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Research Article
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SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF BENZOTHIAZOLYL-PYRAZOLINE CARBOXAMIDES

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ABSTRACT

*A new series of benzothiazole-pyrazoline-1- carboxamides (**5a-5p**) were synthesized and these compounds were confirmed by IR, NMR, mass spectroscopy and evaluated for their antimicrobial property. The compounds were tested for their antimicrobial activity against Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 6538P, Salmonella typhimurium ATCC 14028, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027 were used. Antifungal activity was performed against Candida albicans ATCC 10231 and Saccharomyces cerevisiae ATCC 9763 by a diffusion method. Among the synthesized compounds **5b, 5c** and **5d** showed an interesting antimicrobial activity against all the tested strains. The hydroxyl substitutions exhibited good inhibitory activity against bacterial and fungal strains, whereas the compounds with methoxy and/or methyl and/or chloro and/or nitro substitutions showed less activity. Hence, these results revealed that the inhibitory activity of synthesized compounds against tested strains depends upon not only on the nature of substituents but also on their relative positions.*

KEYWORDS

Benzothiazole-pyrazoline-1- carboxamides, ATTC

INTRODUCTION

The millions of people die yearly due to infections caused by microorganism's resistant to current antibiotics [1]. When an antibiotic is discovered and commercially available, the presence of resistant strains begins to decrease its clinical utility after a period of indiscriminate use, leading to upcoming use restriction [2]. The use of antibiotics with broad spectrum of action and low toxicity can diminish the efficacy of upcoming antimicrobial therapies, leading to the use of drug. A need for new antimicrobial agents is defensible as more microorganisms develop resistance to the present drugs available in the market. Resistance of pathogenic bacteria to antibiotics is quickly becoming a major problem in the community and hospital-based healthcare settings. The search for novel agents to battle resistant bacteria has become one of the most important areas of antibacterial research today [3]. Some microorganisms are resistant to all approved antibiotics and can only are treated with potentially toxic drugs. Pharmaceutical and organic chemists are trying to synthesize new drugs with better pharmacokinetic and dynamic properties. Especially numerous multi-drug resistant Gram-positive bacteria pathogens, which are methicillin-resistant like *Staphylococcus aureus* (MRSA), are growing threat to human health[4]. Similarly, the fungal infections caused by various species, such as, *Candida* and *Aspergillus*, have been rising in prevalence all over the world [5]. This has required new efforts for the development of new powerful antimicrobial agents with broad spectrum of activity that have an important role to control the emerging multi-drug resistance strains of bacteria and fungi [6]. Pyrazoline is a five-membered heterocyclic compound containing two nitrogen atoms in adjacent position and contains two endocyclic double bonds. It is dihydropyrazoline possessing only one endocyclic double bond and unique in their chemical behaviour. Among a wide range of heterocyclic compounds that have been explored for the development new molecules, pyrazolines constitute an interesting class of heterocycles due to their synthetic flexibility and effective biological activities such as anticancer[7], antioxidant[8], antibacterial[9], antifungal[9], antidepressant[10,11], antitubercular[7], anti-inflammatory[8], antimalarial[12], anthelmintic[13], anticonvulsant [11] properties and etc. Benzothiazole belongs to the family of bicyclic heterocyclic compounds having benzene nucleus fused with five-membered ring containing nitrogen and sulfur atoms. Benzothiazole consist of wide variety of biological activities and therapeutic

functions including antitubercular[14], antibacterial[14], antifungal[14], antimalarial[15], anticonvulsant[16], anthelmintic[17], analgesic[18], anti-inflammatory[18], antidiabetic[19] and anticancer[20] activities and etc. In an attempt, to identify new and potent anticancer agents, tried benzothiazole-pyrazole hybrid motif, thus may be exhibit synergistic anticancer effect here to generate new benzothiazolyl-pyrazoline derivatives as anticancer agents using simple methods.

EXPERIMENTAL

Chemistry

Melting points were determined using Thermonik Melting Point Apparatus (Campbell electronics, India) by capillary method and are uncorrected. Infrared (IR) spectra were taken on a Fourier Transform Infrared Spectrophotometer IR-Prestige 21 (Shimatzu Corporation, Japan) from 4000-400 cm^{-1} using KBr discs. ^1H NMR spectra were recorded at 400 MHz in DMSO-d_6 using a Bruker Avance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured at δ units (ppm) relative to tetramethylsilane (TMS). Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer (Jeol Ltd Akishima, Tokyo, Japan) using argon/xenon (6 kV, 10 mA) as FAB gas, m-nitrobenzyl alcohol as matrix, and 10 kV as accelerating voltage at room temperature. Elemental analysis was performed on a Vario EL III Elemental Analyser (Elementar, Germany) using sulfanilamide as standard. All chemicals were purchased from Merck, Spectrochem or CDH, India. Solvents were of reagent grade and were purified and dried by standard procedure. Reactions were monitored by thin-layer chromatography on silica gel plates in either iodine or UV chambers. Intermediates were characterized by IR spectroscopic analysis and elemental analysis for CHN. In the elemental analysis, the observed values were within ±0.4% of the calculated values. Final compounds were characterized by ^1H NMR and FAB mass spectrometry (MS). The final yields and the physicochemical data of the compounds **5a-5p** are presented in **Table 1.**

General procedure for synthesis of 2-amino-benzothiazoles:

A solution of aniline (0.03 M) in 95% acetic acid (20 ml) was added to a solution of KSCN (0.12 M) in 95% acetic acid (20 ml). The reaction mixture was cooled to 0 °C and a solution of Br_2 (1.6 ml) in acetic acid (10 ml) was added over 90 minutes; during the addition, the temperature should not raise to 5 °C. After addition, continued the stirring for about 3 hr at 10-15 °C, and then poured into hot water (300 ml). Separated hydrogen bromide salt was filtered, washed with acetic acid and dried. It was dissolved in hot water and neutralizes with 26% ammonium hydroxide solution, filtered the solid product, washed with water and recrystallized from ethanol[21].

*General procedure for the synthesis of chalcones (**2a-2p**)*

To a solution of suitably substituted benzaldehyde (0.01 M) and acetophenone (0.01 M) in ethanol (10 ml) was added aqueous solution of potassium hydroxide (60%) drop wise with continuous stirring at 0 °C over a period of 15 minutes. The reaction mixture was kept at room temperature for about 48 h with occasional shaking. After 48 h it was poured into ice-cold water, and then neutralized to pH 2 using 6 N hydrochloric acid. The yellow precipitate obtained was filtered, washed, dried, and recrystallized from dry methanol. The intermediates **2a-2p** were obtained.

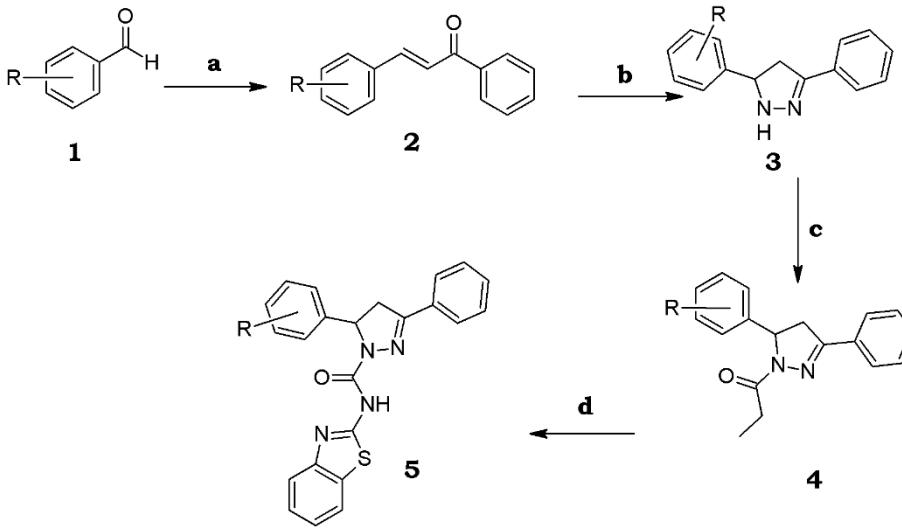
*General procedure for the synthesis of 3,5-diaryl-4,5-dihydro-1H-pyrazole (**3a-3p**)*

Appropriate chalcone (1–2) was treated with 10 times excess of hydrazine hydrate in dry ethanol and refluxed for 3–6 h. The hot reaction mixture was then poured into ice-cold water. The solid separated out was filtered, washed, dried and recrystallized from ethanol to afford respective pyrazoline (**3a-3p**).

General procedure for the synthesis of N-(1,3-benzothiazol-2-yl)-3-(substituted phenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5a-5p**):

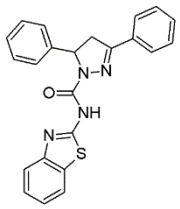
Phenyl chloroformate (0.001 M) and triethylamine (0.001 M) were added to an ice-cooled solution of appropriate 3,5-diaryl-4,5-dihydro-1H-pyrazole derivative (**3a-3p**, 0.001 M) in dry THF and the mixture was stirred for 1 h. The solid obtained was filtered off and to the filtrate was added freshly prepared solution of 2-amino benzothiazole in THF. After stirring at room temperature for 3 h, the solid obtained was filtered, dried and recrystallized from suitable solvent to afford respective pyrazolines (**5a-5p**).

Scheme. 1

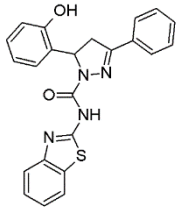


Reagents and condition: (a). Acetophenone, KOH (60%), stirring at 0 °C, 15 min, 48 hr, RT; (b) NH_2NH_2, ethanol, reflux 3–6 h; (c). Phenyl chloroformate, trimethylamine, THF, stirring at below 5 °C, 1 hr; (d). 2-amino benzothiazole, THF, stirring, RT, 3 hrs.

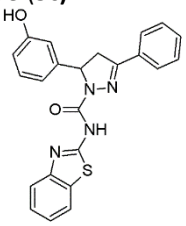
N-(1,3-benzothiazol-2-yl)-3,5-diphenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5a**):
IR (KBr, cm^{-1}): 3148 (Ar-H), 3089, 2865 (C-H), 1616 (C=N), 1233 (C-N), 1264 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.36 (d, 2H, CH_2), 5.44 (t, 1H, CH_2), 6.78-7.36 (m, 14H, ArH), 9.02 (s, 1H, NH); FAB-MS (m/z): 399 $[m+1]^+$; Elemental analyses Found (Calcd.): C 69.12; (69.32) H 4.56; (4.55) N 14.03 (14.06)



N-(1,3-benzothiazol-2-yl)-5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5b**):
IR (KBr, cm^{-1}): 3079 (Ar-H), 3056, 2918 (C-H), 1620 (C=N), 1463 (C-N), 1256 (C-S), 1315 (C-O), 2794 (O-H); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.56 (d, 2H, CH_2), 5.88 (t, 1H, CH_2), 6.92-7.18 (m, 4H, ArH), 7.34-7.52 (m, 9H, ArH), 8.82 (Ar-OH), 9.06 (s, 1H, NH); FAB-MS (m/z): 415 $[M+1]^+$; Elemental analyses Found (Calcd.): C 66.40 (66.65); H 4.37 (4.38); N 13.50 (13.52)

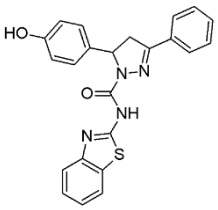


N-(1,3-benzothiazol-2-yl)-5-(3-hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5c**):
IR (KBr, cm^{-1}): 3082 (Ar-H), 3056, 2851 (C-H), 1629 (C=N), 1460 (C-N), 1286 (C-S), 1308 (C-O), 2796 (O-H); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.58 (d, 2H, CH_2), 5.72 (t, 1H, CH_2), 6.96-7.16 (m, 4H, ArH), 7.38-7.44 (m, 9H, ArH), 8.88 (Ar-OH), 9.08 (s, 1H, NH); FAB-MS (m/z): 415 $[M+1]^+$; Elemental analyses Found (Calcd.): C 66.42 (66.65); H 4.39 (4.38); N 13.53 (13.52)

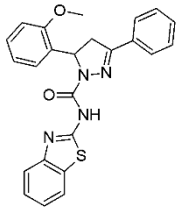


N-(1,3-benzothiazol-2-yl)-5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5d**):

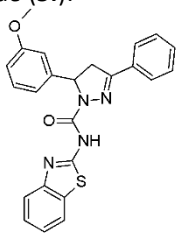
IR (KBr, cm-1): 2925 (Ar-H), 3080, 2865 (C-H), 1630 (C=N), 1440 (C-N), 1276 (C-S), 1298(C-O), 2790 (O-H); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.60 (d, 2H, CH_2), 5.64 (t, 1H, CH_2), 6.94-7.18 (m, 4H, ArH), 7.36-7.48 (m, 9H, ArH), 8.76 (Ar-OH), 9.22 (s, 1H, NH); FAB-MS (m/z): 415 $[M+1]^+$; Elemental analyses Found (Calcd.): C 66.46 (66.65); H 4.41 (4.38); N 13.49 (13.52)



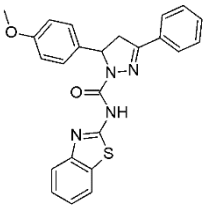
N-(1,3-benzothiazol-2-yl)-5-(2-methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5e**):
IR (KBr, cm^{-1}): 3121 (Ar-H), 3051, 2851 (C-H), 1632 (C=N), 1471 (C-N), 1281 (C-S), 1310 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.50 (s, 3H, -O-CH_3, 3.42 (d, 2H, CH_2), 5.80 (t, 1H, CH_2), 6.82-7.02 (m, 4H, ArH), 7.24-7.36 (m, 9H, ArH), 9.16 (s, 1H, NH); FAB-MS (m/z): 429 $[M+1]^+$; Elemental analyses Found (Calcd.): C 67.32 (67.27); H 4.69 (4.70); N 13.05(13.07)



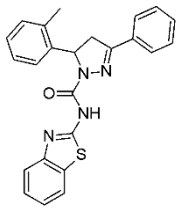
N-(1,3-benzothiazol-2-yl)-5-(3-methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5f**):
IR (KBr, cm^{-1}): 2998 (Ar-H), 3062, 2870 (C-H), 1670 (C=N), 1264 (C-N), 1298 (C-S), 1346(C-O); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.55 (s, 3H, -O-CH_3, 3.48 (d, 2H, CH_2), 5.88 (t, 1H, CH_2), 6.90-7.14 (m, 4H, ArH), 7.32-7.42 (m, 9H, ArH), 9.18 (s, 1H, NH) ; FAB-MS (m/z): 429 $[M+1]^+$; Elemental analyses Found (Calcd.): C 67.34 (67.27); H 4.71 (4.70); N 13.09 (13.07)



N-(1,3-benzothiazol-2-yl)-5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5g**):
IR (KBr, cm-1): 3082 (Ar-H), 3056, 2844 (C-H), 1684 (C=N), 1436 (C-N), 1236 (C-S), 1287 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.60 (s, 3H, -OCH_3, 3.54 (d, 2H, CH_2), 5.66 (t, 1H, CH_2), 6.84-7.08 (m, 4H, ArH), 7.24-7.38 (m, 9H, ArH), 9.16 (s, 1H, NH); FAB-MS (m/z): 429 $[M+1]^+$; Elemental analyses Found (Calcd.): C 67.39 (67.27); H 4.69 (4.70); N 13.10 (13.07)

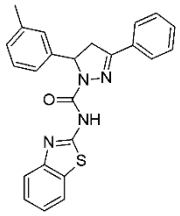


N-(1,3-benzothiazol-2-yl)-5-(2-methylphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5h**):
IR (KBr, cm-1): 3130 (Ar-H), 3084, 2919 (C-H), 1636 (C=N), 1398 (C-N), 1264 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.22 (s, 3H, -CH_3, 3.20 (d, 2H, CH_2) , 5.28 (t, 1H, -CH), 6.72-6.84 (m, 4H, ArH), 6.98-7.14 (m, 9h, ArH), 9.12 (s, 1H, NH); FAB-MS (m/z): 413 $[M+1]^+$; Elemental analyses Found (Calcd.): C 69.72 (69.88); H 4.88 (4.89); N 13.56 (13.58)

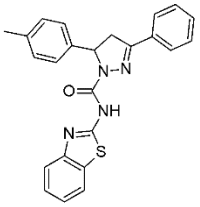


N-(1,3-benzothiazol-2-yl)-5-(3-methylphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5i**)*:*

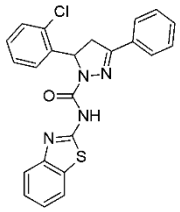
IR (KBr, cm^{-1}): 3094 (Ar-H), 3060, 2941 (C-H), 1662 (C=N), 1468 (C-N), 1270 (C-S); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 2.28 (s, 3H, $-CH_3$), 3.24 (d, 2H, CH_2), 5.26 (t, 1H, -CH), 6.50-6.74 (m, 4H, ArH), 6.92-7.10 (m, 9h, ArH), 9.06 (s, 1H, NH); FAB-MS (m/z): 413 $[M+1]^+$; Elemental analyses Found (Calcd.): C 69.76 (69.88); H 4.90 (4.89); N 13.59 (13.58)



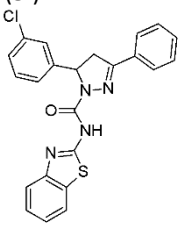
N-(1,3-benzothiazol-2-yl)-5-(4-methylphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5j**):
IR (KBr, cm^{-1}): 3024 (Ar-H), 3070, 2954 (C-H), 1676 (C=N), 1454 (C-N), 1263 (C-S); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 2.22 (s, 3H, $-CH_3$, 3.30 (d, 2H, CH_2) , 5.28 (t, 1H, -CH), 6.54-6.78 (m, 4H, ArH), 7.12-7.39 (m, 9h, ArH), 9.04 (s, 1H, NH) ; FAB-MS (m/z): 413 $[M+1]^+$; Elemental analyses Found (Calcd.): C 69.80 (69.88); H 4.87 (4.89); N 13.60 (13.58)



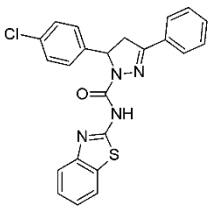
N-(1,3-benzothiazol-2-yl)-5-(2-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5k**):
IR (KBr, cm^{-1}): 3040 (Ar-H), 3048, 2846(C-H), 1624 (C=N), 1284 (C-N), 1200 (C-S), 812 (C-Cl); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.42 (d, 2H, CH_2), 5.90 (t, 1H, CH), 7.33-7.44 (m, 4H, ArH), 7.92-8.08 (m, 9H, ArH), 9.24 (s, 1H, NH); FAB-MS (m/z): 433 $[M+1]^+$; Elemental analyses Found (Calcd.): C 63.62 (63.81); H 3.95 (3.96); N 12.92 (12.94)



N-(1,3-benzothiazol-2-yl)-5-(3-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5l**):
IR (KBr, cm^{-1}): 3060, (Ar-H), 3028, 2952 (C-H), 1620 (C=N), 1285 (C-N), 1216 (C-S), 818 (C-Cl); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.34 (d, 2H, CH_2), 5.82 (t, 1H, CH), 7.26-7.58 (m, 4H, ArH), 7.94-8.30 (m, 9H, ArH), 9.26 (s, 1H, NH); FAB-MS (m/z): 433 $[M+1]^+$; Elemental analyses Found (Calcd.): C 63.76 (63.81); H 3.97 (3.96); N 12.96 (12.94)

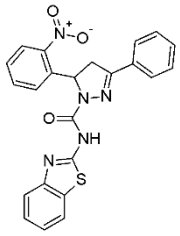


N-(1,3-benzothiazol-2-yl)-5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5m**):
IR (KBr, cm^{-1}): 3042 (Ar-H), 3082, 2842 (C-H), 1634(C=N), 1278 (C-N), 1216 (C-S) 816 (C-Cl); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.42 (d, 2H, CH_2), 6.06 (t, 1H, CH), 7.46-7.70 (m, 4H, ArH), 8.18-8.26 (m, 9H, ArH), 9.28 (s, 1H, NH); FAB-MS (m/z): 433 $[M+1]^+$; Elemental analyses Found (Calcd): C 63.79 (63.81); H 3.95 (3.96); N 12.93 (12.94)

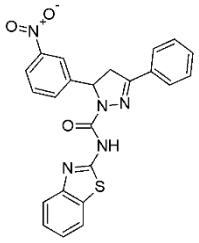


N-(1,3-benzothiazol-2-yl)-5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5n**):

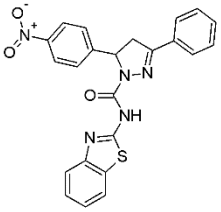
IR (KBr, cm^{-1}): 3080 (Ar-H), 3060, 2860 (C-H), 1634 (C=N), 1466 (C-N), 1240 (C-S); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.26 (d, 2H, CH_2) , 5.34 (t, 1H, -CH), 6.96-7.26 (m, 9h, ArH), 8.14-8.28 (m, 4H, ArH), 9.16 (s, 1H, NH); FAB-MS (m/z): 444 $[M+1]^+$; Elemental analyses Found (Calcd.): C 62.26 (62.29); H 3.85 (3.86); N 15.76 (15.79)



N-(1,3-benzothiazol-2-yl)-5-(3-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5o**):
IR (KBr, cm^{-1}): 3120 (Ar-H), 3058, 2900 (C-H), 1624 (C=N), 1460 (C-N), 1244 (C-S); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.30 (d, 2H, CH_2) , 5.30 (t, 1H, -CH), 6.98-7.14 (m, 9h, ArH), 7.98-8.26 (m, 4H, ArH), 9.18 (s, 1H, NH); FAB-MS (m/z): 444 $[M+1]^+$; Elemental analyses Found (Calcd.): C 62.40 (62.29); H 3.87 (3.86); N 15.80 (15.79)



N-(1,3-benzothiazol-2-yl)-5-(4-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**5p**):
IR (KBr, cm^{-1}): 3098 (Ar-H), 3062, 2940 (C-H), 1626 (C=N), 1464 (C-N), 1260 (C-S); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 3.32 (d, 2H, CH_2) , 5.28 (t, 1H, -CH), 6.92-7.29 (m, 9h, ArH), 7.88-8.29 (m, 4H, ArH), 9.16 (s, 1H, NH); FAB-MS (m/z): 444 $[M+1]^+$; Elemental analyses Found (Calcd.): C 62.52 (62.29); H 3.85 (3.86); N 15.82 (15.79)



Pharmacology

Antimicrobial activity

The synthesized compounds **5a-p** were screened for their *in vitro* antimicrobial activity against five strains of bacteria and two fungal strains using a disk diffusion assay and dilution method. For antibacterial screening various bacteria, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 were used. Antifungal activity was performed against *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763. Each tested strain was previously grown in Triptic Soy Broth at 25°C for 24 h, and bacterial and fungi suspensions were adjusted to the turbidity of 0.5 McFarland (1.5 x 108 CFU/mL) with a sterile saline solution (0.85% NaCl). The screening results were compared with chloramphenicol for antibacterial and ketoconazole for antifungal activities respectively and dimethyl sulfoxide treated group served as a control. Mueller Hinton and Sabouraud medium were used for determination of antimicrobial activity (diffusion method) benzothiazolyl-pyrazoline carboxamides derivatives by disc diffusion method results were presented as the inhibition zones, given in millimeters (mm). When using the diffusion method, the test samples were dissolved in 30% dimethyl sulfoxide (DMSO) to obtain a 1000 µg/ml stock solution. Bacteria inhibition zones were measured in millimeters at the end of an incubation period of 18 h at 37°C, and fungi zones after 48 h at 25°C [22].

Results and Discussion:

Chemistry

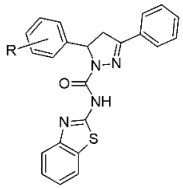
The compounds were synthesized as shown in **Scheme 1** according to previously reported method[23]. The synthesis of chalcones (**2a-2p**) was carried out at room temperature by reacting with different substituted acetophenone and benzaldehyde in the presence of base by conventional Claisen–Schmidt condensation. These chalcones were then reacted with hydrazine in ethanol using catalytic amount of concentrated sulphuric acid offered **3a-3p.** The solid compound so obtained was filtered and purified by recrystallization from ethanol. The final pyrazoline derivatives **5a-5p** were obtained by the reaction of appropiriate pyrazoline **3a–3p** with phenyl chloroformate followed by 2-amino benzothiazole in THF at room temperature. The pyrazoline derivatives were

characterized by their spectral studies using IR, ^{1}H NMR, and FAB-MS. All of the synthesized pyrazoline compounds gave satisfactory analytical and spectroscopic data, which were in full consistent with their depicted structures. The structures of pyrazolines (**5a-5p**) were confirmed through the IR, ^{1}H NMR, FAB-MS spectral data. In the elemental analysis of CHN, the observed values were within ±0.4% of the calculated values.

Antimicrobial activity

The antimicrobial activity, *i.e*. antibacterial and antifungal activity of pyrazolines **5a–5p**, was studied *in vitro* by disc diffusion methods respectively against five bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal strains (*Candida albicans* and *Saccharomyces cerevisiae*) at the concentration of 100 µL. The screening results indicated that all the compounds exhibited moderate to good antimicrobial activities against tested strains. It was noticed that the pyrazolines with only hydroxyl substitution, **5b, 5c** and **5d** exhibited good inhibitory activity against bacteria and fungal strains, whereas the compounds with methoxy and/or methyl and/or chloro and/or nitro substitutions showed less activity. Hence, these results revealed that the inhibitory activity of synthesized compounds against tested strains depends upon not only on the nature of substituents but also on their relative positions. From the structure-activity analysis, it is very clear that methoxy groups are responsible for decrease the antibacterial activity of compounds [24]. The results of diameter of zone of inhibition (in mm) of synthetic pyrazolines have been incorporated in **Table 2**.

Table. 1: Physical data of **5a-5p**



Code	R	MF	MW	% Yield
5a	H	$C_{23}H_{18}N_4OS$	398	63.40
5b	2-OH	$C_{23}H_{18}N_4O_2S$	414	65.28
5c	3-OH	$C_{23}H_{18}N_4O_2S$	414	64.81
5d	4-OH	$C_{23}H_{18}N_4O_2S$	414	65.49
5e	2-OCH_3	$C_{24}H_{20}N_4O_2S$	428	72.01
5f	3-OCH_3	$C_{24}H_{20}N_4O_2S$	428	74.49
5g	4-OCH_3	$C_{24}H_{20}N_4O_2S$	428	72.96
5h	2-CH_3	$C_{24}H_{20}N_4OS$	412	69.83
5i	3-CH_3	$C_{24}H_{20}N_4OS$	412	68.27
5j	4-CH_3	$C_{24}H_{20}N_4OS$	412	69.56
5k	2-Cl	$C_{23}H_{17}ClN_4OS$	432	87.23
5l	3-Cl	$C_{23}H_{17}ClN_4OS$	432	86.92
5m	4-Cl	$C_{23}H_{17}ClN_4OS$	432	82.74
5n	2-NO_2	$C_{23}H_{17}N_5O_3S$	443	79.68
5o	3-NO_2	$C_{23}H_{17}N_5O_3S$	443	86.25
5p	4-NO_2	$C_{23}H_{17}N_5O_3S$	443	76.63

Table 2: Antibacterial and Antifungal activities of 5a-5p derivatives and zone of inhibition (in mm) at 100 µL.

Compound	*B. subtilis*	*S. aureus*	*S. typhimurium*	*E. coli*	*P.aeruginosa*	*C. albicans*	*S. cerevisiae*
5a	11	12	-	13	12	10	11
5b	18	20	19	17	16	14	15
5c	18	20	19	18	15	15	14
5d	18	20	19	19	17	16	15
5e	14	15	-	11	-	9	10
5f	13	-	12	-	13	11	12
5g	14	15	-	14	-	9	8
5h	12	-	13	12	10	10	11
5i	11	12	-	13	11	12	12
5j	11	13	14	-	10	9	8
5k	13	-	14	-	11	12	11
5l	12	14	-	13	12	9	8
5m	14	15	15	-	12	11	13
5n	9	-	10	9	-	8	6
5o	10	10	-	11	9	8	9
5p	8	-	10	12	13	7	8
Chloramphenicol	24	22	23	21	20	-	-
Ketoconazole	-	-	-	-	-	16	14

CONCLUSION:

The present investigation synthesized 16 molecules (**5a–5p**) and characterized based on its physical and spectral data. The synthesized compounds were exhibited moderate to good antimicrobial activities against tested strains. Furthermore, our preliminary results which support the antimicrobial potential of the synthesized compounds, suggest that generating hybrid compounds containing N-benzothiazole-pyrazolines are a promising new approach of developing an effective antimicrobial agent.

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