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ORIGINAL ARTICLE

Synthesis of 1-(4-methoxybenzyl)-3-cyclopropyl-1 *H*-pyrazol-5-amine derivatives as antimicrobial agents

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Abstract

A series of novel substituted 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine benzamides **9(a–h)** were synthesized to determine their antibacterial and antifungal activities as well as possible structure–activity relationships (SARs) to improve therapeutic efficacy. The pyrazol-5-amine benzamides were screened for their antibacterial activity against standard strains of Gram-positive (*Streptococcus pyogenes* NCIM 2608, *Staphylococcus aureus* ATCC 29737, *Bacillus subtilis* NCIM 2010) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 20852, *Klebsiella pneumoniae* MTCC 618) bacteria by using streptomycin as positive control. They were also tested for their antifungal activities against mycotoxic strains of *Fusarium verticillioides*, *Aspergillus ochraceous*, *Aspergillus flavus*, *Alternaria alternata*, and *Penicillium chrysogenum* using nystatin as positive control. Among the synthesized compounds, **9d**, **9g**, and **9h** showed potent antimicrobial activities.

Keywords: *4-methoxybenzaldehyde; 3-cyclopropyl-3-oxopropanenitrile; acid chlorides; antibacterial; antifungal*

Introduction

Despite the progress made in medical sciences and health care, morbidity and mortality following nosocomial infections are still commonplace. It is important to underline the role of opportunistic pathogens, which may be part of the normal human bacterial flora and cause diseases especially when the host immunity becomes impaired. There are serious concerns that untreatable pathogens may develop at an alarming rate in the near future. Further, in recent years, increasing rates of antimicrobial resistance among community and nosocomial pathogens has severely limited the therapeutic options for treating infections caused by such organisms. In view of these considerations, further efforts are needed to develop a new group of antimicrobial compounds. In recent years, there has been an alarming increase in life-threatening microbial infections especially in immunocompromised individuals suffering from acquired immune deficiency syndrome (AIDS), cancer, etc.[1–4]. Despite the development of several new antimicrobial agents, their clinical value is limited to treating an increasing array of life-threatening systemic infections because of their relatively high risk of toxicity, emergence of drug resistant strains, pharmacokinetic differences, and/or insufficiencies in their antimicrobial activity.

Pyrazoles and several *N*-substituted pyrazoles are known to possess numerous chemical, biological, medicinal, and agricultural applications because of their versatile biological activities appearing as antimicrobial activity[5–8], antitumor and antileukemia activity[9], antidepressant and anticonvulsant activities[10,11], antifungal activity[12] and antitubercular activity against *Mycobacterium tuberculosis*[13]. β-Lactamase is generally considered to be responsible for microbial resistance against a broad spectrum of β-lactam antibiotics. In 1935, Domargk showed the therapeutic value of a group of compounds known as sulfonamides. These are not specific for a special group of organisms, but are effective against a large variety of pathogenic organisms.

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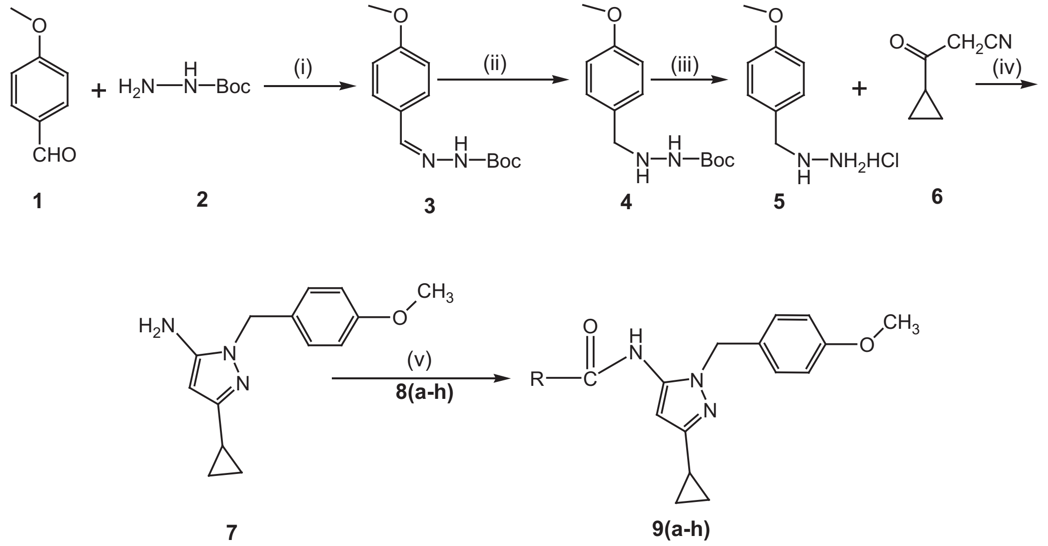
Bezenesulfonamide derivatives were also reported as elastase inhibitors[14], carbonic anhydrase and cyclo-oxygenase-2 (COX-2) inhibitors[15,16], herbicides, and plant growth regulators[17]. They show affinities for endothelin ETA and ETB receptors in the low nanomolar range and high functional antagonistic potency *in vitro*[18], and also exhibit a dual action to inhibit the thromboxane receptor and thromboxane synthase in cardiovascular and renal diseases[19]. Antiproliferative, antiviral, and antifungal activities have been similarly evaluated[20]. Compounds containing an amide group can alter the chemical properties, disposition, and biological activities of drugs[21]. Amides are currently used as antidepressants, anti-inflammatory agents, antimalarial agents, antipsychotic agents, antiviral agents, steroids, and general anesthetics[22]. Amide functional groups are also found in many antibacterial agents, as for example benzimidazole carboxamides, peptide, penicillin, cephalosporins, and thiozolidinones[23].

Our earlier work on the synthesis of different heterocyclic systems containing high antimicrobial activity prompted us to synthesize a new class of heterocyclic carboxamides and sulfonamides and study their antibacterial activities[24]. Recently, we reported the synthesis and antimicrobial studies of bioactive heterocyclic sulfonamides and benzamides[24–26]. In continuation of our research work on the synthesis of bioactive heterocycles, the activities of the synthesized compounds were tested on bacterial and fungal strains by the cup-borer method, microwell dilution assay, and turbidometric method.

Materials and methods

Synthesis

Melting points were determined using a Selco-650 hot stage melting apparatus and were uncorrected. Infrared (IR) spectra were recorded using a Jasco FTIR-4100 series spectrometer. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on Bruker, 400 MHz spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard (chemical shift in δ ppm). Spin multiples are given as br s (broad singlet), d (doublet), t (triplet), and m (multiplet). Mass and purity were recorded on an LC/MSD-Trap-XCT apparatus. Elemental (CHN) analyses were obtained on a Vario EL III Elementar analyzer. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck thin layer chromatography (TLC) plates. 1-(4-Methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine derivatives **9(a–h)** were synthesized as per the method summarized in Scheme 1. Initially, mono-Boc protected 1-(4-methoxybenzylidene) hydrazine (3) was synthesized by the condensation reaction of 4-methoxybenzaldehyde **(1)** with mono-Boc protected hydrazine **(2)**. The subsequent double bond reduction was done using 10% Pd/C in ethanol, which yielded mono-Boc protected 1-(4-methoxybenzyl) hydrazine **(4)**. Deprotection of the amine group was carried out using HCl in ether, which gave the free amine compound **(3)**. Finally, the key intermediate, 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** by cyclization of 1-(4-methoxybenzyl)-2-methylhydrazine salt **(5)** (1.0 g, 5.36 mol) and 3-cyclopropyl-3-oxopropanenitrile **(6)** (0.85 g, 5.36 mol) was taken in ethanol, and then sodium ethoxide (1.09 g, 16.0 mol) was added. The reaction mixture was refluxed for 2–3 h. The progress of the reaction was monitored by TLC. When the reaction was complete, water was added to the reaction mixture and extraction was done with ethyl acetate. The organic layer was washed with 10% ammonium chloride solution followed by a water wash, and dried with anhydrous sodium sulfate. The solvent was evaporated, and the crude product obtained was purified by column chromatography over silica gel (60–120 mesh)

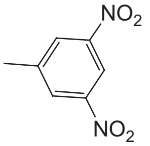
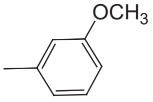
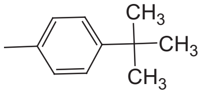
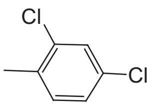
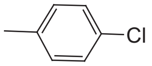
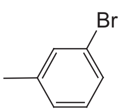
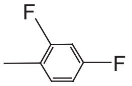
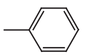


Scheme 1. Synthesis of compounds **9(a–h)**. Reactions and reagent conditions: (i) EtOH/r.t, 2–3 h; (ii) 10% Pd/C/H_2EtOAc, r.t, 3 h; (iii) dichloromethane, ether in HCl, 3 h; (iv) EtOH/EtCOONa, reflux 80°C, 2–3 h; (v) triethylamine, dichloromethane, **8(a–h)**, r.t, 4–5 h; where R-CO-Cl are: **(8a)** 3,5-dinitrobenzoyl chloride; **(8b)** 3-methoxybenzoyl chloride; **(8c)** 4-*tert*-butylbenzoyl chloride; **(8d)** 2,4-dichlorobenzoyl chloride; **(8e)** 4-chlorobenzoyl chloride; **(8f)** 3-bromobenzoyl chloride; **(8g)** 2,4-difluorobenzoyl chloride; **(8h)** benzoyl chloride.

using hexane:ethyl acetate (8:2) as eluent. The product obtained was a white crystalline solid and the yield was found to be 85%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.95 (d, 2H, *J* = 6.52 Hz, Ar-H), 6.92 (d, 2H, *J* = 6.2 Hz, Ar-H), 5.18 (s, 1H, Ar-H), 5.02 (s, 2H, $-CH_2$), 3.95 (br s, 2H, $-NH_2$), 3.85 (s, 3H, $-OCH_3$), 1.85 (m, 1H, -CH), 0.92 (m, 2H, $-CH_2$), 0.71 (m, 2H, $-CH_2$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 165.4 (C), 157.7 (C), 148.5 (C), 130.1 (2C), 128.6 (C), 114.2 (2C), 89.3 (C), 55.9 (C), 51.5 (C), 9.3 (C), 8.2 (2C). MS (ESI) *m/z*: 243.3. IR (KBr, cm^{-1}): 3360, 1685, 1602, 1355, 1277, 1225, 865. Anal. calcd. for $C_{14}H_{17}N_3O$ (in %): C-69.11; H-7.04; N-17.27. Found C-69.10; H-7.02; N-17.23.

The nucleophilic substitution reactions of 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** with different substituted aromatic acid chlorides (R-CO-Cl) **(8)** were carried out in the presence of triethylamine and dichloromethane (DCM) as solvents. The presence of an N-H proton peak at δ = 10.51 ppm in carboxamide **9(a-h)** in proton NMR confirmed our products. They were also confirmed by IR data for the carboxamide series **9(a–h)**, which showed an asymmetric stretching frequency of R-CO-Cl in the range 1650–1710 cm^{-1} and symmetric stretching frequency at 1640–1720 cm^{-1}. All the compounds obtained were in good yield with high purity. The structures and physical data of the synthesized molecules are tabulated in Table 1.

Table 1. Chemical structures and physical data of the synthesized compounds **9(a–h)**.

Compound	R	Yield (%)	M.P. (°C)
9a		89	254–256
9b		91	245–246
9c		88	263–265
9d		85	254–255
9e		81	248–249
9f		84	255–258
9g		79	273–275
9h		80	267–268

General procedure for the synthesis of 1-(4-methoxybenzyl)-3-cyclopropyl-1H-pyrazol-5-amine derivatives 9(a–h)

A solution of 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** (1.0 eq) in dry dichloromethane was taken and cooled to 0–5°C in an ice bath. Triethylamine (3.0 eq) was added to the cold reaction mixture and stirred for 10 min, and different acid chlorides (1.0 eq) were added; the mixture was stirred at room temperature for 4–5 h and progress of the reaction was monitored by TLC. When the reaction was complete, water was added to the reaction mixture and extraction was done with ethyl acetate. The organic layer was washed with 10% ammonium chloride solution followed by a water wash, and dried with anhydrous sodium sulfate. The solvent was evaporated, and the crude product obtained was purified by column chromatography over silica gel (60–120 mesh) using hexane:ethyl acetate (8:2) as eluent. The product obtained was a white solid with good yield and high purity.

*Synthesis of N-(1-(4-methoxybenzyl)-3-cyclopropyl-1H-pyrazol-5-yl)-3,5-dinitrobenzamide (**9a**)* The product as a yellow colored solid was obtained from 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** (0.25 g, 1.13 mmol), 3,5-dinitrobenzoyl chloride **(8a)** (0.28 g, 1.13 mmol), and triethylamine (0.46 g, 3.39 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 11.37 (s, 1H, Ar-H), 11.27 (s, 2H, Ar-H), 10.51 (s, 1H, -NH), 6.95 (d, 2H, *J* = 6.52 Hz, Ar-H), 6.92 (d, 2H, *J* = 6.8 Hz, Ar-H), 5.18 (s, 1H, Ar-H), 5.02 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 1.85 (m, 1H, -CH), 0.92 (m, 2H, $-CH_2$), 0.71 (m, 2H, $-CH_2$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 165.4 (C), 164.8 (C), 157.7 (C), 149.4 (2C), 139.2 (C), 136.0 (C), 130.1 (2C), 128.6 (C), 128.5 (2C), 122.1 (C), 114.2 (2C), 89.3 (C), 55.9 (C), 51.5 (C), 9.3 (C), 8.2 (2C). MS (ESI) *m/z*: 437.41. IR (KBr, cm^{-1}): 3250, 1683, 1604, 1353, 1274, 1222, 863. Anal. calcd. For $C_{21}H_{19}N_5O_6$ (in %): C-57.66; H-4.38; N-16.01. Found C-57.64; H-4.32; N-16.00.

*Synthesis of N-(1-(4-methoxybenzyl)-3-cyclopropyl-1H-pyrazol-5-yl)-3-methoxybenzamide (**9b**)* The product as a yellow colored solid was obtained from 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** (0.25 g, 1.13 mmol), 3-methoxybenzoyl chloride **(8b)** (0.21 g, 1.13 mmol), and triethylamine (0.46 g, 3.39 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.51 (s, 1H, -NH), 7.53 (s, 2H, Ar-H), 7.46 (s, 2H, Ar-H), 7.33 (d, 1H, *J* = 7.4 Hz, Ar-H), 7.02 (d, 1H, *J* = 7.6 Hz, Ar-H), 6.95 (d, 2H, *J* = 6.52 Hz, Ar-H), 6.92 (d, 2H, *J* = 6.2 Hz, Ar-H), 5.18 (s, 1H, Ar-H), 5.02 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.83 (s, 3H, $-OCH_3$), 1.85 (m, 1H, -CH), 0.92 (m, 2H, $-CH_2$), 0.71 (m, 2H, $-CH_2$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 165.4 (C), 164.8 (C), 160.8 (C), 157.7 (C), 139.2 (C), 135.2 (C), 130.1 (2C), 129.9 (C), 128.6 (C), 119.8 (C), 117.7 (2C), 114.2 (2C), 89.3 (C), 55.9 (2C), 51.5 (C), 9.3 (C), 8.2 (2C). MS (ESI) *m/z*: 377.44. IR (KBr, cm^{-1}): 3290, 1681, 1601, 1353, 1274, 1223, 864. Anal. calcd. for $C_{22}H_{23}N_3O_3$ (in %): C-70.01; H-6.14; N-11.13. Found C-69.01; H-6.04; N-11.10.

*Synthesis of N-(1-(4-methoxybenzyl)-3-cyclopropyl-1H-pyrazol-5-yl)-4-tert-butylbenzamide (**9c**)* The product as a yellow colored solid was obtained from

1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** (0.25 g, 1.13 mmol), 4-*tert*-butylbenzoyl chloride **(8c)** (0.24 g, 1.13 mmol), and triethylamine (0.46 g, 3.39 mmol). ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 10.51 (s, 1H, -NH), 7.87 (d, 2H, J = 7.9 Hz, Ar-H), 7.47 (s, 1H, Ar-H), 6.95 (d, 2H, J = 6.52 Hz, Ar-H), 6.92 (d, 2H, J = 6.2 Hz, Ar-H), 5.18 (s, 1H, Ar-H), 5.02 (s, 2H, -CH$_2$), 3.85 (s, 3H, -OCH$_3$), 1.85 (m, 1H, -CH), 1.36 (s, 3H, -CH$_3$), 1.34 (s, 3H, -CH$_3$), 1.32 (s, 3H, -CH$_3$), 0.92 (m, 2H, -CH$_2$), 0.71 (m, 2H, -CH$_2$). ^{13}C NMR (CDCl$_3$, 400 MHz) δ: 165.4 (C), 164.8 (C), 157.7 (C), 153.4 (C), 139.2 (C), 131.1 (C), 130.1 (2C), 128.6 (C), 127.1 (2C), 125.2 (2C), 114.2 (2C), 89.3 (C), 55.9 (C), 51.5 (C), 40.7 (C), 31.4 (3C), 9.3 (C), 8.2 (2C). MS (ESI) *m/z*: 403.52. IR (KBr, cm^{-1}): 3350, 1683, 1601, 1353, 1274, 1223, 863. Anal. calcd. For $C_{25}H_{29}N_3O_2$ (in %): C-74.41; H-7.24; N-10.41. Found C-74.35; H-7.20; N-10.35.

*Synthesis of N-(1-(4-methoxybenzyl)-3-cyclopropyl-1H-pyrazol-5-yl)-2,4-dichlorobenzamide (**9d**)* The product as a yellow colored solid was obtained from 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** (0.25 g, 1.13 mmol), 2,4-dichlorobenzoyl chloride **(8d)** (0.25 g, 1.13 mmol), and triethylamine (0.46 g, 3.39 mmol). ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 10.51 (s, 1H, -NH), 7.83 (s, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 7.33 (t, 1H, J = 7.4 Hz, Ar-H), 6.95 (d, 2H, J = 6.52 Hz, Ar-H), 6.92 (d, 2H, J = 6.2 Hz, Ar-H), 5.18 (s, 1H, Ar-H), 5.02 (s, 2H, -CH$_2$), 3.85 (s, 3H, -OCH$_3$), 1.85 (m, 1H, -CH), 0.92 (m, 2H, -CH$_2$), 0.71 (m, 2H, -CH$_2$). ^{13}C NMR (CDCl$_3$, 400 MHz) δ: 165.4 (C), 164.8 (C), 157.7 (C), 139.2 (2C), 133.7 (C), 130.5 (2C), 129.3 (3C), 128.6 (C), 127.1 (C), 114.2 (2C), 89.3 (C), 55.9 (C), 51.5 (C), 9.3 (C), 8.2 (2C). MS (ESI) *m/z*: 416.3. IR (KBr, cm^{-1}): 3320, 1683, 1601, 1353, 1272, 1223, 864. Anal. calcd. For $C_{21}H_{19}Cl_2N_3O_2$ (in %): C-60.59; H-4.60; N-10.09. Found C-60.49; H-4.50; N-10.06.

*Synthesis of N-(1-(4-methoxybenzyl)-3-cyclopropyl-1H-pyrazol-5-yl)-4-chlorobenzamide (**9e**)* The product as a yellow colored solid was obtained from 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** (0.25 g, 1.13 mmol), 4-chlorobenzoyl chloride **(8e)** (0.21 g, 1.13 mmol), and triethylamine (0.46 g, 3.39 mmol). ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 10.51 (s, 1H, -NH), 7.89 (d, 2H, J = 8.2 Hz, Ar-H), 7.45 (d, 2H, J = 7.6 Hz, Ar-H), 6.95 (d, 2H, J = 6.52 Hz, Ar-H), 6.92 (d, 2H, J = 6.2 Hz, Ar-H), 5.18 (s, 1H, Ar-H), 5.02 (s, 2H, -CH$_2$), 3.85 (s, 3H, -OCH$_3$), 1.85 (m, 1H, -CH), 0.92 (m, 2H, -CH$_2$), 0.71 (m, 2H, -CH$_2$). ^{13}C NMR (CDCl$_3$, 400 MHz) δ: 165.4 (C), 164.8 (C), 157.7 (C), 139.2 (C), 137.7 (C), 132.3 (C), 130.1 (2C), 129.4 (2C), 128.9 (2C), 127.6 (C), 114.2 (2C), 89.3 (C), 55.9 (C), 51.5 (C), 9.3 (C), 8.2 (2C). MS (ESI) *m/z*: 381.86. IR (KBr, cm^{-1}): 3364, 1683, 1600, 1353, 1273, 1222, 862. Anal. calcd. For $C_{21}H_{20}ClN_3O_2$ (in %): C-66.05; H-5.28; N-11.00. found C-66.02; H-5.26; N-11.03.

*Synthesis of N-(1-(4-methoxybenzyl)-3-cyclopropyl-1H-pyrazol-5-yl)-3-bromobenzamide (**9f**)* The product as a yellow colored solid was obtained from 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** (0.25 g, 1.13 mmol), 3-bromobenzoyl chloride **(8f)** (0.27 g, 1.13 mmol), and triethylamine (0.46 g, 3.39 mmol). ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 10.58 (s, 1H, Ar-H), 10.51 (s, 1H, -NH), 7.89 (d, 1H, J = 8.2 Hz, Ar-H), 7.68 (d, 1H, J = 7.52 Hz, Ar-H), 7.33(t, 1H, J = 7.3 Hz, Ar-H), 6.95 (d, 2H, J = 6.52 Hz, Ar-H), 6.92 (d, 2H, J = 6.2 Hz, Ar-H), 5.18 (s, 1H, Ar-H), 5.02 (s, 2H, -CH$_2$), 3.85 (s, 3H, -OCH$_3$), 1.85 (m, 1H, -CH), 0.92 (m, 2H, -CH$_2$), 0.71 (m, 2H, -CH$_2$). ^{13}C NMR (CDCl$_3$, 400 MHz) δ: 165.4 (C), 164.8 (C), 157.7 (C), 139.2 (C), 136.4 (C), 135.1 (C), 131.1 (2C), 130.1 (2C), 128.6 (C), 126.5 (C), 123.2 (C), 114.2 (2C), 89.3 (C), 55.9 (C), 51.5 (C), 9.3 (C), 8.2 (2C). MS (ESI) *m/z*: 426.31. IR (KBr, cm^{-1}): 3260, 1684, 1603, 1354, 1276, 1224, 864. Anal. calcd. for $C_{21}H_{20}BrN_3O_2$ (in %): C-59.15; H-4.73; N-9.86. Found C-59.17; H-4.70; N-9.83.

*Synthesis of N-(1-(4-methoxybenzyl)-3-cyclopropyl-1H-pyrazol-5-yl)-2,4-difluorobenzamide (**9g**)* The product as a yellow colored solid was obtained from 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** (0.25 g, 1.13 mmol), 2-fluorobenzoyl chloride **(8g)** (0.19 g, 1.13 mmol), and triethylamine (0.46 g, 3.39 mmol). ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 10.51 (s, 1H, -NH), 7.93 (t, 1H, J = 7.58 Hz, Ar-H), 7.49 (t, 1H, J = 7.42 Hz, Ar-H), 7.21 (t, 1H, J = 7.12 Hz, Ar-H), 6.95 (d, 2H, J = 6.52 Hz, Ar-H), 6.92 (d, 2H, J = 6.2 Hz, Ar-H), 5.18 (s, 1H, Ar-H), 5.02 (s, 2H, -CH$_2$), 3.85 (s, 3H, -OCH$_3$), 1.85 (m, 1H, -CH), 0.92 (m, 2H, -CH$_2$), 0.71 (m, 2H, -CH$_2$). ^{13}C NMR (CDCl$_3$, 400 MHz) δ: 167.9 (C), 165.4 (C), 164.8 (C), 159.6 (C), 157.7 (C), 139.2 (C), 130.1 (3C), 128.6 (C), 120.7 (C), 114.2 (2C), 111.2 (C), 104.8 (C), 89.3 (C), 55.9 (C), 51.5 (C), 9.3 (C), 8.2 (2C). MS (ESI) *m/z*: 365.4. IR (KBr, cm^{-1}): 3260, 1685, 1602, 1355, 1277, 1225, 865. Anal. calcd. for $C_{21}H_{19}F_2N_3O_2$ (in %): C-69.03; H-5.52; N-11.50. Found C-69.01; H-5.50; N-11.50.

*Synthesis of N-(1-(4-methoxybenzyl)-3-cyclopropyl-1H-pyrazol-5-yl) benzamide (**9h**)* The product as a yellow colored solid was obtained from 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine **(7)** (0.25 g, 1.13 mmol), benzoyl chloride **(8h)** (0.17 g, 1.13 mmol), and triethylamine (0.46 g, 3.39 mmol). ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 10.51 (s, 1H, -NH), 7.95 (d, 2H, J = 7.62 Hz, Ar-H), 7.51 (t, 1H, J = 7.86 Hz, Ar-H), 7.44 (d, 2H, J = 7.2 Hz, Ar-H), 6.95 (d, 2H, J = 6.52 Hz, Ar-H), 6.92 (d, 2H, J = 6.2 Hz, Ar-H), 5.18 (s, 1H, Ar-H), 5.02 (s, 2H, -CH$_2$), 3.85 (s, 3H, -OCH$_3$), 1.85 (m, 1H, -CH), 0.92 (m, 2H, -CH$_2$), 0.71 (m, 2H, -CH$_2$). ^{13}C NMR (CDCl$_3$, 400 MHz) δ: 165.4 (C), 164.8 (C), 157.7 (C), 139.2 (C), 134.2 (C), 132.2 (C), 130.1 (2C), 129.4 (C), 128.6 (2C), 127.5 (2C), 114.2 (2C), 89.3 (C), 55.9 (C), 51.5 (C), 9.3 (C), 8.2 (2C). MS (ESI) *m/z*: 347.41. IR (KBr, cm^{-1}): 3328, 1682, 1601, 1352, 1274, 1223, 864. Anal. calcd. for $C_{21}H_{21}N_3O_2$ (in %): C-72.60; H-6.09; N-12.10. Found C-72.58; H-6.05; N-12.07.

Antibacterial activity

Microorganisms used were Gram-positive (*Streptococcus pyogenes* NCIM 2608, *Staphylococcus aureus* ATCC 29737, *Bacillus subtilis* NCIM 2010) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 20852, *Klebsiella pneumoniae* MTCC 618) bacteria. A single colony of each of the cultures was inoculated in 5 mL of Luria–Bertani (LB) broth and incubated under shaking conditions overnight at 37°C. Each of the overnight cultures (500 µL) was inoculated into 25 mL of LB broth and incubated at 37°C at 0.2 optical density (OD; $\lambda_{max} = 600$ nm). Each of these cultures (500 μL) was inoculated into a conical flask containing 30 mL of nutrient broth. The contents of the flasks were poured into petri plates and allowed to solidify. The plates were appropriately labeled. On solidifying, 10 wells were made on each plate using a cork-borer first for the negative control (dimethylsulfoxide, DMSO), second for the positive control (streptomycin), and the remaining third and fourth wells for the compounds. The plates were kept at room temperature for 30 min and then at 37°C for 24 h. After 24 h, the zone of inhibition was measured and recorded. The minimum inhibitory concentration (MIC) values were also determined for the microorganisms that were determined as sensitive to the compound disk diffusion assay. The inocula of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity and the compounds were dissolved in DMSO. MIC values of compounds against each bacterial strain were determined based on the microwell dilution method with some modification. The 96-well plates were prepared by dispensing 95 μL of nutrient broth and 5 μL of the inoculum into each well. Initially, prepared compound (100 μL) at the concentration of 600 μg/mL was added into the first well. Then, 100 μL from the serial dilution was transferred into six consecutive wells. The last well containing 195 μL of nutrient broth without compound and 5 μL of inoculum on each strip was used as the negative control. The final volume in each well was 200 μL. Streptomycin and DMSO at the concentration of 100 μL were prepared in nutrient broth and used as standard drugs for the positive and negative controls, respectively. The plates were covered with a sterile plate sealer. The contents of each well were mixed on a plate shaker at 300 rpm for 30 min and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by the absorbance at 610 nm using an ELx800 universal microplate reader (Bio-Tek Instruments Inc., Highland Park, Vermont, USA) and confirmed by plating 5 μL samples from clear wells on nutrient agar medium. The compounds used in this study were screened twice against each organism. The MIC was defined as the lowest concentration of each compound required to inhibit the growth of a particular organism.

Antifungal activity

Synthesized compounds were screened *in vitro* for their antifungal activities against mycotoxigenic strains of *Fusarium verticillioides*, *Aspergillus ochraceous*, *Aspergillus flavus*, *Alternaria alternata*, and *Penicillium chrysogenum*, which are capable of producing toxins that have proved to be toxic to both animals and plants. Potato dextrose agar (PDA) medium was used as the growth medium for *F. verticillioides* and *A. alternata*, and Czapek–Dox agar (CDA) medium for *Aspergillus* and *Penicillium* spp. A spore suspension of pathogen was prepared in 50 mL sterile distilled water. The spore concentration was adjusted to 1×10^6 spores/mL using a hemocytometer. A suspension of 2 mL was spread on PDA and CDA plates uniformly. After solidifying, four wells were bored in each petri plate using a cork borer (0.5 cm diameter). DMSO (negative control) in the first well, nystatin (positive control) in the second well, and synthesized compounds in the remaining two wells were taken in each plate. The plates were incubated at 23 ± 2°C under alternate cycles of 12/12 h NUV (near-ultraviolet light) and darkness. After 4 days of incubation, plates were evaluated based on the zone of inhibition caused by the compounds. Compounds were screened for their antifungal activity at different concentrations, using nystatin as positive control and DMSO as negative control. To the culture tubes containing 1.9 mL of sterile medium, 0.1 mL of test compound was added under sterile conditions. Fresh inoculum was added to all the tubes including standard and controls, with a spore concentration adjusted to 1×10^6 spores/mL. After incubating all tubes at 37°C for 48 h, the absorbance was recorded at 610 nm. Percentage inhibition was calculated according to the formula:

$$\% \text{ Inhibition} = 100(P - Q)/P$$

where P is absorbance without test sample and Q is absorbance with test sample. The MIC was recorded in μg/mL. All determinant tests were performed in duplicate and the results are reported as the mean of these values.

Results and discussion

The synthesized compounds showed significant and moderate inhibition against the bacterial strains. Among the compounds **9(a–h)**, **9d**, **9g**, and **9h** showed significant inhibitory activity (zones of inhibition in the order **9d** > **9g** > **9h**, 4.9–8.0 mm, 5.1–7.3 mm, and 5.8–7.2 mm, respectively) against bacterial strains. The remaining compounds showed moderate inhibitory activity compared to the standard, streptomycin. Minimum inhibitory concentration was 06 μg/mL against *K. pneumoniae* MTCC 618, 07 μg/mL against *B. subtilis* NCIM 2010, and 09 μg/mL against *P. aeruginosa* ATCC 20852. The antibacterial activities of the tested compounds are shown in Tables 2 and 3. As for the antifungal activity of compounds against the fungal pathogens, among the series **9(a–h)**, compounds **9d**, **9g**, and **9h** showed significant inhibitory activity in the order of **9d** > **9g** > **9h** (zones of inhibition 2.3–4.4 mm, 2.3–3.5 mm, and 2.1–3.2 mm, respectively). The other compounds showed moderate inhibitory activity compared to the standard drug, nystatin. Minimun inhibitory concentration for antifungal activity was 09 μg/mL against *Aspergillus ochraceous*, 11 μg/mL against *A. flavus*, and 12 μg/mL against *A. alternata*, respectively. The antifungal activities of the tested compounds are shown in Tables 4 and 5. From the results obtained, it is evident that the presence of two chloro groups at the 2nd and 4th positions in **9d**, the presence of two fluorine atoms at the 2nd and 4th positions in **9g**, and the presence of a benzoyl group in **9h** might be the reason for the significant inhibitory activity. These results confirm our previous reports[27,28]

Table 2. Inhibition zone (diameter) in mm of synthesized compounds tested against bacterial strains.

	Gram-positive bacteria			Gram-negative bacteria		
Compound	*S. pyogenes* NCIM 2608	*B. subtilis* NCIM 2010	*S. aureus* ATCC 29737	*E. coli* ATCC25922	*P. aeruginosa* ATCC 20852	*K. pneumoniae* MTCC 618
9a	4.1	3.6	6.2	3.5	4.7	5.9
9b	4.3	3.9	5.9	5.8	6.6	6.9
9c	3.7	3.2	—	4.2	5.4	6.1
9d	5.8	4.9	5.4	5.8	6.6	8.0
9e	5.4	3.7	5.2	5.0	6.3	6.2
9f	5.3	3.9	—	5.0	—	4.8
9g	6.2	5.1	5.9	5.8	6.4	7.3
9h	6.1	5.8	6.7	5.9	6.1	7.2
Streptomycin	5.9	4.9	5.2	5.6	6.4	5.3

Table 3. Minimum inhibitory concentration (MIC) in μg/mL of synthesized compounds tested against bacterial strains.

	Gram-positive bacteria			Gram-negative bacteria		
Compound	*S. pyogenes* NCIM 2608	*B. subtilis* NCIM 2010	*S. aureus* ATCC 29737	*E. coli* ATCC25922	*P. aeruginosa* ATCC 20852	*K. pneumoniae* MTCC 618
9a	51	61	56	72	66	58
9b	42	51	58	54	55	58
9c	65	59	73	49	63	73
9d	33	32	37	31	39	06
9e	43	51	60	43	52	53
9f	63	50	53	77	56	53
9g	27	07	21	31	42	38
9h	29	32	35	25	09	22
Streptomycin	39	34	42	39	44	47

Table 4. Inhibition zone (diameter) in mm of synthesized compounds tested against fungal pathogens.

	Microorganism used				
Compound	*F. verticilloides*	*A. alternata*	*A. flavus*	*A. ochraceous*	*P. chrysogenum*
9a	1.6	0.9	1.2	1.4	0.9
9b	1.4	1.1	2.0	2.2	1.1
9c	1.6	1.4	2.1	1.4	1.3
9d	4.1	3.6	3.4	4.4	2.3
9e	2.9	1.2	3.4	3.2	2.1
9f	1.9	2.4	—	0.4	—
9g	3.3	2.4	2.3	3.5	2.9
9h	3.2	2.1	2.5	3.2	2.1
Nystatin	1.7	1.4	2.1	2.1	1.9

Table 5. Minimum inhibitory concentration (MIC) in μg/mL of synthesized compounds tested against fungal pathogens.

	Microorganism used				
Compound	*F. verticilloides*	*A. alternata*	*A. flavus*	*A. ochraceous*	*P. chrysogenum*
9a	56	29	42	54	59
9b	54	51	50	22	41
9c	46	44	41	34	43
9d	13	14	23	09	39
9e	19	12	54	32	41
9f	49	34	—	44	—
9g	18	20	11	24	21
9h	21	12	31	32	23
Nystatin	32	24	37	39	39

which indicated that chlorine and fluorine atoms possess potent inhibitory activity. In this connection, different electron withdrawing groups attached to the phenyl ring as substituents linked to the benzoyl group were studied for antimicrobial efficacy. On the other hand, compound **9g** showed higher activity against bacterial strains, with an electron withdrawing fluorine atom at the 2nd and 4th positions. A further structure-activity relationship (SAR) study revealed that compound **9h**, without a substituent on the phenyl ring, also showed relatively significant inhibitory activity. We have briefly investigated the different SARs of the carboxamide **9(a-h)** functionalized derivatives with different groups added on the phenyl ring. These modifications change the potency of the antibacterial and antifungal activity profile of the synthesized compounds.

Declaration of interest

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