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GREEN APPROACH FOR THE FACILE CONSTRUCTION OF PYRAZOLYLPYRAZOLINE BEARING BENZOTHIAZOLE DERIVATIVES AND ITS BIOLOGICAL EVALUATION

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Abstract A new, facile and environment friendly protocol for the synthesis of 1,3-diphenyl-1*H*-pyrazolyl-2-benzo[d]thiazole-2-pyrazoline derivatives **5a-r** have been achieved from the more reactive pyrazole-chalcone and hydrazinobenzothiazole in presence of NaOH in EtOH at room temperature. The reaction proceeded efficiently to get the 2-pyrazoline in excellent yields (85-94%). While reaction medium was simple conventional method, mild reaction condition, easy isolation of product and short reaction times are additional process for the green purpose. In addition, target compounds were screened for their *in vitro* antibacterial, antifungal and antituberculosis activity and some of them shows good to excellent activity as compare to standard drugs.

Keywords : pyrazole, benzothiazole, pyrazoline, NaOH, green approach

Introduction

Tuberculosis (TB), an infectious disease stand as a major health issue in developing countries, affecting millions of people around the world. Mycobacteria are immanent organisms that are becoming gradually more important intracellular pathogens that establish an infection in oxygen-rich macrophage of the lung[I]. Nascent infectious diseases and the increasing number of multi-drug resistant microbial pathogens still make the treatment of infectious diseases an important and imperative global health problem. Therefore, there is an important need to discover new antimicrobial agents to prevent the growth of pathogens and aiming is to short the therapeutic time duration[II, III]. Furthermore, the synthesis and discover a highly potential antimicrobial agents has been intimately considered during the decennium. Different heterocyclic compounds containing hetero atoms have been investigated for the development of new antimicrobial agents[IV-VI]. In view of these facts, it is momentous to develop more efficient antimicrobial agents.

Pyrazole derivatives have attracted continuing attention over the years because of their strong efficacy and broad-spectrum biological activities such as agrochemical, pharmaceutical industries and antibacterial[VII, VIII]. In many laboratories dealt mainly with benzothiazole is known to cover a large domain of pharmacological activities serving as antitumor, anti-microbial, analgesic and anti-inflammatory agents[IX-XIII]. The pyrazoline motif has the distinction of being the parent ring in numerous derivatives of biological relevance. The study of pyrazolines has become of much interest because of their diverse biological properties such as antibacterial, antioxidant and antimycobacterial properties[XIV-XVII].

Synthesis of 2-pyrazolines from substituted chalcone treated with different hydrazines in the presence of acidic as well as basic catalysts under reflux condition[XVIII-XXII]. However, these methods suffer from some crude drawbacks[XXIII-XXVI]. Our aiming is to development of a more direct and green process. In present study, we have used some acid as well as base catalyst like CAN, CH_3COOH, $InCl_3$, piperidine, K_2CO_3 and p-TsOH use for the synthesis of 2-pyrazoline derivatives, however these catalysts have some drawbacks such as higher temperature, longer reaction time, formation of a side product, poor yield, environmentally hazardous and typical workup process. Consequently, to overcome these drawbacks, we have used NaOH as a catalyst for the synthesis of 2-pyrazoline derivatives.

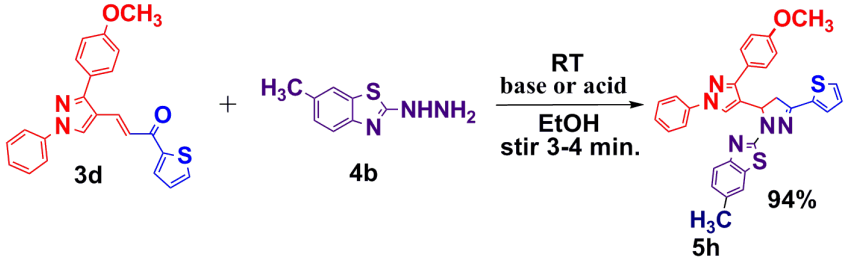
Herein we wish to report a simple, mild and efficient conventional method which is very rapid and relatively less toxic that promoted by the NaOH for the synthesis of 2-pyrazolines derivatives **5a-r** and investigated for their biological activity such as antimicrobials and antituberculosis.

Results and Discussion

Analytical results

In the present study, the new series of pyrazolylpyrazoline bearing benzothiazole derivatives **5a-r** were prepared as shown in **Scheme 1**. The starting compounds 1*H*-pyrazole-4-carbaldehyde derivatives **1a-c** were prepared by Vilsmeier–Haack reaction, according to literature procedure[XXVII]. The key intermediate, chalcones **3a-i** were synthesized in good to excellence yields by a base catalyzed Claisen–Schmidt condensation reaction of substituted aromatic aldehydes **1a-c** and 2-acetyl thiophene/furan/pyrrole compound **2a-c** in the presence of NaOH (5 mol%) in ethanol/water at room temperature. Substituted 2-pyrazolines **5a-r** were prepared from the compounds **3a-i** were cyclized with substituted hydrazinobenzothiazole using NaOH as catalyst in ethanol at room temperature. The reaction was performed in acidic as well as basic conditions. In acid catalyst, $InCl_3$ more preferable as compare to *p*-TsOH and CH_3COOH, it gave good yield (CH_3COOH<*p*-TsOH<$InCl_3$). While in base catalyst, strong base is more preferable as compare to other (CAN<K_2CO_3<Piperidine<NaOH), Also NaOH is inorganic base use as a green purpose, it was reduced the reaction time, mild reaction and gave an excellent yield. Therefore, we select NaOH as a catalyst is summarized in **Table 1**.

Table 1. Optimization of reaction conditions for the synthesis of pyrazolylpyrazoline **5h**



Entry[a]	Catalyst (0.125 mmol)	Temperature (°C)	Time (min.)	Yield[b] (%)
1	CAN	Reflux	60	82
2	CH_3COOH	Reflux	120	72
3	$InCl_3$	70	40	83
4	**NaOH**	**rt**	**3-4**	**94**
5	piperidine	rt	30	88
6	K_2CO_3	60	40	86
7	*p*-TsOH	80	80	74

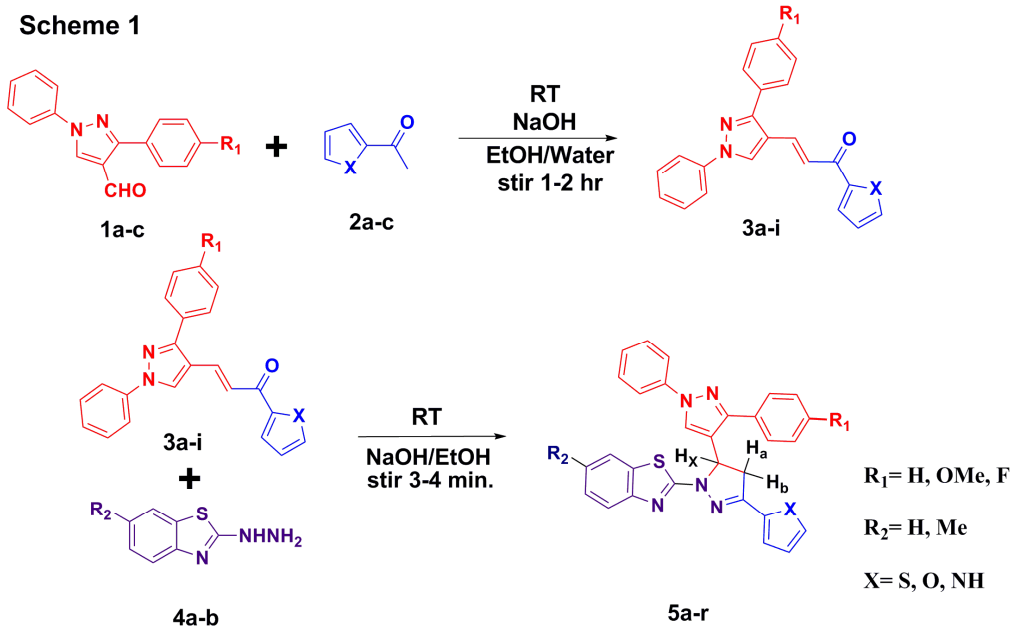
[a]Reaction conditions: Chalcone **3d** (2.5 mmol), hydrazinobenzothiazole **4b** (5.25 mmol), catalyst (0.125 mmol), ethanol (5 mL); [b]Isolated yield

The reaction between (*E*)-3-(3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-(thiophen-2-yl)prop-2-en-1-one **3d** and hydrazinobenzothiazole **4b** was chosen as a model reaction for optimization of the reaction condition. For these, mixture of reactant and 0.125 mmol of NaOH were use, it gave 94% yield in 3-4 min. at room temperature (Table 2, Entry 1). In same reaction condition, using a dosage of NaOH (0.025 mmol), it gave 83% yield in 20 min. (Table 2, Entry 2). While in using a dosage of NaOH (0.250 and 0.375 mmol) it gave 88% and 78% yield in 15 and 20 min. respectively (Table 2, Entry 3, 4). When the reaction was carried out without NaOH, no product was obtained, even after 1 h (Table 2, Entry 5). If we change the solvent factor in same manner, we did not improve the product yield 86% and 76% respectively (Table 2, Entry 6, 8). After set up the mmol ratio of NaOH in ethanol, we get the excellent yield and less time reaction.

Table 2. Catalyst screening of compound **5h** with different solvent,

Entry[a]	Dosage of catalyst (mmol)	Solvent	Time (min.)	Yield[b] (%)
1	**0.125**	**EtOH**	**3-4**	**94**
2	0.025	EtOH	20	83
3	0.250	EtOH	15	88
4	0.375	EtOH	20	78
5	-	EtOH	1 h	-
6	0.125	AcCN	10	86
7	0.250	AcCN	25	79
8	0.125	THF	15	76
9	0.250	THF	30	71

[a]Reaction conditions: Chalcone **3d** (2.5 mmol), hydrazinobenzothiazole **4b** (5.25 mmol), NaOH (0.025-0.375 mmol), solvent (5 mL); [b]Isolated yield.



Scheme 1. Synthetic strategies adopted for the preparation of key precursors **3a-f** and title compounds **5a-r**

The structures of compounds **5a–r** were characterized by elemental analyses, FT-IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. All compounds gave satisfactory elemental analysis. In the IR spectra of compounds **5a-r,** the C-H stretching band was seen around 2925-3025 cm^{-1} whereas the –C=N stretching was observed around 1587-1610 cm^{-1}. In the 400 MHz ^{1}H NMR spectrum of compounds, the C_4 protons of the pyrazoline ring resonated as multiplet at 3.29–3.45 ppm (H_a), 4.05-4.17 ppm (H_b). The C_5 (H_x) proton of pyrazoline appeared as multiplet at 5.88–5.99 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring. In addition, synthesized compounds were further confirmed by mass spectrometry. All compounds showed a particular molecular ion peak which was in conformity with the respective molecular formula of compounds.

Table 3. Substituent pattern, reaction time and % yield of compounds **5a-r.**

Entry[a]	Product	R_1	X	R_2	Time (min.)	Yield[b] (%)
1	5a	H	S	H	3	85
2	5b	H	S	CH_3	3	89
3	5c	H	O	H	4	86
4	5d	H	O	CH_3	3.5	91
5	5e	H	NH	H	4	89
6	5f	H	NH	CH_3	4	90
7	5g	OMe	S	H	3	85
8	5h	OMe	S	CH_3	3.5	94
9	5i	OMe	O	H	3.5	90
10	5j	OMe	O	CH_3	4	92
11	5k	OMe	NH	H	4	90
12	5l	OMe	NH	CH_3	3	89
13	5m	F	S	H	3	88
14	5n	F	S	CH_3	3	90
15	5o	F	O	H	4	85
16	5p	F	O	CH_3	3.5	87
17	5q	F	NH	H	4	92
18	5r	F	NH	CH_3	4	90

[a]Reaction conditions: Chalcone **3a-i** (2.5 mmol), hydrazinobenzothiazole **4a, b** (5.25 mmol), NaOH (0.125 mmol), solvent (5 mL); [b]Isolated yield

Biological Results

Antimicrobial activity

Upon exploration of antimicrobial activity data **Table 4**, revealed that some compounds showed good to excellent antibacterial and antifungal activity against the representative species when compared with the standard drugs such as ampicillin, ciprofloxacin, norfloxacin, nystatin and griseofulvin.

Against Gram-positive bacteria *B. subtilis*, compound **5b** (MIC=100 µg/mL) were shown equipotent as compare to ampicillin (MIC=100 µg/mL). Against *C. tetani*, compound **5b** (MIC=50 µg/mL) elicited excellent activity as compare to ampicillin (MIC=250 µg/mL), ciprofloxacin (MIC=100 µg/mL) and comparable activity to that norfloxacin. Compounds **5k** and **5m** (MIC=100 µg/mL) were elicited similar potency as compare to ciprofloxacin

(MIC=100 µg/mL). Against *S. aureus*, compound **5g** (MIC=100 µg/mL) and **5h** (MIC=62.5 µg/mL) have shown more potency as compare to ampicillin (MIC=100 µg/mL).
Against Gram-negative bacteria *E. coli*, compound **5d** (MIC=100 µg/mL) have shown equal activity and **5m** (MIC=62.5 µg/mL) have shown excellent activity as compare to ampicillin (MIC=100 µg/mL). Against *S. typhi*, compounds **5g**, **5m**, **5n**, **5q** (MIC=100 µg/mL) were found equipotent to ampicillin (MIC=100 µg/mL). Against *V. Cholerae*, compounds **5b**, **5n**, **5r** (MIC=100 µg/mL) were elicited more potent as compare to ampicillin (MIC=250 µg/mL), and comparable activity to that norfloxacin (MIC=100 µg/mL).
It has been observed that against *C. albicans*, compounds **5g**, **5m**, **5n**, **5q** (MIC=250 µg/mL) were found excellent activity and compounds **5b**, **5d**, **5j**, **5p**, **5r** (MIC=500 µg/mL) have shown equally active as compare to griseofulvin (MIC=500 µg/mL). Against *T. rubrum*, Compound **5n** (MIC=100 µg/mL) were found equipotent as compare to nystatin and griseofulvin (MIC=100 µg/mL).

Table 4. *In vitro* antimicrobial activity of **5a-r** MICs (µg/mL).

Sr. No.	Sample code	Gram-positive bacteria			Gram-negative bacteria			Fungi	
		BS MTCC 441	CT MTCC 449	SA MTCC 96	EC MTCC 443	ST MTCC 98	VC MTCC 3906	CA MTCC 227	TR MTCC 296
1	5a	250	**250**	500	200	200	**250**	>1000	>1000
2	5b	**100**	**50**	500	500	500	**100**	**500**	500
3	5c	250	500	500	250	200	**250**	1000	1000
4	5d	250	500	250	**100**	125	**250**	1000	**250**
5	5e	200	**200**	500	200	250	**200**	**500**	1000
6	5f	250	**100**	250	500	500	**250**	1000	>1000
7	5g	200	**250**	**100**	125	**100**	**250**	**250**	1000
8	5h	200	**250**	**62.5**	250	250	**200**	1000	1000
9	5i	500	**250**	250	250	250	500	1000	1000
10	5j	500	**200**	200	200	200	500	**500**	500
11	5k	250	**100**	125	200	250	**200**	1000	500
12	5l	250	**200**	250	200	250	**250**	1000	>1000
13	5m	250	**100**	250	**62.5**	**100**	**250**	**250**	500
14	5n	125	500	500	125	**100**	**100**	**250**	**100**
15	5o	500	**250**	500	200	125	500	1000	500
16	5p	500	500	500	200	250	500	**500**	1000
17	5q	250	**250**	250	125	**100**	**200**	**250**	1000
18	5r	500	**200**	200	100	200	**100**	**500**	>1000
Ampicillin		100	250	100	100	100	250	-	-
Chloramphenicol		50	50	50	50	50	50	-	-
Ciprofloxacin		50	100	25	25	25	50	-	-
Norfloxacin		10	50	10	10	10	100	-	-
Nystatin		-	-	-	-	-	-	100	100
Griseofulvin		-	-	-	-	-	-	500	100

BS: *Bacillus subtilis*; CT: *Clostridium tetani*; SA: *Staphylococcus aureus*; EC: *Escherichia coli*; ST: *Salmonella typhi*; VC: *Vibrio cholerae*; CA: *Candida albicans*; TR: *Trichophyton rubrum*. MTCC: Microbial Type Culture Collection. Bold numbers indicate more or equivalent potent compounds compared to standard drugs

Antituberculosis activity

The preliminary screening of the target compounds for their *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv bacteria in **Table 5**. It is most interesting part that fluorine substituted five membered heteroatom (S) effectively inhibits the growth of *M. tuberculosis* activity. Compound **5n** (MIC=12.5 µg/mL) have shown highest potency as compare to Rifampicin against *M. tuberculosis* with 99% inhibition. Other compounds **5a** (MIC=100 µg/mL), **5b** (MIC=50 µg/mL), **5g** (MIC=62.5 µg/mL) and **5m** (MIC=50 µg/mL) exhibited moderate inhibition of 94% in **Table 6**.

Table 5. *In vitro* antituberculosis activity (% Inhibition) of **5a-r** against *M. tuberculosis* H37Rv (at concentration 250 µg/mL)

Compounds	%Inhibition	Compounds	%Inhibition
5a	**96**	5k	25
5b	**98**	5l	40
5c	79	**5m**	**96**
5d	67	**5n**	**99**
5e	88	5o	35
5f	65	5p	40
5g	**96**	5q	62
5h	86	5r	88
5i	47	Rifampicin	98
5j	65	Isoniazid	99

Table 6. *In vitro* antituberculosis activity of **5a**, **5b, 5g**, **5m**, **5n** compounds exhibiting higher %inhibition against *M. tuberculosis* H37Rv (MICs, µg/mL).

compounds	**%Inhibition**	**MIC(µg/mL)**
5a	96	100
5b	98	50
5g	94	62.5
5m	96	50
5n	99	12.5
Rifampicin	98	40
Isoniazid	99	0.20

Experimental

All reactions were performed with commercially available reagents and were used without further purification. Organic solvents were purified by standard methods and stored over molecular sieves. All reactions were monitored by thin-layer chromatography (TLC, on aluminum plates coated with silica gel $^{60}F_{254}$, 0.25 mm thickness, Merck) carried on fluorescent coated plates and detection of the components was made by exposure to iodine vapors or UV light. Melting points of all the title compounds were determined by open tube capillary method (using silicon oil 350 cst) and are uncorrected. The IR spectra were recorded on a Perkin Elmer Spectrum GX FT-IR Spectrophotometer (Perkin Elmer, USA) using potassium bromide pellets in the range 4000-400 cm^{-1} and frequencies of only characteristic peaks are expressed in cm^{-1}. ^{1}H NMR, and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using TMS as an internal standard at 400 MHz Chemical shifts are reported in parts per million (ppm). Splitting patterns are designated as s, for singlet; d, for doublet; m, for multiplet. Elemental analysis (% C, H, N) was carried out using Perkin Elmer

2400 series-II elemental analyzer (Perkin Elmer, USA) and all compounds are within ±0.4% of the theoretical compositions.

General procedure for the synthesis of compounds 3a-i

In a round bottomed flask, an equimolar mixture of substituted carbaldehydes **1a-c** (1 mmol), substituted acetyl moiety (1 mmol) and NaOH (5 mol %) were added into water/ethanol (1:2), stir for 1-1.5h at room temperature. Progress of the reaction was monitored by TLC. After completion of reaction, the solid mass separated was collected by filtration, and recrystallized from methanol to afford pure compounds **3a-i**.

General procedure for synthesis of pyrazolylpyrazoline derivatives 5a-r

A 100 ml round bottomed flask, was charge with a mixture of compound **3a-i** (2.5 mmol), hydrazinobenzothiazole **4a**, **b** (5.25 mmol) and NaOH (0.125 mmol) in ethanol (5mL) under simple conventional method stir for 2-3 min. at room temperature, completion of the reaction was confirmed by TLC. Easy isolation of the product obtain were purified by recrystallization with methanol and dried. The products were received quantitatively (85-94% yield) with excellent purity.

2-(5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[d]thiazole 5a

Yield: 85%; m.p 226 °C, IR (KBr, νmax, cm^{-1}): 3017 (Ar-CH), 1599 (C=N), 1567 (C=C); ^{1}H NMR (400MHz, DMSO-d_6, δ): 3.45 (dd, 1H, H_a), 4.17 (dd, 1H, H_b), 5.99 (dd, 1H, H_x), 7.08-8.58 (m, 18H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 44.76, 56.62, 118.68, 119.90, 121.77, 122.34, 122.91, 126.34, 126.80, 127.68, 128.54, 128.57, 128.70, 129.09, 129.92, 130.51, 131.60, 132.51, 133.50, 134.38, 139.73, 150.27, 150.60, 152.49, 163.35; ESI-MS (m/z): Calcd.: 503.12, Found: 504.05 $(M+1)^+$, Anal. Calcd. for $C_{29}H_{21}N_5S_2$: C,69.16; H, 4.20; N, 13.91%. Found C, 69.37; H, 4.37; N, 13.68%.

2-(5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-6-methylbenzo[d]thiazole 5b

Yield: 89%; m.p 235 °C; IR (KBr, νmax, cm^{-1}): 3012 (Ar-CH), 1609 (C=N), 1560 (C=C); ^{1}H NMR (400MHz, DMSO-d_6, δ): 2.33 (s, 3H, -CH_3 of benzothiazole), 3.40 (dd, 1H, H_a), 4.12 (dd, 1H, H_b), 5.94 (dd, 1H, H_x), 7.02-8.54 (m, 17H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 21.14, 44.76, 56.63, 118.68, 119.76, 121.70, 122.34, 122.80, 126.25, 126.80, 127.56, 128.50, 128.57, 128.67, 129.09, 129.90, 130.63, 131.71, 132.51, 133.61, 134.40, 139.70, 150.30, 150.60, 152.45, 163.12; ESI-MS (m/z): Calcd.: 517.14, Found: 518.10 $(M+1)^+$, Anal. Calcd. for $C_{30}H_{23}N_5S_2$: C, 69.60; H, 4.48; N, 13.53%. Found C, 69.42; H, 4.13; N, 13.79%.

2-(5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[d]thiazole 5c

Yield: 86%; m.p 182 °C; IR (KBr, νmax, cm^{-1}): 3019 (Ar-CH), 1601 (C=N), 1564 (C=C); ^{1}H NMR (400MHz, DMSO-d_6, δ): 3.34 (dd, 1H, H_a), 4.07 (dd, 1H, H_b), 5.89 (dd, 1H, H_x), 6.75-8.52 (m, 18H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 43.90, 56.12, 112.80, 114.24, 118.81, 119.64, 121.67, 122.90, 126.84, 127.44, 127.68, 128.60, 128.70, 129.12, 130.02, 131.70, 133.46, 139.71, 145.35, 145.98, 146.70, 150.40, 150.71, 152.87, 162.80; ESI-MS (m/z): Calcd.: 487.57, Found: 488..05 $(M+1)^+$, Anal. Calcd. for $C_{29}H_{21}N_5OS$: C, 71.44; H, 4.34; N, 14.36%. Found: C, 71.24; H, 4.56; N, 14.12%.

2-(5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-6-methylbenzo[d]thiazole 5d

Yield: 91%; m.p 194 °C; IR (KBr, νmax, cm^{-1}): 3010 (Ar-CH), 1594 (C=N), 1566 (C=C); ^{1}H NMR (400MHz, DMSO-d_6, δ): 2.33 (s, 3H, -CH_3 of benzothiazole), 3.29 (d, 1H, H_a), 4.05 (dd, 1H, H_b), 5.88 (dd, 1H, H_x), 6.67- 8.53 (m, 17H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 21.26, 43.80, 56.03, 112.74, 114.08, 118.63, 119.53, 121.61, 122.86, 126.77, 127.39, 127.58, 128.57, 128.68, 129.09, 129.92, 131.62, 133.45, 139.71, 145.32, 145.95, 146.59,

150.34, 150.59, 152.82, 162.71; ESI-MS (m/z): Calcd.: 501.16, Found: 502.10 $(M+1)^+$, Anal. Calcd. for $C_{30}H_{23}N_5OS$: C, 71.83; H, 4.62; N, 13.96%. Found: C, 71.68; H, 4.86; N, 13.65%.

2-(5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(1*H*-pyrrol-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[d]thiazole 5e

Yield: 89%; m.p 210 °C; IR (KBr, νmax, cm^{-1}): 3012 (Ar-CH), 1603 (C=N), 1570 (C=C); ^1H NMR (400MHz, DMSO-d_6, δ): 3.38 (dd, 1H, H_a), 4.03 (dd, 1H, H_b), 5.20 (s, 1H, -NH of pyrrole), 5.85 (dd, 1H, H_x), 6.66-8.52 (m, 18H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 43.84, 56.03, 116.34, 118.12, 118.70, 119.62, 121.26, 121.60, 122.80, 126.30, 126.80, 127.37, 127.65, 128.55, 128.67, 129.08, 131.68, 133.45, 139.70, 145.88, 146.68, 150.34, 150.67, 152.84, 162.73; ESI-MS (m/z): Calcd.: 486.57, Found: 487.10 $(M+1)^+$, Anal. Calcd. for $C_{29}H_{22}N_6S$: C, 71.58; H, 4.56; N, 17.27%. Found: C, 71.34; H, 4.23; N, 17.54%.

2-(5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(1*H*-pyrrol-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-6-methylbenzo[d]thiazole 5f

Yield: 90%; m.p 230 °C; IR (KBr, νmax, cm^{-1}): 3019 (Ar-CH), 1598 (C=N), 1568 (C=C); ^1H NMR (400MHz, DMSO-d_6, δ): 2.34 (s, 3H, $-CH_3$ of benzothiazole), 3.35 (dd, 1H, H_a), 4.06 (dd, 1H, H_b), 5.23 (s, 1H, -NH of pyrrole), 5.86 (dd, 1H, H_x), 6.64-8.51 (m, 17H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 21.25, 43.78, 56.02, 116.30, 118.10, 118.68, 119.50, 121.24, 121.60, 122.84, 126.80, 127.52, 128.55, 128.65, 129.06, 129.90, 131.40, 133.43, 139.70, 145.30, 145.92, 146.56, 150.32, 150.57, 152.81, 162.68; ESI-MS (m/z): Calcd.: 500.16, Found: 501.05 $(M+1)^+$, Anal. Calcd. for $C_{30}H_{24}N_6S$: C, 71.98; H, 4.83; N, 16.79%. Found: C, 71.69; H, 4.96; N, 16.51%.

2-(5-(3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[d]thiazole 5g

Yield: 85%; m.p 237 °C; IR (KBr, νmax, cm^{-1}): 3013 (Ar-CH), 1604 (C=N), 1561 (C=C); ^1H NMR (400MHz, DMSO-d_6, δ): 3.44 (dd, 1H, H_a), 3.84 (s, 3H, $-OCH_3$), 4.16 (dd, 1H, H_b), 5.92 (dd, 1H, H_x), 7.04-8.56 (m, 17H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 44.56, 55.66, 56.72, 118.95, 119.97, 122.12, 122.50, 123.11, 126.32, 126.62, 127.17, 127.82, 128.67, 128.84, 129.12, 129.52, 129.87, 131.61, 132.56, 133.53, 133.87, 139.70, 150.31, 150.61, 152.47, 163.61; ESI-MS (m/z): Calcd.: 533.67, Found: 534.10 $(M+1)^+$, Anal. Calcd. for $C_{30}H_{23}N_5OS_2$: C, 67.52; H, 4.34; N, 13.12%.Found: C, 67.74; H, 4.58; N, 13.34%.

2-(5-(3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-6-methylbenzo[d]thiazole 5h

Yield: 94%; m.p 220 °C; IR (KBr, νmax, cm^{-1}): 3015 (Ar-CH), 1601 (C=N), 1567 (C=C); ^1H NMR (400MHz, DMSO-d_6, δ): 2.33 (s, 3H, $-CH_3$ of benzothiazole), 3.39 (dd, 1H, H_a), 3.84 (s,3H, $-OCH_3$), 4.12 (dd,1H, H_b), 5.88 (dd, 1H, H_x), 7.07- 8.51 (m, 16H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 21.27, 44.56, 55.66, 56.72, 118.62, 119.80, 121.74, 122.30, 122.85, 126.32, 126.78, 127.62, 128.48, 128.52, 128.70, 129.02, 129.85, 130.46, 131.54, 132.44, 133.45, 134.33, 139.68, 150.22, 150.56, 152.44, 163.28; ESI-MS (m/z): Calcd.: 547.69, Found: 548.05 $(M+1)^+$, Anal. Calcd. for $C_{31}H_{25}N_5OS_2$: C, 67.98; H, 4.60; N, 12.79%. Found: C, 67.78; H, 4.36; N, 12.74%.

2-(3-(furan-2-yl)-5-(3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[d]thiazole 5i

Yield: 90%; m.p 226 °C; IR (KBr, νmax, cm^{-1}): 3017 (Ar-CH), 1599 (C=N), 1563 (C=C); ^1H NMR (400MHz, DMSO-d_6, δ): 3.35 (dd, 1H, H_a), 3.83 (s, 3H, $-OCH_3$), 4.09 (dd, 1H, H_b), 5.89 (dd, 1H, H_x), 6.77-8.50 (m, 17H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 43.86, 56.62, 56.62, 112.75, 114.12, 118.68, 120.09, 121.63, 121.89, 126.81, 127.42, 127.64, 128.32, 128.70, 129.43, 130.12, 131.23, 133.54, 140.12, 145.40, 146.13, 146.70, 150.37,

150.67, 152.80, 162.73; ESI-MS (m/z): Calcd.: 517.60, Found: 518.10 $(M+1)^+$, Anal. Calcd. For $C_{30}H_{23}N_5O_2S$**:** C, 69.61; H, 4.48; N, 13.53%. Found: C, 69.91; H, 4.67; N, 13.22%.

2-(3-(furan-2-yl)-5-(3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-6-methylbenzo[d]thiazole 5j

Yield: 92%; m.p 210 °C; IR (KBr, νmax, cm^{-1}): 3012 (Ar-CH), 1605 (C=N), 1561 (C=C); ^{1}H NMR (400MHz, DMSO-d_6, δ): 2.33 (s, 3H, -CH_3 of benzothiazole), 3.31 (dd, 1H, H_a), 3.83 (s, 3H, -OCH_3), 4.06 (dd, 1H, H_b), 5.88 (dd, 1H, H_x), 6.79-8.55 (m, 16H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 21.30, 43.92, 55.50, 56.43, 112.34, 113.89, 118.23, 119.12, 121.97, 123.12, 126.45, 127.13, 127.97, 128.46, 128.70, 129.13, 129.87, 131.59, 133.39, 140.07, 145.14, 145.70, 146.56, 150.57, 151.13, 152.76, 162.32; ESI-MS (m/z): Calcd.: 531.63, Found: 532.05 $(M+1)^+$, Anal. Calcd. for $C_{31}H_{25}N_5O_2S$: C, 70.04; H, 4.74; N, 13.17%. Found: C, 70.06; H, 4.96; N, 13.35%.

2-(5-(3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(1H-pyrrol-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[d]thiazole 5k

Yield: 90%; m.p 220 °C; IR (KBr, νmax, cm^{-1}): 3019 (Ar-CH), 1602 (C=N), 1567 (C=C); ^{1}H NMR (400MHz, DMSO-d_6, δ): 3.38 (dd, 1H, H_a), 3.84 (s, 3H, -OCH_3), 4.04 (dd, 1H, H_b), 5.22 (s, 1H, -NH of pyrrole), 5.84 (dd, 1H, H_x), 6.56-8.51 (m, 17H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 43.86, 55.52, 56.59, 112.31, 113.78, 118.68, 119.60, 121.23, 121.57, 122.76, 126.28, 126.78, 127.35, 127.64, 128.51, 128.65, 129.06, 131.63, 133.35, 139.85, 145.85, 146.65, 150.32, 150.65, 152.83, 162.89; ESI-MS (m/z): Calcd.: 516.60, Found: 517.10 $(M+1)^+$, Anal. Calcd. for $C_{30}H_{24}N_6OS$: C, 69.75; H, 4.68; N, 16.27%. Found: C, 69.87; H, 4.45; N, 16.56%.

2-(5-(3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(1H-pyrrol-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-6-methylbenzo[d]thiazole 5l

Yield: 89%; m.p 230 °C; IR (KBr, νmax, cm^{-1}): 3010 (Ar-CH), 1599 (C=N), 1569 (C=C); ^{1}H NMR (400MHz, DMSO-d_6, δ): 2.34 (s, 1H, -CH_3 of benzothiazole), 3.34 (dd, 1H, H_a), 3.84 (s, 3H, -OCH_3), 4.06 (dd, 1H, H_b), 5.23 (s, 1H, -NH of pyrrole), 5.85 (dd, 1H, H_x), 6.64-8.54 (m, 16H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 21.24, 43.75, 55.50, 56.55, 112.29, 113.70, 118.65, 119.58, 121.20, 122.72, 126.75, 127.30, 128.05 128.50, 128.63, 129.02, 130.12, 131.60, 133.23, 140.02, 145.10, 145.80, 146.62, 150.30, 150.64, 152.80, 162.85; ESI-MS (m/z): Calcd.: 530.62, Found: 531.10 $(M+1)^+$, Anal. Calcd. for $C_{31}H_{26}N_6OS$: C, 70.17; H, 4.94; N, 15.84%. Found: C, 70.46; H, 4.68; N, 15.93%.

2-(5-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[d]thiazole 5m

Yield: 88%; m.p 196 °C; IR (KBr, νmax, cm^{-1}): 3014 (Ar-CH), 1597 (C=N), 1570 (C=C); ^{1}H NMR (400MHz, DMSO-d_6, δ): 3.44 (dd, 1H, H_a), 4.14 (dd, 1H, H_b), 5.89 (dd, 1H, H_x), 7.08-8.61 (m, 17H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 44.76, 56.62, 118.65, 119.78, 121.77, 122.32, 122.83, 126.31, 126.81, 127.79, 128.54, 128.56, 129.91, 129.96, 130.85, 130.94, 131.56, 134.35, 135.10, 139.67, 149.90, 150.30, 152.43, 160.42, 163.21; ESI-MS (m/z): Calcd.: 521.11, Found: 522.05 $(M+1)^+$, Anal. Calcd. for $C_{29}H_{20}FN_5S_2$: C, 66.77; H, 3.86; N, 13.43%. Found: C, 66.59; H, 4.07; N, 13.71%.

2-(5-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-6-methylbenzo[d]thiazole 5n

Yield: 90%; m.p 233 °C; IR (KBr, νmax, cm^{-1}): 3011 (Ar-CH), 1603 (C=N), 1566 (C=C); ^{1}H NMR (400MHz, DMSO-d_6, δ): 2.33 (s, 3H, -CH_3 of benzothiazole), 3.40 (dd, 1H, H_a), 4.10 (dd, 1H, H_b), 5.88 (dd, 1H, H_x), 7.09-8.57 (m, 16H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 44.74, 56.50, 118.40, 119.47, 121.52, 122.21, 123.09, 126.22, 127.21, 127.65, 128.52, 128.58, 129.90, 129.95, 130.40, 130.86, 131.53, 134.34, 135.10, 139.65, 150.12, 150.33,

152.34, 160.53 162.70; ESI-MS (m/z): Calcd.: 535.66, Found: 536.10 $(M+1)^+$, Anal. Calcd. for $C_{30}H_{22}FN_5S_2$: C, 67.27; H, 4.14; N, 13.07%. Found: C, 67.61; H, 3.98; N, 12.75%.

2-(5-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[d]thiazole 5o

Yield: 85%; m.p 203 °C; IR (KBr, νmax, cm^{-1}): 3016 (Ar-CH), 1600 (C=N), 1561 (C=C); ^1H NMR (400MHz, DMSO-d_6, δ): 3.34 (dd, 1H, H_a), 4.08 (dd, 1H, H_b), 5.90 (dd, 1H, H_x), 6.74-8.53 (m, 17H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 43.78, 56.10, 112.75, 114.23, 118.68, 119.21, 122.10, 122.78, 126.54, 127.12, 127.43, 128.54, 129.60, 129.91, 131.20, 133.34, 139.70, 145.32, 145.95, 146.65, 150.31, 150.67, 153.04, 160.21, 162.78; ESI-MS (m/z): Calcd.: 519.63, Found: 520.05 $(M+1)^+$, Anal. Calcd. for $C_{29}H_{20}FN_5OS$: C, 68.90; H, 3.99; N, 13.85. Found: C, 68.84; H, 3.74; N, 14.21%.

2-(5-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-6-methylbenzo[d]thiazole 5p

Yield: 87%; m.p 199 °C; IR (KBr, νmax, cm^{-1}): 3019 (Ar-CH), 1598 (C=N), 1562 (C=C); ^1H NMR (400MHz, DMSO-d_6, δ): 2.32 (s, 3H, -CH_3 of benzothiazole), 3.30 (dd, 1H, H_a), 4.06 (dd, 1H, H_b), 5.88 (dd, 1H, H_x), 6.76-8.51 (m, 16H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 21.31, 43.70, 56.04, 112.68, 114.12, 118.60, 119.14, 121.80, 123.51, 127.30, 127.56, 128.52, 128.60, 128.96, 129.92, 131.58, 133.45, 139.68, 145.40, 145.70, 146.50, 150.20, 150.40, 152.80, 160.12, 162.50; ESI-MS (m/z): Calcd.: 535.66, Found: 536.05 $(M+1)^+$, Anal. Calcd. for $C_{30}H_{22}FN_5OS$: C, 69.35; H, 4.27; N, 13.48%. Found: C, 69.15; H, 4.52; N, 13.59%.

2-(5-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(1*H*-pyrrol-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[d]thiazole 5q

Yield: 92%; m.p 210 °C; IR (KBr, νmax, cm^{-1}): 3013 (Ar-CH), 1602 (C=N), 1566 (C=C); ^1H NMR (400MHz, DMSO-d_6, δ): 3.37 (dd, 1H, H_a), 4.05 (dd, 1H, H_b), 5.21 (s, 1H, -NH of pyrrole), 5.84 (dd, 1H, H_x), 6.68-8.54 (m, 17H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 43.54, 56.12, 116.20, 118.20, 118.67, 119.60, 121.24, 121.56, 122.78, 126.27, 126.79, 127.35, 127.64, 128.52, 128.65, 131.64, 133.43, 139.65, 145.86, 146.76, 150.30, 150.64, 152.78, 160.10, 162.56; ESI-MS (m/z): Calcd.: 518.63, Found: 519.10 $(M+1)^+$, Anal. Calcd. for $C_{29}H_{21}FN_6S$: C, 69.03; H, 4.19; N, 16.66%. Found: C, 69.28; H, 4.03; N, 16.43%.

2-(5-(3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(1*H*-pyrrol-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-6-methylbenzo[d]thiazole 5r

Yield: 90%; m.p 235 °C; IR (KBr, νmax, cm^{-1}): 3014 (Ar-CH), 1605 (C=N), 1568 (C=C); ^1H NMR (400MHz, DMSO-d_6, δ): 2.35 (s, 3H, -CH_3 of benzothiazole), 3.34 (dd, 1H, H_a), 4.07 (dd, 1H, H_b), 5.22 (s, 1H, -NH of pyrrole), 5.84 (dd, 1H, H_x), 6.67-8.52 (m, 16H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 21.24, 43.73, 56.12, 116.27, 118.17, 118.64, 119.54, 121.23, 121.57, 122.80, 126.75, 127.32, 127.65, 128.50, 128.62, 130.23, 131.62, 133.40, 139.63, 145.84, 146.73, 150.26, 150.59, 152.76, 160.13, 162.64; ESI-MS (m/z): Calcd.: 534.66, Found: 535.05 $(M+1)^+$, Anal. Calcd. for $C_{30}H_{23}FN_6S$: C, 69.48; H, 4.47; N, 16.20%. Found: C, 69.52; H, 4.24; N, 16.25%.

Biological Assay

Antimicrobial screening

Antimicrobial activity of all the synthesized compounds was carried out by broth microdilution method [National Committee for Clinical Laboratory Standards (NCCLS) (2002)][XXVIII]. Mueller–Hinton broth was used as the nutrient medium for the test bacteria and Sabouraud dextrose broth was for the test fungi. Inoculum size for the test strains was adjusted to 108 CFU [Colony Forming Unit] per milliliter by comparing the Mac Fernald standard turbidity. The strains used for [MTCC—Microbial Type Culture Collection]

antimicrobial activity were procured from the Institute of Microbial Technology, Chandigarh, India. Each synthesized compound was diluted with DMSO to have the stock solution of 2000 µg/mL concentration as a stock solution. The results were recorded in the form of primary and secondary screening. The compounds **5a-r** were screened for their antibacterial activity against *Bacillus subtilis* (MTCC 441), *Clostridium tetani* (MTCC 449), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 443), *Salmonella typhi* (MTCC 98), *Vibrio cholerae* (MTCC 3906), *Candida albicans* (MTCC 227) and *Trichophyton rubrum* (MTCC 296) at concentrations of 1000, 500, and 250 µg/mL for primary screening. Dimethyl sulfoxide (DMSO) was used as the medium to get the desired concentrations of compounds. The compounds showing activity against microbes in the primary screening were further screened in a second set of dilution at concentrations of 200, 100, 62.5, 50, and 25 µg/mL. The deferment of 10 µL was further inoculated in a 96-well plate and growth was noted after 24 and 48 h. The lowest concentration, which showed no visible growth (turbidity) after spot subculture, was considered as the minimum inhibitory concentration (MIC) for each compound. In the present study, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Griseofulvin were used as standard antibacterial drugs, whereas Nystatin and griseofulvin was used as standard antifungal drug. The values of MIC are summarized in **Table 4**.

Antituberculosis screening

In vitro antituberculosis activity of all the newly synthesized compounds against *M. tuberculosis* H37Rv strain was determined using Lowenstein–Jensen medium (conventional method) as described by Rattan (2000) and the observed results are presented in **(Table 5)** in the form of % inhibition, relative to that of standard antitubercular drugs INH and RIF. Of the compounds studied, six compounds those exhibited the highest % inhibition, were again screened to get their MIC values **(Table 6)**. Drug susceptibility and determination of antituberculosis activity of the test compounds against *M. tuberculosis* H37Rv were performed by Lowenstein–Jensen method with slight modification where 6.25 µg/mL dilution of each test compound were added liquid Lowenstein–Jensen medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein–Jensen medium was harvested in 0.85 % saline in bijou bottles.

All test compound make solution of 6.25 µg/mL concentration of compounds was prepared in DMSO. These tubes were then incubated at 37^0C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 9 104 bacilli per tube). These tubes were then incubated at 37^0C. Growth of bacilli was seen after 12, 22 days, finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv.The concentration at which no development of colonies occurred or 20 colonies was taken as MIC concentration of test compound. The screening results are summarized as % inhibition relative to standard drugs INH and RIF.

Conclusion

We have to develop a simple, eco-friendly and efficient method for synthesis of novel 1,3-diphenyl-1*H*-pyrazolyl-2-benzo[d]thiazole-2-pyrazoline derivatives **5a-r**. This synthetic approach allows the integration of three promising bioactive nuclei in single scaffold through an easy way, for aiming their potent antimicrobial and antitubercular activities. Compounds **5b**, **5g**, **5h**, **5m** and **5n** were found to be most efficient antimicrobials and compound **5n** have shown excellent %inhibition of the series, while other compounds **5a**, **5b**, **5g** and **5m** displayed moderated to low inhibition of antituberculosis.

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