(5-(2-((4-chlorophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl)-4-methyl benzamide (5f):

White solid powder; yield 42 %, mp.= 251-253 °C, IR (KBr, cm^{-1}): 3305, 3120 (NH), 3050 (CH *arom.*), 2927, 2858 (CH-*aliph.*),1718 (C=O *asym.*),1678 (C=O *sym.*),1602 (C=O *amide*),1533, 1490 (C=C),1452 C=S),1400 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.22 (s, 1H, H_d), 10.21 (s, 1H, H_e), 7.80-7.29 (m, 8H, H-*Ar*), 4.59 (t, *J* = 4.1 Hz, 1H, H_c), 4.50-4.45 (m, 1H, H_f), 3.04-2.96 (m, 2H, H_a,H_b), 2.36 (s, 3H, CH_3), 2.23-2.15 (m, 2H, H-*cyclohexyl*), 1.83-1.63 (m, 5H, H-*cyclohexyl*), 1.28-1.13 (m, 3H, H-*cyclohexyl*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 185.12 (C=S),172.46 (PhNHC=O), 167.06 (C=O *thioimidazole*), 165.90 (NNHC=O), 143.05, 129.50, 129.14, 129.07, 128.17, 127.21, 121.00 (C-*Ar*), 59.55 (CH_c), 56.07 (CH_f), 35.22 (CH_aH_b), 29.04, 28.31, 26.05, 25.90, 25.38 (C-*cyclohexyl*), 21.53 (CH_3); MS *m/z* (%): 499 (M^+, 8), 368 (18), 202 (27), 160 (45), 119 (100), 91 (70), 43 (13).

N-(5-(2-((4-bromophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl)-4-methylbenzamide (5g):

White solid powder; yield 46 %, mp.= 255-256 °C; IR (KBr, cm^{-1}): 3305, 3115 (NH), 2924, 2856 (CH-*aliph.*),1718 (C=O *asym.*),1678 (C=O *sym.*),1598

C=O *amide*), 1531, 1489 (C=C *arom.*), 1450 (C=S), 1396 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.23 (s, 1H, H_d), 10.21 (s, 1H, H_e), 7.80 (dd, *J* = 8.3, 2.6 Hz, 2H, H-*Ar*), 7.58 – 7.44 (m, 4H, H-*Ar*), 7.38–7.26 (m, 2H, H-*Ar*), 4.60 (t, *J* = 4.3 Hz, 1H, H_c), 4.53–4.41 (m, 1H, H_f), 3.04–2.96 (m, 2H, H_a, H_b), 2.37 (s, 3H, CH_3), 2.26-2.16 (m, 2H, H-*cyclohexyl*), 1.84–1.63 (m, 5H, H-*cyclohexyl*), 1.28–1.13 (m, 3H, H-*cyclohexyl*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 185.13 (C=S), 172.45 (PhNHC=O), 167.09 (C=O *thioimidazole*), 165.87 (NNHC=O), 143.03, 138.75, 131.99, 129.50, 129.16, 128.17, 121.38, 115.21 (C-*Ar*), 59.54 (CH_c), 56.06 (CH_f), 35.25 (CH_aH_b), 29.05, 28.31, 26.05, 25.91, 25.38 (C-*cyclohexyl*), 21.54 (CH_3); M *m/z* (%): 544 (M^+, 32), 408 (10), 236 (18), 210 (37), 119 (100), 91 (100), 55 (100), 41 (65).

N-(3-cyclohexyl-4-oxo-5-(2-oxo-2-(p-tolylamino) ethyl)-2-thioxoimidazolidin-1-yl)-4-methyl benzamide (5h):

4. White solid powder; yield 81 %, mp.= 225-227 °C; IR (KBr, cm^{-1}): 3305 (NH), 3043 (CH *arom.*), 2939, 2860 (CH-*aliph.*),1720 (C=O *asym.*),1693 C=O *sym.*),1610 (C=O *amide*),1531, 1494 (C=C),1458 (C=S); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.24 (s, 1H, H_d), 9.96 (s, 1H, H_e), 7.80 (d, *J* = 8.2 Hz, 2H, H-*Ar*), 7.40 (d, *J* = 8.4 Hz, 2H, H-*Ar*), 7.31 (d, *J* = 8.3 Hz, 2H, H-*Ar*), 7.08 (d, *J* = 8.4 Hz, 2H, H-*Ar*), 4.58 (t, *J* = 4.2 Hz, 1H, H_c), 4.49-4.44 (m, 1H, H_f), 2.97-2.96 (m, 2H, H_a, H_b), 2.36 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.20-2.15 (m, 2H, H-*cyclohexyl*), 1.83-1.62 (m, 5H, H-*cyclohexyl*), 1.31-1.13 (m, 3H, H-*cyclohexyl*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 185 (C=S), 172.55 (PhNHC=O), 166.58 (C=O *thioimidazole*), 165.91 (NNHC=O), 143.02, 136.90, 132.54, 129.51, 129.49, 128.18, 119.48 (C-*Ar*), 59.61 (CH_c) , 56.05 (CH_f), 35.15 (CH_aH_b), 29.05, 28.31, 26.05, 25.91, 25.38 (C-*cyclohexyl*), 21.53 (CH_3), 20.89 (CH_3); MS *m/z* (%): 479 ($[M + H]^+$, 26), 341 (20), 119 (100), 91 (30), 45 (35).

Antibacterial activity

The diffusion method was used to determine the minimum inhibitory concentration (MIC) of the new compounds toward three bacteria pathogens, Gram–positive and Gram-negative (*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa (*ATCC 9027)*)*. Briefly, four concentrations (25, 50, 75, and 100) mg/ml of the compounds were added to each Petri dish (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). The Petri dishes were incubated for 24 h at 36 ± 1 ºC, under aerobic conditions. After incubation, confluent bacterial growth was observed. Zones of inhibition were measured in millimeters (mm) to identify MIC values [29]. The positive antibiotics used for reference were spiromycin and cefotaxime.

Antifungal activities

The diffusion method on Potato Dextrose Agar (PDA) growth medium was used to determine MICs of the compounds against two fungal species, *Candida albicans and Aspergillus niger*. Briefly, standardized the fungal suspension to 10^6 conidia/mL in sterile saline solution (0.85%) and 100 µl of each fungal suspension was spread onto the surface of the Petri dishes. After a 10 minutes wait period, 6-mm-diameter holes were punched and filled with four concentrations (25, 50, 75, and 100) mg/ml. As control compounds for each experiment, dimethyl sulfoxide (DMSO) as a hydroethanolic solution was used at 70% and the fungicide tebuconazole (TEB) (Folicur 20EC) was used at 0.1%. The Petri dishes were incubated at 28 ± 2 °C. After 72 h, they measured the diameter of fungal mycelial growth inhibition (clear zones of inhibition formed around the holes were considered indicative of antifungal activity) [29]. The positive antibiotic used for reference was fluconazole. The experiments were in triplicates, and measure the zones of inhibition in mm. The activity index of antibacterial and antifungal potent compounds have been estimated using standard drugs following the equation bellow [30]:

5. Activity index (AI)= Zones of inhibition $_{(sample)}$ / Zones of inhibition $_{(standard)}$

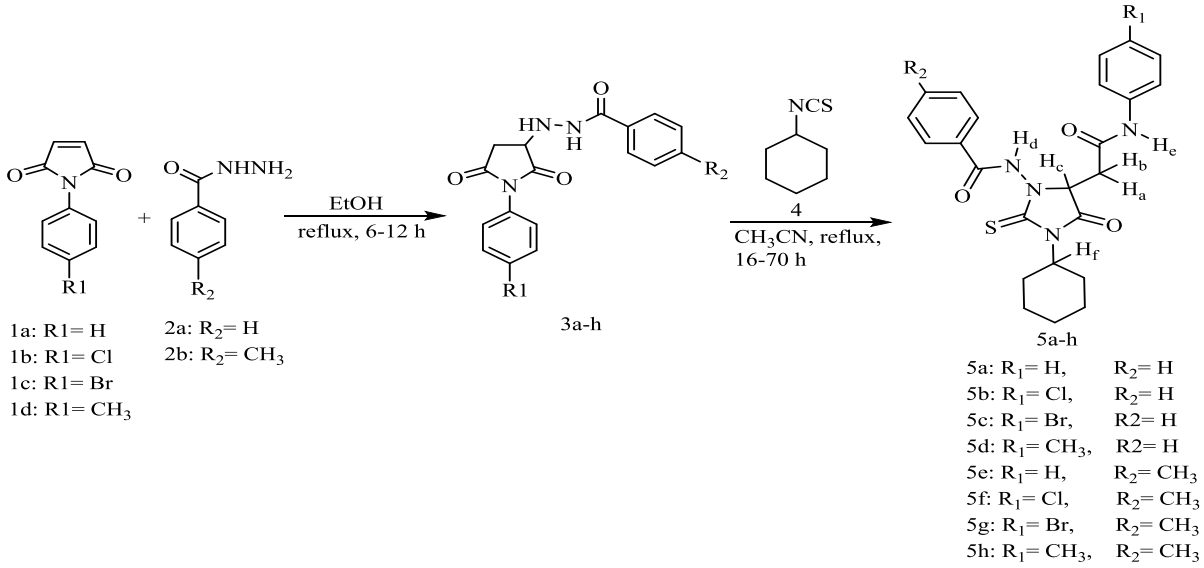
Results and discussion

Synthesis

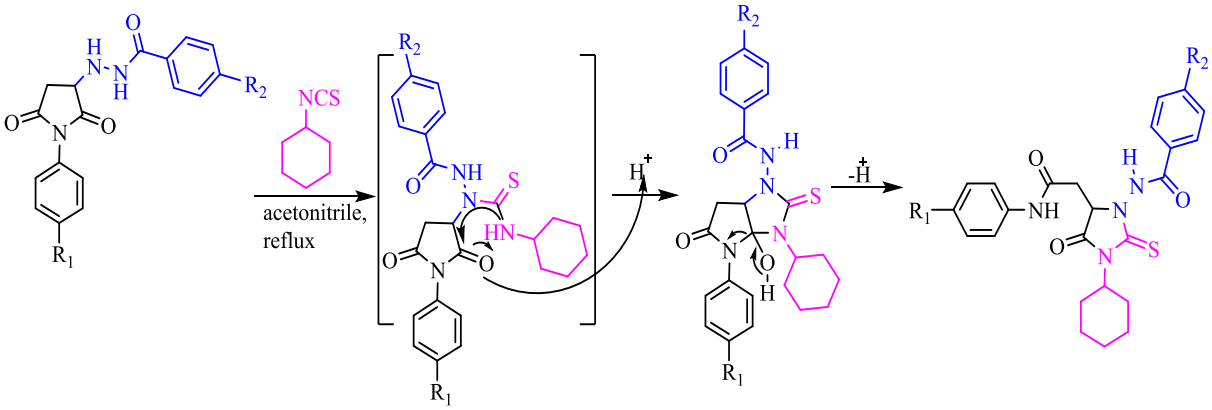
This study involved the preparation of eight new 2-thioxo-imidazolidinones (**5a-h**) with various functional groups, represented in scheme (1).

The compounds were synthesized via two steps: The first step could be easily obtained by Micheal's addition of phenyl hydrazide (2a), 4-methyl phenyl hydrazide 2b on N-substituted maleimides 1a-d in ethanol, followed by heating for 6 to 12 hours led to maleimide derivatives 3a-h. The second step is the reaction of maleimide derivatives with cyclohexyl isothiocyanate 4 in acetonitrile under reflux for a period of 16 to 70 hours, allowing the complete conversion of the starting material to the products (5a-h) with good yield. The mechanism of the reaction between compounds 3a-h and cyclohexyl isothiocyanate 4 was carried out in the presence of glacial acetic acid, scheme (2). The results indicated that the cyclization reaction times of compounds 5a-h were longer (16-70 hours) than those of derivatives 3a-h (6-12 hours). The structures of synthesized compounds 5a-h were elucidated by ^{1}H-NMR,^{13}C-NMR, FTIR, and mass spectra data, and all spectral

data were in accordance with assumed structures. The ^{1}H-NMR spectra of compounds 5a-h showed characteristic patterns of an ABX system corresponding to a $COCH_2CH$ fragment, with the presence of two signals as a triplet at 4.64-4.58 ppm for H_c showed coupling with H_a and H_b. Another signal appeared multiplet at 3.06-2.96 ppm corresponding to protons H_a and H_b because they attached to the carbon adjacent to the chiral center. The ^{1}H-NMR spectrum of compounds 5a-h showed two singlets 11.36-11.22 ppm and 10.21-9.96 ppm, which were attributed to H_d and H_e protons, respectively. The two signals showed a doublet in the region of 7.92-7.80 ppm and a multiplet at 7.80-7.01 ppm belonging to the aromatic ring protons (H_k, H_l, H_m, H_n). Signals of a multiplet were observed 4.50-4.41 ppm for H_f, while protons of the cyclohexyl group (H_g, H_i, H_j) showed a multiplet signal in the region of 2.26-1.13 ppm. The presence of all carbon atoms for compounds 5a-h was confirmed by ^{13}C-NMR spectra. Signals appearing in carbon's aliphatic range 59.61-20.90 ppm were observed. The thiocarbonyl carbons and the carbonyl carbons appeared in the regions of 185.14-185 and 172.55-165.57 ppm, respectively. The FT-IR spectra of 2-thioxo-4-imidazolidinones 5a-h show characteristic absorptions 3277-3115, 1751-1598, and 1458-1404 cm^{-1}, which correspond to the presence of (NH amide, NHPh), carbonyl (C=O), and thiocarbonyl (C=S) groups, respectively. The mass spectra of 5a-h showed a molecular ion (m/z): 450 $[M]^+$, 485 $[M]^+$, 530 $[M]^+$, 464 $[M]^+$, 465 $[M + H]^+$, 499 $[M]^+$, 544 $[M]^+$, and 479 $[M + H]^+$, respectively. The mass spectra confirmed the structure of new compounds.



Scheme 1: The general synthesis of compounds (5a-h)



Scheme 2: Synthetic mechanistic of compounds (5a-h)

Antimicrobial activities

The natural and synthetic heterocyclic compounds have significant biological properties, which increase their medicinal importance [31-33]. Hence, low and high doses (treatment dose and treatment duration) play a role in resistant management and pharmacogentic factors. The intense of resistance can imposed by high doses, while low doses slow the ability of resistance, and reducing doses might increase the mutation. Thus, low doses acquire resistance infection, and high doses equalize the risk of the genotype [34,35]. The present study involved the evaluation of the antibacterial activities of eight new compounds (**5a-h**) against *S. aureus*, *E. coli*, and *P. aeuroginosa*. All compounds were inactive against *E. coli*. Among these compounds, only compound 5b showed zones of inhibition (12 mm) with MIC values equal to 25 mg/ml for both pathogens *S. aureus* and *P. aeuroginosa*. The activity index (AI) was estimated for compound **5b** and was 0.52, compared with the used positive control spiromycin and cefotaxime (zones of inhibition 23 mm) toward both pathogens (*S. aureus* and *P. aeuroginosa)*. AI value is less than 1.0, which means compound **5b** has a moderate effect on both pathogens, Table 1. Compound **5b** has R_1=Cl and R_2= H groups, and the chlorine group in the para position might affect both bacteria. Two methyl groups (an electron-donating group) were present in compound 5h, which was inactive toward the pathogens [36]. The antibacterial activity of compound **5b** may be due to the hydrophilic properties of this compound, which might enhance its ability to break through the cell walls of both pathogens [37].

The antifungal activity of these compounds against *C. albicans* and *A. niger* has also been studied. The compounds **5a, 5b, 5c**, and **5e** showed zones of inhibition (20, 20, 20, and 15 mm, respectively) toward *C. albicans*, the activity index values (1.0, 1.0, 1.0, and 0.75, respectively), while compounds 5a-g showed zones of inhibition (15, 15, 15, 15,12, 13, and 13 mm, respectively) toward *A. niger* species with various MICs, the activity index values (0.83, 0.83, 0.83, 0.83, 0.62, 0.72, and 0.72, respectively), Table 2. The values of activity index is between 0.5-1, which means moderate effect of potent compounds toward *C. albicans* and *A. niger*. No activity of compounds **5d, 5f,** and **5g** toward *C. albicans*, and compound 5h was inactive toward *C. albicans* and *A. niger* [38, 39]. These results might be due to the methyl group's function in enhancing the Cl, Br, and H groups' activities toward *C. albicans*. Compound 5h has two electron-donating groups (CH_3), which reduce the function of the compound compared with other compounds that have one electron-withdrawing group and one electron-donate group. Compound 5h did not show any activity against all bacterial and fungal species because of the bulky pulled group in its aromatic hydrazide part.

Table 1: **Antibacterial activity of the compounds (5a-h), estimated the zones of inhibition in millimeter and MIC. The activity index (AI) was estimated for the potent compounds. The compounds were negative to *E. coli.***

	Gram positive (S. aureus)			***Gram negative (P. aeruginosa)***		
Compound symbol	**MIC**	**ZI**	**AI**	**MIC**	***ZI***	***AI***
5a	-	NA	-	-	NA	-
5b	25	12	0.52	25	12	0.52
5c	-	NA	NA	-	NA	-
5d	-	NA	NA	-	NA	-
5e	-	NA	NA	-	NA	-
5f	-	NA	NA	--	NA	--
5g	-	NA	NA	-	NA	-
5h	-	NA	NA	-	NA	-
***Spiromycin**	-	23	-	-	No tested	-
***Cefotaxime**	-	No tested	No tested	-	23	-

MIC: Minimum inhibition concentration in mg/ml; **ZI**: Zones of inhibition in millimetre, **AI:** Activity index**;** **NA**: No activity**;** * : Standard drug

Table 2: Antifungal activity of compounds (5a-h) against *C. albican* and *A. niger*, estimated the zones of inhibition, MIC, and AI for the active compounds.

	Candida albican			*Aspergillus niger*		
Compound symbol	***MIC***	**ZI**	**AI**	***MIC***	**ZI**	**AI**
5a	75	20	1	75	15	0.83
5b	25	20	1	25	15	0.83
5c	75	20	1	75	15	0.83
5d	-	NA	NA	75	15	0.83
5e	100	15	**0.75**	100	12	0.66
5f	-	NA	NA	100	13	0.72
5g	-	NA	NA	100	13	0.72
5h	-	NA	NA	-	NA	-
***Fluconazole**	-	20	-	-	18	-

MIC: Minimum inhibition concentration in mg/mL; **ZI**: Zones of inhibition in millimeter;
AI: Activity index**;** **NA**: No activity**;** * : Standard drug

Conclusion

The aim of this work is to prepare a series of 2-thioxo-4-imidazolidinone derivatives which involved various substituent at R_1 and R_2. A comparison was made between the substituent groups to target their structure relationship and activity. It is a first study of these compounds (**5a-h**) to evaluate their antibacterial and antifungal activities. Only compound N-(5-(2-((4-chlorophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl) benzamide (5b) exhibited moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Compound N-(3-cyclohexyl-4-oxo-5-(2-oxo-2-(p-tolylamino)ethyl)-2-thioxoimi dazolidin-1-yl)-4-methyl benzamide (**5h**) was significantly inactive as an antibacterial and antifungal agent Compounds **5a-g** exhibited significant antifungal activities toward *C. albican*, while significant effect of compounds **5a, 5b, 5c**, toward *A. niger*. Furthermore, the activity index value was less than or equal to 1, which means a significant effect for the potent compounds.

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