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Original Research Article

Nematicidal, Larvicidal and Antimicrobial Activities of Some New Mannich Base Imidazole Derivatives

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Abstract

Purpose: *To synthesize Mannich base imidazole derivatives, and evaluate their antimicrobial, nematicidal and larvicidal properties .*
Methods: *Compounds 1a-g and 2a-g were prepared using a Mannich condensation method. The chemical structures of compounds 2a-g were confirmed by Fourier transform infrared spectroscopy (IR), proton nuclear magnetic resonance (^{1}H-NMR), carbon nuclear magnetic resonance (^{13}C-NMR), and mass spectrometry (MS) and elemental analyses. Compounds 1a-f and 2a-f were screened for antimicrobial properties using an agar diffusion method. The nematicidal activity of the compounds was evaluated against juvenile Meloidogyne javanica as test organism while larvicidal activity was assessed against the urban mosquito, Culex. Quinquefasciatus, using a standard bioassay protocol.*
Results: *Compounds 1b, 1g, 2e and 2g were highly active against a few bacterial organisms compared with the reference antibacterial, ciprofloxacin while the antifungal activity of compound 2d was high compared with the reference, clotrimazole. Compounds 1c, 1e, 1g, and 2e showed high toxicity levels of larvicidal activity based their half maximal lethal dose (LD_{50}) values. Compounds 1d, 1e, 1f, 1g, 2d and 2e were highly toxic to nematodes.*
Conclusion: *Compounds 1b, 1g, 2e and 2g may be useful as lead molecules for the development of new classes of larvicidal, nematicidal and antimicrobial agents.*

Keywords: *Imidazole, Thiosemicarbazide, Semicarbazide, Condensation, Antimicrobial, Nematicidal, Larvicidal, Structure-activity relationship.*

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INTRODUCTION

Culex is an important genus of mosquito that acts as a vector for several serious diseases, including filariasis, west Nile virus, dangue fever, yellow fever, chikungunya and other diseases caused by encephalitides. Nematodes are tiny worms, some of which are plant parasites. It is thought that nematode infections play an important role in the predisposition of a host plant to invasion by secondary pathogens. Edible plants attacked by nematodes show retarded growth and development, resulting in loss of quality and quantity of the harvest.

Currently employed nematicides are slated for tighter regulations and less use due to environmental problems and human and animal health concerns. The optimum methods of

controlling mosquito larvae and nematodes involve the use of insecticides such as various organophosphates, and natural and synthetic heterocyclic products. New environmentally safe and biodegradable insecticides that specifically target mosquitoes and nematodes are urgently needed.

Naturally occurring and synthetic imidazole derivatives are an important class of heterocycles that are known to exhibit various biological activities [1]. The imidazole nucleus is a major component in a variety of drugs, including angiotensin II receptor antagonists, anti-inflammatory agents, protein kinase inhibitors, and fungicides [2]. Imidazole also plays important roles in biochemical processes [3]. Many substituted imidazoles are used as fungicides and herbicides, plant growth regulators and therapeutic agents [4]. Imidazole is common compounds of a large number of biologically and medicinally significant substances [5,6], including anticonvulsant [7] and monoamine oxidase (MAO) inhibitors [8].

The Mannich reaction is commonly employed to develop agricultural chemicals such as plant growth regulators [9] and is an important tool in the synthesis and modification of biologically active compounds [10]. It provides a convenient access to many useful synthetic building blocks because amino groups can be easily converted into a variety of other functionalities [11]. Mannich bases often exhibit significant biological properties including antimicrobial [12], cytotoxic [13] and anticancer [14].

The present investigation focuses on a series of imidazole compound in a single molecular framework and examines their larvicidal, nematicidal, antibacterial and antifungal activities.

EXPERIMENTAL

Materials

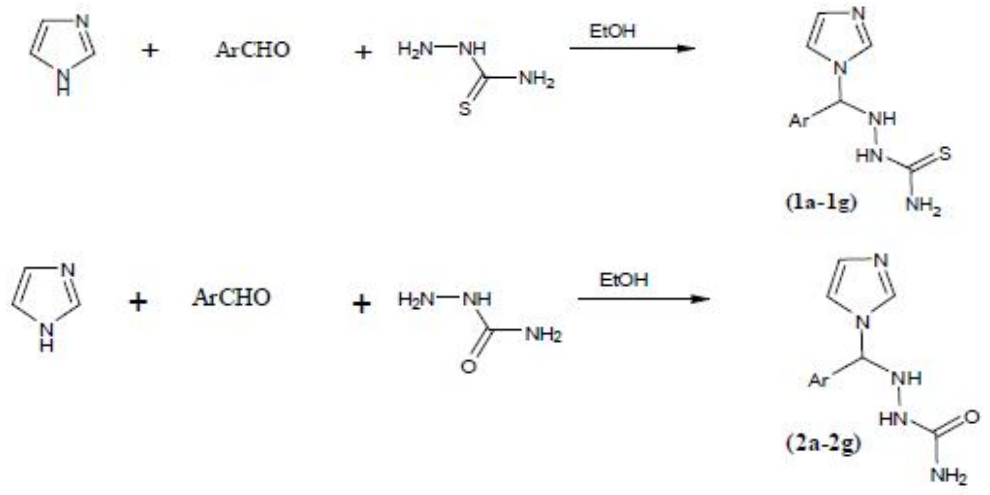
All the melting points were recorded in open capillary tubes and are uncorrected. IR spectra were recorded in KBr on a FT–IR Shimadzu 8201pc and ^{1}H NMR spectra were recorded on a Brucker DRX-300 MHZ. Elemental analysis (C, H and N) were undertaken using an Elementer analyzer model vario EL III. The purity of the compounds was checked by thin layer chromatography (TLC) with silica gel plates.

General procedure for the synthesis of 2-[1*H*-imidazol-1-yl (phenyl) methyl] hydrazinecarbo thioamide (1a)

Imidazole (0.1 mol), thiosemicarbazide hydrochloride (0.1 mol), and benzaldehyde (0.1 mol) were added in ethanol solvent (20 mL). The reaction mixture was refluxed 5h with temperature 60 ^{o}C. Then the mixture was poured over crushed ice. The precipitate was obtained in few min. then the precipitate was collected by filtration. The precipitate was dried and recrystallized from suitable alcohols. The above procedure was followed by all the remaining compounds **1a-1g**.

2-[1*H*-imidazol-1-yl (phenyl) methyl] hydrazinecarboxamide (2a)

Imidazole, (0.1 mol), semicarbazide hydro-chloride (0.1 mol) and benzaldehyde (0.1 mol) were added in ethanol solvent (20 mL). The reaction mixture was refluxed 5 h with 60 ^{o}C. Then the mixture was poured over crushed ice. The precipitate was obtained in few min. then the precipitate was collected by filtration.



Scheme 1: Synthetic route of compounds **1a-1g** and **2a-2g**

The precipitate was dried and recrystallized from suitable alcohols. The above procedure was followed by all the remaining compounds **2a-2g**.

In vitro antibacterial screening

Compounds **1a-1g** and **2a-2g** were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *Enterococcus feacalis, Pseudomonas aeruginosa* (ATCC-27853), *Klebsiella pneumoniae* (recultured) by agar diffusion method [15,16] was performed using Mueller–Hinton agar (Hi-Media) medium.

Each compound was tested at a concentration of 100 µg/mL in DMSO. Ciprofloxacin was used as the standard. The zone of inhibition was measured after 24 h incubation at 37 ºC (Table 2). The minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited growth on agar plates.

In vitro antifungal screening

Compounds **1a-1g** and **2a-2g** were evaluated for their *in vitro* antifungal activity *Aspergillus niger, Candia albicans, Aspergillus fumigatus, Cryptococcus neoformans* and *Microsporum audouinii* (recultured) using an agar diffusion method [17,18] with Sabouraud's dextrose agar (Hi-Media). Each compound was tested at a concentration of 100 µg/mL in DMSO. Clotrimazole was used as the standard. The zone of inhibition was measured incubated at 37 °C for 24 h and MICs were determined.

Evaluation of larvicidal activity

The assessment of larvicidal activity of synthesized test compounds **1a-1g** and **2a-2g** was tested against the urban mosquitoes *Culex. Quinquefasciatus* using standard bioassay protocol [19]. Egg rafts of mosquito were obtained from a drainage system. The eggs were reared under standard insectary conditions at ambient temperature (29 ± 3 °C) and relative humidity (80 ± 5 %), 12:12 light: dark photoperiod and fed with ground shrimp feed daily.

Larval development was monitored for 7 days. The second and third stage larvae were collected at the tip of a pasture pipette and placed in cotton bud to remove excess water and transferred gently to the test vial (10/vial) by tapping. The larval mortality was observed using various concentrations of synthesized compounds (10, 20, 30, and 40 µg/mL).

Assessment of nematicidal activity

For the determination of nematicidal activity, juveniles of *Meloidogyne javanica* were used as test organism [19]. Assay system was prepared with 2 ml Milli Q water containing different concentrations (10, 20, 30 and 40 µg/mL) of synthesized test compounds 1-6 in glass tubes.

Ten juveniles of *M. javanica* were transferred in test, positive (with 2 % methanol) and negative (without vehicle) control tubes. Mortality was observed under a zoom stereomicroscope after 24 h of exposure.

Statistical analysis

The mean of the results was calculated based on at least 3 independent evaluations and the standard deviations (SD) were also calculated using Microsoft Excel. All LD_{50} values were calculated from the corresponding sigmoidal dose–response curve according to best fit shapes based on at least five reaction points using the Microsoft Office Excel 2007 software (Microsoft, Redmond, WA, USA).

RESULTS

Chemistry

2-[1*H*-Imidazol-1-yl (phenyl) methyl] hydrazinecarbothioamide (1a)

IR(KBr,cm^{-1}): 3292.26(NH_2), 2916.17(NH), 1436.87(C=S), 811.98(Ar-H); 460(CH) ^{1}HNMR (DMSO-d_6, 300MHz): 9.53(s, 2H, NH_2), 7.83(s, 1H, CH), 7.26-7.65(m, 5H, Phenyl), 6.77(s, 1H, Imidazole-CH), 6.84(s, 1H, CH), 2.2(1H, NH), 2.0(s, 1H, NH) ^{13}C NMR (DMSO-d_6, 300MHz); 182.7 (C=S), 128.7, 126.6, 128.1, 132.5 (Pheny), 128.6 (Imidazole ring CH=CH), 137.8 (HC=N), 75.3 (CH). EI-MS, m/z (Relative intensity %): [247.28^+, 42 %].

2-((4-Chlorophenyl) (1*H*-imidazol-1-yl) methyl) hydrazinecarbothioamide (1b)

IR(KBr,cm^{-1}): 3291.44(NH_2), 2912.34(NH), 1440.88(C=S), 837(C-Cl), 819.18(Ar-H), 456(CH); ^{1}H NMR (DMSO-d_6, 300MHz): 9.43(s, 2H, NH_2), 7.62(s, 1H, CH), 7.36 - 7.16(tt, 4H, Phenyl), 6.55(s, 1H, Imidazole-CH), 6.72(s, 1H, CH), 2.3(1H,NH), 2.1(s,1H,NH); ^{13}C NMR(DMSO-d_6, 300MHz); 1810.7 (C=S), 136.6, 132.3, 128.6, 128.3 (Ph-Cl), 128.0 (Imidazole ring CH=CH), 138.1 (HC=N), 76.8(CH). EI-MS, m/z (Relative intensity %): [281.76^+, 37 %].

2-((4-Hydroxyphenyl) (1*H*-imidazol-1-yl) methyl) hydrazinecarbothioamide (1c)

IR (KBr,cm^{-1}): 3290.21(NH_2), 2914.16(NH), 1435.83(C=S), 809.97(Ar-H), 458(CH); ^{1}HNMR (DMSO-d_6, 300MHz): 9.52(s, 2H, NH_2), 9.47 (1H, *s*, C–OH), 9.43 (s, 1H, 9.43), 7.80 (s, 1H, CH), 6.63 – 7.08 (tt, 4H, Phenyl), 6.73 (s, 1H, Imidazole-CH), 6.78 (s, 1H, CH), 2.4 (1H,NH), 2.1 (s,1H,NH); ^{13}C NMR(DMSO-d_6, 300MHz); 1821.4 (C=S), 115.7, 128.3, 131.2 (Ph - OH), 127.9 (Imidazole ring CH=CH), 138.2 (HC=N), 74.2(CH). EI-MS, m/z (Relative intensity %): [263.31$^+$, 26 %].

2-((1*H*-Imidazol-1-yl) (4-nitrophenyl) methyl) hydrazinecarbothioamide (1d)

IR(KBr,cm^{-1}): 3294.29 (NH_2), 2915.15 (NH), 1435.88 (C=S), 814.90 (Ar-H), 1536 (C–NO2), 458.92 (CH); ^{1}H NMR (DMSO-d_6, 300MHz): 9.51 (s, 2H, NH_2), 7.80 (s, 1H, CH), 7.22-7.64 (m, 5H, Phenyl), 6.77 (s, 1H, Imidazole-CH), 6.84 (s, 1H, CH), 2.4 (s, 1H, NH), 2.1 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300MHz); 181.8 (C=S), 155.2, 131.6, 128.3, 127.9 (Ph-NO_2),, 128.2 (Imidazole ring CH=CH), 136.3 (HC=N), 74.8 (CH); EI-MS, m/z (Relative intensity %): [292.31$^+$, 36 %].

2-((1*H*-imidazol-1-yl) (4-methoxyphenyl) methyl) hydrazinecarbothioamide (1e)

IR (KBr, cm^{-1}): 3288.20 (NH_2), 2912.14 (NH), 1432.80 (C=S), 812.95 (Ar-H), 458.67 (CH); ^{1}H NMR (DMSO-d_6, 300MHz): 9.51 (s, 2H, NH_2), 7.88 (s, 1H, CH), 6.88 – 7.10 (tt, 4H, Ph – OCH_3), 6.68 (s, 1H, Imidazole-CH), 6.80 (s, 1H, CH), 3.84 (s, 3H, –OCH3), 2.1 (s, 1H, NH), 2.0 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300MHz): 1818.6 (C=S), 158.6, 130.9, 114.0, 126.0(Ph - OH), 128.1 (Imidazole ring CH=CH), 55.7 (Ph–OCH3), 134.2 (HC=N), 74. 8(CH); EI-MS, m/z (Relative intensity %): [277.34$^+$, 28 %].

2-((4-(dimethylamino) phenyl) (1*H*-imidazol-1-yl) methyl) hydrazinecarbothioamide (1f)

IR (KBr, cm^{-1}): 3282.16 (NH_2), 2912.12 (NH), 1438.82 (C=S), 808.92 (Ar-H), 460 (CH); ^{1}H NMR (DMSO-d_6, 300MHz): 9.53 (s, 2H, NH_2), 7.83 (s, 1H, CH), 7.02 – 6.64 (tt, 4H, Ph – $N(CH_3)_2$), 6.70 (s, 1H, Imidazole-CH), 3.12 (*s*, 6H, –$N(CH_3)_2$), 6.84 (s, 1H, CH), 2.2(1H,NH), 2.0 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300Mhz): 180.6 (C=S), 148.3, 128.1, 127.2, 112.8, 40.2 (Ph – N $(CH_3)_2$), 126.2 (Imidazole ring CH=CH), 136.8 (HC=N), 40.8 (–$N(CH_3)_2$), 74.0 (CH); EI-MS, m/z (Relative intensity %): [290.38$^+$, 37 %].

2-((1*H*-Imidazol-1-yl) (p-tolyl) methyl) hydrazinecarbothioamide (1g)

IR(KBr,cm^{-1}): 3280.22 (NH_2), 2914.13 (NH), 1430.82 (C=S), 814.96 (Ar-H), 454.89(CH); ^{1}H NMR (DMSO-d_6, 300MHz): 9.50 (s, 2H, NH_2), 7.82 (s, 1H, CH), 7.11 - 7.14 (tt, 4H, Ph – CH_3), 6.56 (s, 1H, Imidazole-CH), 6.82 (s, 1H, CH), 2.42 (s, 1H, NH), 2.33 (s, 3H, -CH_3), 2.21 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300MHz); 184.5 (C=S), 135.9, 135.4, 128.5, 126.8 (Ph – CH_3), 128.5 (Imidazole ring CH=CH), 137.2 (HC=N), 74.6 (CH); EI-MS, m/z (Relative intensity %): [261.34$^+$, 22 %].

2-[1*H*-Imidazol-1-yl (phenyl) methyl] hydrazinecarboxamide (2a)

IR(KBr,cm^{-1}) : 3287.20 (NH_2), 2932.97 (NH), 1623.21 (C=O), 819.78 (Ar-H), 456.98 (CH) ^{1}H NMR (DMSO d_6,300 MHz): 9.50 (s, 2H, NH_2), 7.86 (s, 1H, CH), 7.26-7.65 (m, 5H, Phenyl), 6.89 (s, 1H, Imidazole-CH), 6.76 (s, 1H, CH), 2.21 (1H, NH), 2.09 (s, 1H, NH) ^{13}C NMR (DMSOd_6, 300MHz); 157.9 (C=O), 126.1, 126.8, 128.5, 138.9 (Phenyl), 128.1 (Imidazole ring CH=CH), 137.1 (HC=N), 75.9 (CH). EI-MS, m/z (Relative intensity %): [231.28$^+$, 51 %].

2-((4-Chlorophenyl) (1*H*-imidazol-1-yl) methyl) hydrazinecarboxamide (2b)

IR (KBr, cm^{-1}) : 3281.18 (NH_2), 2930.92 (NH), 1621.18 (C=O), 837(C-Cl), 818.71 (Ar-H), 455.91 (CH); ^{1}H NMR (DMSO d_6, 300 MHz): 9.52 (s, 2H, NH_2), 7.82 (s, 1H, CH), 7.36 - 7.16 (tt, 4H, Phenyl), 6.86 (s, 1H, Imidazole-CH), 6.74 (s, 1H, CH), 2.20 (1H, NH), 2.12 (s, 1H, NH); ^{13}C NMR (DMSOd_6, 300MHz); 157.2 (C=O), 136.6, 132.3, 128.6, 128.3 (Ph-Cl) 128.4 (Imidazole ring CH=CH), 137.6 (HC=N), 76.5 (CH); EI-MS, m/z (Relative intensity %): [265.70$^+$, 38 %].

2-((4-Hydroxyphenyl) (1*H*-imidazol-1-yl) methyl) hydrazinecarboxamide (2c)

IR (KBr,cm^{-1}) : 3280.24 (NH_2), 2930.94 (NH), 1624.23 (C=O), 816.76 (Ar-H), 1472 (C–OH), 457.91(CH); ^{1}H NMR (DMSO d_6, 300 MHz): 9.46 (s, 2H, NH_2), 9.41 (1H, *s*, OH), 7.84 (s, 1H, CH), 6.63 – 7.08 (tt, 4H, Phenyl), 6.86 (s, 1H, Imidazole-CH), 6.74 (s, 1H, CH), 2.19 (1H, NH), 2.12 (s, 1H, NH); ^{13}C NMR (DMSOd_6, 300MHz); 155.6 (C=O), 125.2, 126.6, 128.2, 138.4 (Phenyl), 128.4 (Imidazole ring CH=CH), 136.5 (HC=N), 115.7, 128.3, 131.2 (Ph - OH), 75.2 (CH); EI-MS, m/z (Relative intensity %): [247.25$^+$, 33 %].

2-((1*H*-Imidazol-1-yl) (4-nitrophenyl) methyl) hydrazinecarboxamide (2d)

IR(KBr,cm^{-1}) : 3278.10 (NH_2), 2930.91 (NH), 1622.18 (C=O), 813.18 (Ar-H), 1530 (C–NO2), 455.91(CH); ^{1}H NMR (DMSO d_6,300 MHz): 9.46 (s, 2H, NH_2), 7.84 (s, 1H, CH), 7.26-7.65(m, 5H, Phenyl), 6.88 (s, 1H, Imidazole-CH), 6.74 (s, 1H, CH), 2.18 (1H, NH), 2.12 (s, 1H, NH); ^{13}C NMR (DMSOd_6, 300MHz); 156.8 (C=O), 155.2, 131.6, 128.3, 127.9 (Ph-NO_2),, 128.6 (Imidazole ring CH=CH), 136.4 (HC=N), 75.2 (CH). EI-MS, m/z (Relative intensity %): [276.25$^+$, 30 %].

2-((1*H*-Imidazol-1-yl) (4-methoxyphenyl) methyl) hydrazinecarboxamide (2e)

IR (KBr,cm^{-1}) : 3276.10 (NH_2), 2936.87 (NH), 1628.22 (C=O), 816.72 (Ar-H), 456.94 (CH) ^{1}H NMR (DMSO-d_6, 300 MHz): 9.54 (s, 2H, NH_2), 7.85 (s, 1H, CH), (tt, 4H, Ph – OCH_3), 6.86 (s, 1H, Imidazole-CH), 6.74 (s, 1H, CH), 3.81 (3H, *s*, –OCH3), 2.18 (1H, NH), 2.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300MHz); 158.6 (C=O), 158.6, 130.9, 114.0, 126.0 (Ph -OH), 128.2 (Imidazole ring CH=CH), 137.3 (HC=N), 55.9 (–OCH3); 74.8 (CH); EI-MS, m/z (Relative intensity %): [261.28$^+$, 54 %].

2-((4-(Dimethylamino) phenyl) (1*H*-imidazol-1-yl) methyl) hydrazinecarboxamide (2f)

IR(KBr,cm^{-1}) : 3281.21 (NH_2), 2929.91 (NH), 1627.27 (C=O), 817.71 (Ar-H), 453.96 (CH); ^{1}H NMR (DMSO-d_6,300 MHz): 9.47 (s, 2H, NH_2), 7.81(s, 1H, CH), (tt, 4H, Ph – $N(CH_3)_2$), 6.82 (s, 1H, Imidazole-CH), 6.72 (s, 1H, CH), 3.06 (1H, *s*, –N (CH3)2), 2.27 (1H, NH), 2.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300MHz); 156.2 (C=O), 148.3, 128.1, 127.2, 112.8, 40.2 (Ph – $N(CH_3)_2$), 128.6(Imidazole ring CH=CH), 136.5(HC=N), 40.8 (N(CH3)2), 76.2 (CH). EI-MS, m/z (Relative intensity %): [274.32$^+$, 23 %].

2-((1*H*-Imidazol-1-yl)(p-tolyl) methyl) hydrazinecarboxamide (2g)

IR (KBr,cm^{-1}) : 3266.21(NH_2), 2930.70(NH), 1622.19(C=O), 818.18(Ar-H), 455.18(CH); ^{1}H NMR (DMSO-d_6, 300 MHz): 9.52(s, 2H, NH_2), 7.81(s, 1H, CH), 7.26-7.65(tt, 4H, Phenyl), 6.87 (s, 1H, Imidazole-CH), 6.75 (s, 1H, CH), 2.34 (s, 3H, CH_3), 2.26 (1H, NH), 2.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 300MHz); 155.6 (C=O), 135.9, 135.4, 128.5, 126.8 (Ph –CH_3), 128.8 (Imidazole ring CH=CH), 136.9(HC=N), 75.1 (CH). EI-MS, m/z (Relative intensity %): [245.28$^+$, 56 %].

Antibacterial activity

Compounds **1a-g** and **2a-2g** were evaluated for *in vitro* antibacterial activity against *Staphylococcus aureus, Escherichia coli, Enterococcus. faecalis. Pseudomonas aeruginosa* and *Klebsiella pneumoniae* using conventional agar dilution procedures. Ciprofloxacin was used as a positive control. Inhibition zones were measured and compared against those of controls. The bacterial zones of inhibition are given in Table 2.

Compared with ciprofloxacin, compound **1b** was highly active against *S. aureus*, and **1g** exhibited an equivalent activity (0.5 µg/mL) against *E. faecalis* and greater activity (0.25 µg/mL) against *P. aeruginosa*. Compound **2e** was highly active against *K. pneumonia* and **2f** showed equivalent activities (0.5 µg/mL) against *E. coli* and *E. feacalis*. Compound **2g** was highly active (0.5 µg/mL) against *P. aeruginosa*. The MIC values are summarised in Table 3.

Antifungal activity

Compounds **1a-g** and **2a-g** were evaluated in terms of their *in vitro* antifungal activity against *Aspergillus niger, Candida albicans, Aspergillus fumigatus, Cryptococcus neoformans* (recultured), and *Microsporum audouinii* using an agar diffusion method. The fungal activity of each compound was compared with that of clotrimazole as positive control. The fungal zones of inhibition are given in Table 2. Compounds **2d** and **2g** (0.25 µg/mL) were highly active against *C. albicans*. Compound **2d** was equipotent active (1 µg/mL) against *M. audouinii* compared with clotrimazole (MIC, 2 µg/ml).

Larvicidal activity

The larvicidal activity of the test compounds is listed in Table 4. Larvicidal activity was determined for compounds **1a-g** and **2a-g** by exposing second instar larvae for 24 h at room temperature. With the exception of **1g**, the compounds exhibited moderate larvicidal activity against mosquito. Compounds **1a, 1b, 1d, 1f, 2a, 2b, 2c, 2d, 2f** and **2g**) yielded 100 % mortality at 40 µg/mL. Compound **1g** was particularly toxic with an LD_{50} of 9.5 µg/mL.

Nematicidal activity

Compounds **1a-g** and **2a-g** were alao evaluated in terms of their *in vitro* nematicidal activity against *Meloidogyne javanica* at various aqueous concentrations. Compound **1g** was the most effective nematicide as evidenced by its LD_{50} of

8.9 µg/mL. Compounds (**1d, 1e, 1f, 1g, 2d** and **2e**) were more potent then compounds (**1a, 1b, 2a, 2b, 2c, 2f**, and **2g**), which exhibited 100 % mortality at 30 µg/mL. LD_{50} values are reported in Table 5.

Table 1: Physicochemical data of the compounds **(1a-g)** and (**2a-g**)

Compound no.	R	M.W	M.F	M.P(°C)	Elemental analysis (Calculated & found)			
					C	H	N	S
1[a]	-H	247.31	$C_{11}H_{13}N_5S$	130	53.42 (53.40)	5.30 (5.27)	28.32 (28.30)	12.97 (12.96)
1b	-Cl	281.76	$C_{11}H_{12}Cl\ N_5S$	140	46.89 (46.87)	4.29 (4.28)	24.86 (24.84)	11.38 (11.36)
1c	-OH	263.31	$C_{11}\ H_{13}\ N_5OS$	137	50.17 (50.15)	4.98 (4.97)	26.60 (26.63)	12.18 (12.17)
1d	$-NO_2$	292.31	$C_{11}H_{12}N_6O_2S$	143	45.20 (45.22)	4.14 (4.10)	28.75 (28.72)	10.97 (10.98)
1e	$-OCH_3$	277.34	$C_{12}H_{15}N_5OS$	147	51.97 (51.99)	5.45 (5.41)	25.25 (25.23)	11.56 (11.55)
1f	$-N(CH_3)_2$	290.38	$C_{13}H_{18}N_6S$	164	53.77 (53.78)	6.25 (6.24)	28.94 (28.90)	11.04 (11.08)
1g	$-CH_3$	261.34	$C_{12}H_{15}N_5S$	161	55.15 (55.14)	5.79 (5.78)	26.80 (26.83)	12.27 (12.29)
2[a]	-H	231.25	$C_{11}H_{13}N_5O$	110	57.13 (57.10)	5.67 (5.66)	30.28 (30.26)	-
2b	-Cl	265.70	$C_{11}H_{12}ClN_5O$	122	49.72 (49.70)	4.55 (4.53)	26.36 (26.33)	-
2c	-OH	247.25	$C_{11}H_{13}N_5O_2$	137	53.43 (53.40)	5.30 (5.31)	28.32 (28.30)	-
2d	$-NO_2$	276.25	$C_{11}H_{12}N_6O_3$	146	47.83 (47.80)	4.38 (4.34)	30.42 (30.41)	-
2e	$-OCH_3$	261.28	$C_{12}H_{15}N_5O_2$	137	55.16 (55.13)	5.79 (5.78)	26.80 (26.78)	-
2f	$-N(CH_3)_2$	274.32	$C_{13}H_{18}N_6O$	161	56.92 (56.90)	6.61 (6.60)	30.64 (30.61)	-
2g	$-CH_3$	245.28	$C_{12}H_{15}N_5O$	154	58.76 (58.77)	6.16 (6.18)	28.55 (28.51)	-

Table 2: Antimicrobial activities of compounds **1a-g** and **2a-g** at 100 µg/mL

Compound	*Antibacterial screnning*					*Antifungal screnning*				
	S. a	***E.c***	***E.f***	***P. a***	***K.p***	***A.n***	***C.a***	***A.f***	***C.n***	***M.a***
1a	12	-	-	12	-	7	-	-	-	14
1b	28	8	10	10	12	16	24	15	7	13
1c	16	-	-	16	19	10	6	-	-	8
1d	20	-	12	18	8	-	7	-	16	6
1e	18	12	-	12	10	17	26	-	7	8
1f	15	-	14	14	-	-	15	-	9	-
1g	-	24	25	26	12	9	17	-	16	-
2[a]	10	12	-	-	10	-	7	-	9	-
2b	5	-	-	10	12	15	10	8	10	8
2c	-	-	12	12	8	-	-	-	-	16
2d	12	10	10	15	-	10	16	14	-	20
2e	-	16	-	28	-	-	12	6	8	-
2f	22	24	25	-	10	-	16	-	23	-
2g	12	-	10	26	19	18	28	11	20	10
Ciprofloxacin	26	28	22	15	19	-	-	-	-	-
Clotrimazole	-	-	-	-	-	22	26	16	30	18

Clotrimazole was used as a standard; zone of inhibition was measured in mm

Table 3: Minimum inhibitory concentrations (MIC, µg/mL) of compounds **1a-g** and **2a-g**

Comp. No.	Minimum Inhibitory Concentration (MIC, µg/mL)[a]									
	Antibacterial activity					Antifungal activity				
	S.a	*E.c*	*E.f*	*P.a*	*K.p*	*A.n*	*C. a*	*A.f*	*C. n*	*M. a*
1b	0.25	-	-	32	-	32	4	64	-	>100
1c	16	-	-	16	4	>100	>100	-	-	>100
1d	2	-	64	1	>100	-	>100	-	64	>100
1g	-	1	0.5	0.25	64	32	1	-	>100	>100
2d	64	64	64	16	-	64	0.25	64	-	1
2e	-	4	-		0.5	-	32	>100	>100	-
2f	2	0.5	0.5	-	64	-	16	-	2	-
2g	64	-	64	0.5	2	4	0.5	>100	2	32
Ciproflaxacin	0.5	0.5	0.5	4	2	-	-	-	-	-
Clotrimazole	-	-	-	-	-	2	1	8	0.5	2

-, Not determined

Table 4: Larvicidal profile of compounds **1a-g** and **2a-g** on against second instar larvae of Culex sp

Compound no.	Mortality (%) at room temp concentration (µg/mL)				LD_{50} (µg/mL)
	10	20	30	40	
1[a]	25 ± 4.8	51 ± 3.1	79 ± 8.2	100 ± 0.0	19.5
1b	22 ± 3.7	75 ± 4.8	90 ± 2.5	100 ± 0.0	16.2
1c	29 ± 4.1	54 ± 40	100 ± 0.0	-	16.9
1d	18 ± 1.2	51 ± 1.2	76 ± 4.2	100 ± 0.0	20.8
1e	45 ± 2.9	86 ± 2.5	100 ± 0.0	-	10.1
1f	27 ± 4.8	64 ± 4.0	82 ± 4.2	100 ± 0.0	17.2
1g	52 ± 4.1	73 ± 4.2	100 ± 0.0	-	9.5
2a	20 ± 2.2	59 ± 7.3	74 ± 3.8	100 ± 0.0	19.8
2b	24 ± 2.1	40 ± 3.8	82 ± 5.7	100 ± 0.0	19.1
2c	20 ± 3.2	46 ± 4.8	60 ± 3.9	100 ± 0.0	22.4
2d	10 ± 3.2	32 ± 2.5	66 ± 3.8	100 ± 0.0	24.3
2e	28 ± 3.1	62 ± 4.0	100 ± 0.0	-	16.2
2f	22 ± 3.7	63 ± 3.5	71± 1.7	100 ± 0.0	19.2
2g	30 ± 4.8	58 ± 3.8	92 ± 4.2	100 ± 0.0	16.8

Values are mean ± SD (n = 3)

Table 5: Nematicidial activities of compounds **1a-g** and **2a-g**

Compound no.	Mortality (%) at room temp concentration(µg /mL)				LD_{50} (µg /mL)
	10	20	30	40	
1a	20 ± 3.8	49 ± 3.7	87 ± 3.2	100 ± 0.0	20.6
1b	40 ± 4.1	51 ± 2.3	80 ± 1.9	100 ± 0.0	16.5
1c	39 ± 4.1	76 ± 2.3	100 ± 0.0	-	12.8
1d	43 ± 4.8	71 ± 5.7	100 ± 0.0	-	12.5
1e	42 ± 4.0	61 ± 1.8	100 ± 0.0	-	13.9
1f	42 ± 4.1	53 ± 4.1	100 ± 0.0	-	14.8
1g	54± 4.8	72 ± 2.3	100 ± 0.0	-	8.9
2a	18 ± 2.1	36 ± 4.4	60 ± 2.0	100 ± 0.0	23.7
2b	30 ± 3.2	56 ± 3.2	72 ± 3.9	100 ± 0.0	18.5
2c	38 ± 1.9	40 ± 3.8	60 ± 2.0	100 ± 0.0	20.3
2d	23 ± 5.0	52 ± 4.1	100 ± 0.0	-	17.8
2e	42 ± 4.1	66 ± 2.2	100 ± 0.0	-	13.3
2f	28 ± 3.0	47 ± 2.8	62 ± 2.0	100 ± 0.0	20.9
2g	16 ± 1.2	45 ± 4.1	67 ± 3.9	100 ± 0.0	22.4

Values are the means of three replicates ± SD

DISCUSSION

We synthesised and characterised 14 new Mannich base imidazole derivatives (**1a-g**) and (**2a-g**) as outlined in Scheme 1. The physical data of these compounds are given in Table 1.

The Mannich base condensation reaction proceeds via an attack by benzaldehyde at a secondary amine. During this reaction, one mole of water was eliminated and the resulting thiazolidine-4-one products were purified by column chromatography using an eluent of hexane: chloroform (1:4)

The chemical structure of each new compound was confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass, and elemental analysis. The IR spectrum of compound **1a** showed absorption bands at 3292.26, 2916.17, 1436.87 and 460.03 cm^{-1} corresponding to NH_2, NH, C=S and CH groups. The ^{1}H NMR spectrum of the compounds **1a** showed broad signals at 9.53, 7.83 and 2.20 ppm corresponding to NH_2, CH and NH protons respectively. The ^{13}C NMR spectra of **1a** contained important peaks at 182.7 and 75.3 ppm, corresponding to C=S and CH carbon atoms, respectively. The mass spectrum of **1a** contained a molecular ion peak m/z 247.28, thereby confirming its molecular mass. Similar spectral data and corresponding molecular masses were obtained for compounds (**1b-1g**).

Similarly, the IR spectrum of compound **2a** showed absorption bands at 3287.20, 2932.97, 1623.21 and 456.98 cm^{-1} corresponding to NH_2, NH, C=O and CH groups respectively. The ^{1}H NMR spectrum of **2a** showed broad signals at 9.50, 7.86 and 2.21 ppm, corresponding to NH_2, CH and NH protons respectively. The ^{13}C NMR spectrum of compound **2a** showed important peaks at 157.9 and 75.9 ppm, corresponded to C=O and CH carbon atoms respectively. The mass spectrum of **2a** contained a molecular ion peak m/z 231.28, which is consistent with its molecular mass. Similar spectral data and corresponding molecular masses were obtained for compounds (**2b-2g**).

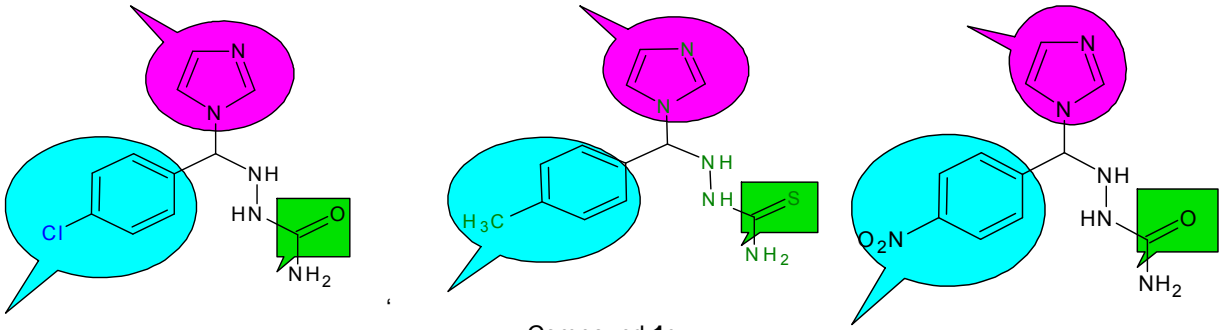
The synthesized imidazole derivatives were evaluated in terms of their antimicrobial, larvicidal and, nematicidal activities. Consistent with their expected structure - activity relationship, compounds **1a-1g** and **2a-2g** were biologically active. The presence of imidazole nucleus and the para substitution of the phenyl ring contribute to the observed activities.

The chemical structure in Figure 1 show that the 4-substituted phenyl ring acts as a lipophilic domain. The C=S group in thiosemicarbazone and the C=O group in semicarbazone form hydrogen bonds with the NH groups in thiosemicarbazone and semicarbazone act as hydrogen bonding domain. Therefore, the imidazole ring is an essential pharmacophore that determine biological activity.

Compound **1b**, which contains a 4-substituted chlorine atom showed significant antibacterial activity against *S. aureus* (MIC, 0.25 µg/mL) relative to that of the positive control ciproflaxiacin (MIC: 0.5 µg/mL).

Compound **1g**, with a 4-substituted methyl group, exhibited a remarkable activity against *P. aeruginosa* (MIC, 0.25 µg/mL) compared with that of ciprofloxacin (MIC, 4 µg/mL).

Compound **2d** containing a 4-NO_2 group exhibited an excellent antifungal activity (MIC, 0.25 µg/mL) against *C. albicans* relative to the activities of the synthesized compounds and the positive control, clotrimazole (MIC, 1 µg/mL).



Compound **1b**
Staphylococcus aureus
MIC:0.25 µg/mL

Compound **1g**
Pseudomonas aeruginosa :MIC:0.25 µg/mL
Nematicidal activity LD_{50} =8.9 µg /mL
Larvicidal activity LD_{50}=9.5 µg /mL

Compound **2d**
Candida albicans,
MIC:0.25 µg/mL

Figure 1: Structure activity relationships of synthesised imiazole derivatives

Compound **1g**, which contains a 4-CH_3-phenyl group with thiosemicarbazone and imidazole moieties, was a potent larvicide (LD_{50}: 9.5 µg/mL) and nematicide (LD_{50}, 8.9 µg/mL) while compound **2g** (4-CH_3-phenyl with semicarbazide and imidazole moieties) showed no significant nematicidal (LD_{50}, 22.9 µg/mL) or larvicidal (LD_{50}, 16.8 µg /mL) activities.

Compound **1e**, which also contains a 4-CH_3O-phenyl with thiosemicarbazone and imidazole moieties, was also a potent larvicide (LD_{50}, 10.1 µg/mL) and nematicide (LD_{50}, 13.9 µg/mL), while compound **2e** was less active against mosquito larvae (LD_{50}, 16.2 µg /mL) and highly active against nematodes (LD_{50}, 13.9 µg/mL). However, compound 1g exhibited the highest activity in both cases.

CONCLUSION

A new series of imidazole derivatives has been synthesized. Some of them possess strong larvicidal, nematicidal, antibacterial and antifungal activities, and thus are capable of serving as potential lead molecules for the development of clinically useful antimicrobial, larvicidal and nematicidal agents.

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