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ORIGINAL PAPER

Synthesis of new pyrazole derivatives via multicomponent reaction and evaluation of their antimicrobial and antioxidant activities

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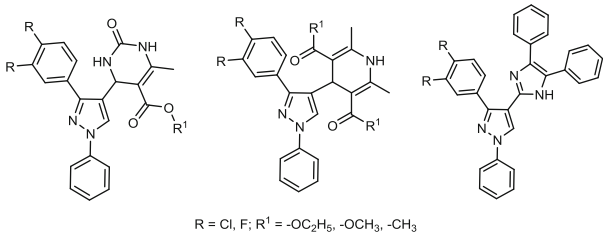
Abstract This work describes a facile synthesis and characterization of a new series of pyrazole containing pyrimidine, 1,4-dihydropyridine, and imidazole derivatives using substituted 4-formylpyrazole as a key intermediate via multicomponent reaction sequence. The structures of these unknown compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, LC–MS, and elemental analysis. The synthesized products were screened for their in vitro antimicrobial and antioxidant properties. Among the tested 3-(3,4-dihalophenyl)-1-phenyl-1*H*-pyrazol-4-yl, incorporated acetyl dihydropyrimidine compounds exhibited promising antimicrobial activity and DPPH radical scavenging activity with levels of inhibition at 89.4 and 83.3 %, respectively.

Graphical abstract



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Introduction

Antimicrobial agents are considered one of the most significant milestones in modern medicines. Infections from resistant bacteria are now relatively usual, and some pathogens have become resistant to multiple types or classes of antibiotics. However, the incidence of systemic microbial infections has increased relentlessly due to increase of immune compromised hosts [1]. Alongside, the side effects of some antibiotics can sometimes prove to be more difficult to tackle than the ailment they are mean to cure. In order to handle this situation, the continued development of new efficient antibiotic agents is more crucial than ever [2]. Much more could be achieved by better and more widespread application of these measures, and there are many promising opportunities for innovation in this area [3]. Many researchers have shown that antioxidants of natural or synthetic origin could have a great importance as therapeutic agents [4]. There are increasing experimental and clinical evidences showing the involvement of oxidative stress induced by reactive oxygen and nitrogen species in a variety of disorders and various other diseases are caused because of their violent reactivity. Antioxidants can minimize or inhibit the oxidative damage byinterrupting the free radical formation or terminating the chain reaction [5]. Moreover, the applications of antioxidants such as food preservatives and skin-protective source in cosmetics have also attracted much interest [6].

Pyrazole and halosubstituted 1,3-diarylpyrazoles [7] are considered to be an important chemical synthon of various physiological significance and pharmaceutical utility [8–

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11]. Antimicrobial and antioxidant-like biological activities have been claimed for compounds comprising pyrazole ring [12–14]. Moreover, pyrazole functionality makes the core structure of medicinally important molecules such as lonazolac, celecoxib, and deracoxib [15]. The incorporation of the aryl system into the pyrazole can severely modify the biological properties of such molecules [16].

Dihydropyrimidinones (DHPMs) and their derivatives have attracted considerable interest because of their pharmacological and therapeutic properties such as antibacterial, antiviral, antitumour, and anti-inflammatory [17]. Hantzsch 4-aryl-1,4-dihydropyridines are biologically and medicinally important class of compounds due to their high potency and selectivity of action as calcium channel blockers [18]. In addition, this class of compounds have been broadly studied for different pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory, and antiulcer [19, 20]. Pyrazole tagged to 1,4-dihydropyridines (DHPs) are found to be potent antibacterial and antioxidant agents [21]. Similar to DHPMs and DHPs, imidazole and its derivatives also possess a broad spectrum of pharmacological activities such as antiinflammatory, analgesic, antitubercular, and antimicrobial [22, 23]. The literature survey revealed that DHPMs, DHPs, and imidazoles are other important pharmacodynamic heterocyclic nuclei, which when incorporated in different heterocyclic templates have been reported to possess potent antimicrobial as well as antioxidant activity [20, 24, 25]. 2,4,5-Triarylimidazole compounds also have gained remarkable importance due to their widespread biological activities [26].

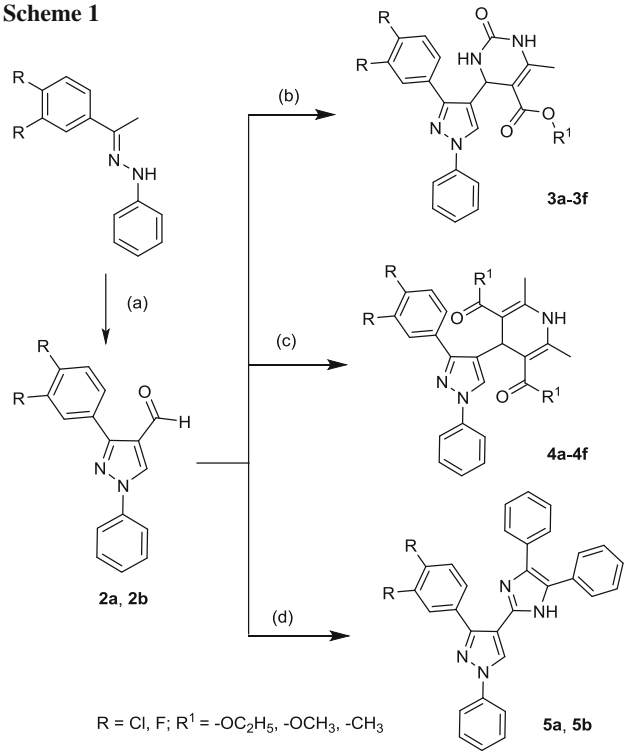
Based on these precedents, we sought to construct hybrid molecular architectures by combining pyrazole with biologically active DHPMs, DHPs, and imidazole pharmocophores through a multicomponent reaction sequences. We envisioned that the resultant conjugates by virtue of the presence of critical structural features might serve as a prototype for new drugs that could be used in antimicrobial and antioxidant research. In continuation of our research work related to pyrazole, imidazole chemistry, and antioxidant activity [27–29], we report here the synthesis, in vitro antimicrobial, and antioxidant activities of new pyrazole tagged multi-functionalized dihydropyrimidinones (DHPMs), dihydropyridines (DHPs), and imidazoles.

Results and discussion

Chemistry

Synthetic strategies for the investigation of new antimicrobial and antioxidant derivatives are illustrated in Scheme 1. The key intermediates used for the present study

Scheme 1



are 4-formylpyrazoles **2a**, **2b**, which were synthesized by the Vilsmeier-Haack reaction from the corresponding hydrazones [27]. In the present study, 4-formylpyrazoles were converted into dihydropyrimidinone derivatives **3a**–**3f** by one-pot three-component Biginelli reaction. A series of pyrazoles bearing 1,4-dihydropyridine in the 4-position was constructed by the classical Hantzsch condensation **4a**–**4f** reaction. The 2,4,5-trisubstituted imidazoles **5a**, **5b** were obtained in excellent yields by refluxing key aldehyde with 1,2-diketone and ammonium acetate in acetic acid for 6–7 h via Debus reaction [28]. The structures of all the compounds were confirmed by their characterization data (Table 1).

Antimicrobial activity

All the synthesized compounds were screened for their antimicrobial activity by disc diffusion method. The compounds were evaluated for their antibacterial activities against *Staphylococcus aureus* MTCC-7443, *Escherichia coli* MTCC-443, *Pseudomonas Aeruginosa* MTCC-424, and *Klebsiella pneumonia* MTCC-139, whereas antifungal activities were tested against *Aspergillus niger* MTCC-281 and *Aspergillus flavus* MTCC-871. The activities and the minimum inhibitory concentration (MIC) of the compounds are presented in Table 2. Streptomycin and itraconozole were used as standards for antibacterial and

Table 1 Characterization data of compounds **3a–3f**, **4a–4f**, and **5a**, **5b**

Comp.	R	R^1	Yield/%	M.p./°C
3a	Cl	OC_2H_5	90	147–149
3b	Cl	OCH_3	88	132–136
3c	Cl	CH_3	77	187–192
3d	F	OC_2H_5	89	169–171
3e	F	OCH_3	80	184–186
3f	F	CH_3	72	167–169
4a	Cl	OC_2H_5	74	112–115
4b	Cl	OCH_3	79	123–126
4c	Cl	CH_3	64	118–121
4d	F	OC_2H_5	72	119–123
4e	F	OCH_3	65	129–131
4f	F	CH_3	62	135–137
5a	Cl	–	86	210–212
5b	F	–	78	197–199

Table 2 Antimicrobial activity of compounds (**3a–f**), (**4a–f**) and **5a, 5b**

Comp.	MIC/μg cm^{-3} (zone of inhibition/mm)					
	Antibacterial activity				Antifungal activity	
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas Aeruginosa*	*Klebsiella pneumonia*	*Aspergillus niger*	*Aspergillus Flavus*
3a	25 (13)	25 (14)	NP	50 (9)	25 (12)	12.5 (10)
3b	12.5 (15)	12.5 (15)	25 (10)	25 (11)	12.5 (14)	12.5 (15)
3c	6.25 (18)	6.25(18)	6.25(19)	12.5(12)	12.5 (14)	25 (11)
3d	12.5 (12)	12.5 (14)	NP	12.5 (11)	12.5 (13)	25 (10)
3e	25 (12)	12.5 (13)	50 (10)	25 (11)	25 (11)	12.5 (12)
3f	12.5 (16)	6.25 (16)	6.25 (17)	6.25(19)	6.25 (19)	12.5 (14)
4a	12.5 (14)	12.5 (14)	25 (11)	25 (12)	25 (10)	12.5 (13)
4b	50 (9)	25 (11)	NP	100 (4)	25 (9)	50 (8)
4c	50 (10)	50 (11)	50 (9)	NP	NP	NP
4d	25 (11)	25 (10)	NP	12.5(12)	50 (8)	25 (11)
4e	12.5 (13)	12.5 (15)	25(10)	12.5(15)	12.5 (14)	12.5 (15)
4f	50 (7)	50 (8)	100 (8)	NP	50 (7)	NP
5a	NP	25(12)	12.5(10)	NP	NP	NP
5b	50 (10)	25(11)	25(9)	100 (5)	NP	100 (7)
Streptomycin	6.25 (20)	6.25 (17)	6.25 (19)	6.25 (18)	–	–
Itraconozole	–	–	–	–	6.25 (19)	6.25 (18)

NP indicates bacterial strains are resistant to the compounds >100 μg cm^{-3}

antifungal drugs for comparison. It is more attractive to speculate on the observation that the result of the antimicrobial activity of the various compounds **3a–3f**, **4a–4f**, **5a**, and **5b** displayed variable inhibitory effects on the growth of the bacteria depending on the nature of the substituents with the pyrazole.

The antibacterial screening results of compounds **3a–3f** revealed that acetyl-substituted pyrimidinone compounds **3c** and **3f** showed broad spectrum of antimicrobial activity against *E. coli*, *P. Aeruginosa* and *K. pneumonia* comparable with the standard streptomycin. With the introduction of ethoxy **3a** and **3d** and methoxy **3b** and **3e** groups in

place of acetyl substituent, gradual decrease in the activity against the tested bacterium was noticed. In classifying antibacterial activity of **3a**–**3f**, generally showed that 1,3-diarylpyrazole incorporated acetyl substituted pyrimidinone emerged as potent molecules. In the pyrazole-substituted 1,4-dihydropyridine series **4a**–**4f**, **4a**, **4d**, and **4e** showed moderate activity against all the bacterial strains, while they were less inhibited by compounds **4c** and **4f**, which contained acetyl substitution on the dihydropyridine. The anti-bacterial data of compounds **5a** and **5b** revealed that this series of compounds are less active against all the tested bacterial strains.

All the series of compounds displayed moderate to good activity against the various fungal pathogens. Majority of the compounds **3a**–**3f** showed appreciable antifungal activity results against *A. niger* and *A. Flavus.* Compound **3f** showed excellent antifungal activity comparable with the standard itraconozole against *A. niger.* However, other compounds **3a**–**3e** showed a good antifungal profile against both fungal strains. In the series of **4a**–**4f**, **4e** showed some degree of inhibition for the fungal strains. The compounds **4a**, **4b**, and **4d** against all the tested organisms, showed less activity. In contrast, compounds with acetyl substituted 1,4-dihydropyridine **4c** and **4d** have not shown significant antifungal activity against both the fungal strains. The results of **5a** and **5b** support the facts that the imidazole ring with pyrazole does not contribute to the antifungal efficacy.

Antioxidant activity

DPPH radical scavenging assay

In the present study, the antioxidant potential of synthesized compounds was studied using the DPPH radical scavenging technique spectrophotometrically. Radical scavenging activities of all compounds were determined by the interacting ability of compounds with stable free radical DPPH. The degree of discolouration indicated the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen-donating ability.

The results of the in vitro antioxidant activity are summarized in Table 3. Anti-oxidant activity for all the series of synthesized compounds showed promising DPPH free radical scavenging ability. The obtained result gives an idea that the substituents appear to be an important factor in their antioxidant results. In the series of newly synthesized compounds, promising antioxidant activity was shown by dihydropyrimidinones (DHPMs) series **3a**–**3f**. The potential antioxidant activity emerged from the acidic proton in the pyrimidinone ring and other groups as a substituent. Compounds **3c** (89.41 %) and **3f** (83.34 %) exhibited excellent DPPH radical scavenging activity as compared to glutathione (89.09 %). In the dihydropyridine series **4a**–**4f**, acetyl-substituted **4c** and **4f** have exhibited maximum radical scavenging activity compared to ester (**4a**, **4b**, **4d**, and **4e**)-substituted compounds. This may be due to the rapid keto-enol tautomerism attributed by these compounds. By comparing the antioxidant results, it is observed that there is a gradual decrease in the activity of acetyl (–CO–CH_3) substituent **3c**, **3f**, **4c**, **4f** followed by methoxy (–CO–OCH_3), **3b**, **3e**, **4b**, **4e** and ethoxy (–CO–OC_2H_5) ester substituents **3a**, **3d**, **4a**, **4d**. This result implies that modulation of the basic structure through ring substituents and/or additional functionalization decreases the antioxidant activity. Compounds with imidazole substituent **5a** and **5b** exhibited poor activity than **3a**–**3f** and **4a**–**4f**.

Reducing power assay

Reducing power is associated with antioxidant activity and may serve as a significant reflection to it [30]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes so that they can act as primary and secondary antioxidants. The substances, which have reduction potential, react with potassium ferricyanide to form potassium ferrocyanide, which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700 nm. Increased absorbance of the reaction mixture indicates the increased reducing power [31]. The results are summarized in Table 3.

Reducing power assay is expressed in effective concentration (mg/cm^3) equivalent of 0.5 absorbance glutathione. Among the tested compounds, compounds **3a**, **3b**, **3e**, and **4a** showed good reducing power capacity while compounds **3c**, **3d**, **4c**, **4f**, and **5b** exhibited moderate reducing power capacity in comparison with the standard glutathione.

Conclusions

In conclusion, we have synthesized a new series and highly functionalized substituted pyrazole structure by one-pot reaction under simple reaction conditions that resulted in high yields with high compatibility. The structures of the derivatives were confirmed by various spectral studies such as IR, ^{1}H NMR, ^{13}C NMR, and LC–MS followed by elemental analysis studies, and screened for their antimicrobial and antioxidant activities. The compounds **3c** and **3f** showed potent antimicrobial and antioxidant agents. From these studies, we have concluded that pyrimidinone-incorporated halo-substituted 1,3-diarylpyrazole is a better molecule from a biological activity point of view and these molecules can be designed as potential drugs with a structural modification.

Table 3 Antioxidant activity of compounds **3a–3f**, **4a–4f**, and **5a**, **5b**

Compounds	% DPPH scavenging	Reducing power assay
3a	61.36 ± 9.64	0.88 ± 0.01
3b	69.54 ± 4.35	1.75 ± 0.19
3c	89.41 ± 1.58	7.07 ± 1.66
3d	57.26 ± 6.42	2.16 ± 0.25
3e	61.36 ± 9.64	1.12 ± 0.07
3f	83.34 ± 2.50	11.32 ± 0.45
4a	42.09 ± 1.42	1.49 ± 0.11
4b	46.81 ± 0.64	25.00 ± 2.78
4c	67.26 ± 6.42	2.15 ± 0.19
4d	28.30 ± 1.12	11.90 ± 0.01
4e	36.81 ± 0.64	22.72 ± 4.67
4f	60.94 ± 3.14	4.94 ± 3.14
5a	29.09 ± 1.42	14.28 ± 2.56
5b	25.34 ± 2.23	8.33 ± 1.98
Glutathione	89.09 ± 1.09	0.595 ± 0.01

Experimental

All the reagents and solvents, purchased from commercial suppliers Sigma-Aldrich, Spectrochem India, were used without further purification. The solvents were all dried and distilled before use. Melting points were determined in an open capillary tube. The progress of each reaction was monitored by ascending thin layer chromatography (TLC) on silica gel G (Merck 1.05570.0001) and visualized by UV light. The IR spectra (in KBr pellets) were recorded on a Shimadzu-FTIR spectrometer. The ^{1}H NMR spectra were recorded ($CDCl_3$/DMSO-d_6 mixture) on a Bruker AMX 400 NMR spectrometer with 5 mm PABBO BB-1H TUBES with TMS as internal standard. LCMS was obtained using Agilent 1200 series LC and Micromass zQ spectrometer. Mass spectra of some compounds were recorded on a Jeol SX-102 (FAB) mass spectrometer. Elemental analyses were carried out using VARIO EL-III (Elementar Analysensysteme GmBH).

*General procedure for the preparation of 4-formylpyrazoles **2a, 2b***

(3,4-Dihalophenyl)ethanone phenylhydrazone (0.01 mol) was added to a mixture of the Vilsmeier-Haack reagent (prepared by dropwise addition of 1.2 cm^3 $POCl_3$ to 10 cm^3 ice-cooled DMF) and refluxed for 6 h. The reaction mixture was poured into crushed ice followed by neutralization using sodium bicarbonate. Crude product was isolated and crystallized from methanol.

3-(3,4-Dichlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**2a**, $C_{16}H_{10}Cl_2N_2O$)

Yield 87 %; m.p.: 150–152 °C; IR (KBr): $\bar{\nu}$ = 3,041 (Ar C–H), 1,678 (C=O), 1,571 (C=N), 1,489 (C=C), 813 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.42–7.83 (m, 9H, Ar–CH), 9.97 (s, 1H, CHO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 119.79, 122.75, 128.43, 129.10, 130.24, 130.61, 131.21, 131.77, 132.34, 137.15, 138.88, 149.98 (Ar–C), 184.87 (aldehyde C=O) ppm; LC–MS: m/z = 317 (M^+), 319 (M^++2).

3-(3,4-Difluorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**2b**, $C_{16}H_{10}F_2N_2O$)

Yield 75 %; m.p.: 95–98 °C; IR (KBr): $\bar{\nu}$ = 3,056 (Ar C–H), 1,675 (C=O), 1,578 (C=N), 1,453 (C = C), 1,086 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.44–9.37 (m, 9H, Ar–CH), 9.96 (s, 1H, CHO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 117.99, 119.71, 122.58, 126.04, 128.33, 129.18, 130.19, 136.83, 138.88, 148.48, 149.19, 150.43, 150.91, 151.65 (Ar–C), 184.87 (aldehyde C=O) ppm; LC–MS: m/z = 285 (M^++1).

*General procedure for the synthesis of pyrazole substituted pyrimidine derivatives **3a–3f***

A mixture of 3-(3,4-dihalophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2** (2.0 mol), ethylacetoacetate (methylacetoacetate/acetylacetone) (2.2 mol), urea (3.0 mol), and 0.5 cm^3 HCl in ethanol medium was heated to reflux for 6 h. Completion of the reaction was monitored by TLC. The resulting solution was cooled to room temperature and poured into cold water with vigorous stirring. The resulting

solid was filtered under suction, washed with 50 % ethanol, and recrystallized from hot ethanol [38].

Ethyl 4-[3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3a**, $C_{23}H_{20}Cl_2N_4O_3$)
IR (KBr): $\bar{\nu}$ = 3,392 (NH), 2,960 (Ar C–H), 1,670 (C=O), 1,602 (C=N), 1,579 (C=C), 840 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.85 (t, 3H, CH_3, J = 7.2 Hz), 2.24 (s, 3H, CH_3), 3.78 (q, 2H, CH_2, J = 7.2 Hz), 5.34 (s, 1H, CH), 7.30–7.95 (m, 9H, Ar–H), 8.39 (s, 1H, NH), 9.15 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.18 (ester CH_3), 18.49 (–CH_3), 46.43 (pyrimidine CH), 60.24 (ester CH_2), 100.90, 119.16, 124.57, 126.87, 126.94, 127.61, 129.42, 130.27, 130.62, 132.48, 132.83, 133.07, 139.53, 146.53, 148.52 (Ar–C), 153.01 (pyrimidine ring C=O), 165.33 (ester C=O) ppm; LC–MS: m/z = 471 (M^+), 473 (M^++2), 475 (M^++4).

Methyl 4-[3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3b**, $C_{22}H_{18}Cl_2N_4O_3$)
IR (KBr): $\bar{\nu}$ = 3,374 (NH), 2,994 (Ar C–H), 1,668 (C=O), 1,598 (C=N), 1,545 (C=C), 864 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.68 (s, 3H, CH_3), 3.32 (s, 3H, OCH_3), 5.47 (s, 1H, CH), 7.48–7.97 (m, 9H, Ar–H), 8.43 (s, 1H, NH), 9.25 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 46.35 (ester CH_3), 18.44 (–CH_3), 48.27 (pyrimidine CH), 101.90, 117.23, 125.71, 126.53, 126.89, 128.52, 129.52, 130.26, 131.04, 132.68, 132.87, 134.12, 138.48, 145.68, 147.52 (Ar–C), 153.50 (pyrimidine ring C=O), 164.76 (ester C=O) ppm; LC–MS: m/z = 457 (M^+), 459 (M^++2), 461 (M^++4).

5-Acetyl-4-[3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-6-methyl-3,4-dihydropyrimidin-2(1H)-one
(**3c**, $C_{22}H_{18}Cl_2N_4O_2$)
IR (KBr): $\bar{\nu}$ = 3,400 (NH), 3,012 (Ar C–H), 1,657 (C=O), 1,606 (C=N), 1,577 (C = C), 862 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.71 (s, 3H, CH_3), 2.56 (s, 3H, –CO–CH_3), 5.43 (s, 1H, CH), 7.49–7.93 (m, 9H, Ar–H), 8.58 (s, 1H, NH), 9.24 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.35 (–CO–CH_3), 17.46 (–CH_3), 47.26 (pyrimidine CH), 102.90, 116.25, 124.79, 125.89, 126.98, 129.02, 129.94, 130.22, 131.08, 132.12, 132.68, 135.45, 139.01, 145.66, 147.54 (Ar–C), 154.34 (pyrimidine ring C=O), 181.72 (ester C=O) ppm; LC–MS: m/z = 441 (M^+), 445 (M^++4).

Ethyl 4-[3-(3,4-difluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3d**, $C_{23}H_{20}F_2N_4O_3$)
IR (KBr): $\bar{\nu}$ = 3,392 (NH), 2,978 (Ar C–H), 1,662 (C=O), 1,602 (C=N), 1,542 (C=C), 1,032 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.93 (t, 3H, CH_3, J = 7.2 Hz), 2.14 (s, 3H, CH_3), 3.86 (q, 2H, CH_2, J = 7.2 Hz), 5.16 (s, 1H, CH), 7.26–7.87 (m, 9H, Ar–H), 8.46 (s, 1H, NH), 9.11 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.56 (ester CH_3), 17.24 (–CH_3), 47.37 (pyrimidine CH), 59.52 (ester CH_2), 101.90, 118.69, 123.58, 125.95, 126.90, 127.78, 129.74, 130.07, 130.98, 131.84, 132.86, 134.96, 138.87, 146.58, 148.63 (Ar–C), 153.05 (pyrimidine ring C=O), 162.52 (ester C=O) ppm; LC–MS: m/z = 438 (M^+).

Methyl 4-[3-(3,4-difluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3e**, $C_{22}H_{18}F_2N_4O_3$)
IR (KBr): $\bar{\nu}$ = 3,406 (NH), 2,956 (Ar C–H), 1,705 (C=O), 1,606 (C=N), 1,581 (C=C), 1,101 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.36 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 5.47 (s, 1H, CH), 7.22–7.81 (m, 9H, Ar–H), 8.01 (s, 1H, NH), 9.06 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 46.35 (ester CH_3), 18.44 (–CH_3), 48.27 (pyrimidine CH), 100.75, 117.46, 119.11, 124.45, 124.56, 124.66, 126.70, 126.87, 129.40, 130.10, 139.54, 146.82, 148.92, 149.15, 151.64 (Ar–C), 153.30 (pyrimidine ring C=O), 165.79 (ester C=O) ppm; LC–MS: m/z = 426 (M^++2).

5-Acetyl-4-[3-(3,4-difluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-6-methyl-3,4-dihydropyrimidin-2(1H)-one
(**3f**, $C_{22}H_{18}F_2N_4O_2$)
IR (KBr): $\bar{\nu}$ = 3,367 (NH), 2,974 (Ar C–H), 1,648 (C=O), 1,587 (C=N), 1,532 (C=C), 1,045 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.63 (s, 3H, CH_3), 2.54 (s, 3H, –CO–CH_3), 5.44 (s, 1H, CH), 7.51–7.97 (m, 9H, Ar–H), 8.39 (s, 1H, NH), 9.18 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.34 (CO–CH_3), 18.24 (–CH_3), 48.02 (pyrimidine CH), 104.23, 114.58, 123.89, 125.08, 126.68, 128.92, 129.31, 130.52, 131.10, 131.88, 132.26, 135.34, 138.92, 145.37, 147.54 (Ar–C), 158.92 (pyrimidine ring C=O), 178.64 (ester C=O) ppm; LC–MS: m/z = 408 (M^+).

General procedure for the synthesis of pyrazole substituted 1,4-dihydropyridine derivatives ***4a–4f***

3-(3,4-Dihalophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2** (1.0 mol), ethylacetoacetate (methylacetoacetate/acetyl acetone) (2.0 mol), and ammonium acetate (1.12 mol) in 20 cm^3 ethanol were refluxed for 8 h in an water bath. After the completion of the reaction, reaction mixture was concentrated and poured into crushed ice. The precipitated product was filtered, washed with water, and recrystallized from hot ethanol.

Diethyl 4-[3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate
(**4a**, $C_{28}H_{27}Cl_2N_3O_4$)
IR (KBr): $\bar{\nu}$ = 3,261 (NH), 3,109 (Ar C–H), 1,666 (C=O), 1,597 (C=N), 1,508 (C=C), 1,230 (C–O), 837 (C–Cl) cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 0.93$ (t, 6H, 2 CH_3, $J = 7.2$ Hz), 2.24 (s, 6H, 2 CH_3), 3.66 (q, 2H, CH_2, $J = 7.2$ Hz), 3.87 (q, 2H, CH_2, $J = 7.2$ Hz), 5.10 (s, 1H, CH), 7.27-8.19 (m, 9H, Ar–H), 8.84 (s, 1H, pyridine-NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 14.25$ (ester CH_3), 19.52 (–CH_3), 29.81 (pyridine CH), 59.79 (ester CH_2), 104.34, 118.89, 126.34, 127.59, 128.24, 129.05, 129.28, 129.85, 130.61, 131.84, 131.33, 134.98, 139.86, 143.39, 148.56 (Ar–C), 167.32 (ester C=O) ppm; LC–MS: $m/z = 540$ (M^+), 542 (M^++2), 544 (M^++4).

Dimethyl 4-[3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4b**, $C_{26}H_{23}Cl_2N_3O_4$)
IR (KBr): $\bar{\nu} = 3{,}267$ (NH), 3,039 (Ar C–H), 1,647 (C=O), 1,558 (C=N), 1,500 (C=C), 1,240 (C–O), 837 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.21$ (s, 6H, 2 CH_3), 1.84 (s, 6H, 2 CH_3), 4.10 (s, 1H, CH), 7.46–8.03 (m, 9H, Ar–H), 8.67 (s, 1H, pyridine-NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 51.65$ (ester CH_3), 18.53 (–CH_3), 30.02 (pyridine CH), 102.36, 118.52, 125.15, 126.89, 128.59, 129.06, 129.54, 129.89, 131.02, 132.33, 135.44, 138.68, 142.58, 146.51, 148.55 (Ar–C), 170.25 (ester C=O) ppm; LC–MS: $m/z = 512$ (M^+), 514 (M^++2), 516 (M^++4).

4-[3-(3,4-Dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-diethanone (**4c**, $C_{26}H_{23}Cl_2N_3O_2$)
IR (KBr): $\bar{\nu} = 3{,}271$ (NH), 2,998 (Ar C–H), 1,665 (C=O), 1,565 (C=N), 1,530 (C=C), 1,252 (C–O), 840 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.75$ (s, 6H, 2 CH_3), 2.68 (s, 6H, 2 CH_3), 5.34 (s, 1H, CH), 7.52–7.93 (m, 9H, Ar–H), 8.72 (s, 1H, pyridine-NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 48.15$ (–CO–CH_3), 16.32 (–CH_3), 30.72 (pyridine CH), 101.31, 117.52, 124.13, 125.99, 127.95, 128.12, 129.12, 130.01, 131.23, 132.35, 134.88, 138.67, 142.57, 145.33, 148.38 (Ar–C), 177.25 (C=O) ppm; LC–MS: $m/z = 480$ (M^+), 482 (M^++2), 484 (M^++4).

Diethyl 4-[3-(3,4-difluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4d**, $C_{28}H_{27}F_2N_3O_4$)
IR (KBr): $\bar{\nu} = 3{,}278$ (NH), 3,005 (Ar C–H), 1,661 (C=O), 1,578 (C=N), 1,512 (C=C), 1,241 (C–O), 1,040 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.53$ (t, 6H, 2 CH_3, $J = 7.3$ Hz), 2.58 (s, 6H, 2 CH_3), 3.56 (q, 2H, CH_2, $J = 7.2$ Hz), 3.74 (q, 2H, CH_2, $J = 7.2$ Hz), 5.17 (s, 1H, CH), 7.34–8.04 (m, 9H, Ar–H), 8.78 (s, 1H, pyridine-NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 14.13$ (ester CH_3), 18.98 (–CH_3), 30.08 (pyridine CH), 58.74 (ester CH_2), 104.52, 117.84, 126.53, 127.64, 128.62, 129.09, 129.32, 129.79, 131.52, 132.38, 134.99, 139.89, 143.82, 145.12, 148.76 (Ar–C), 169.54 (ester C=O) ppm; LC–MS: $m/z = 507$ (M^+).

Dimethyl 4-[3-(3,4-difluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4e**, $C_{26}H_{23}F_2N_3O_4$)
IR (KBr): $\bar{\nu} = 3{,}269$ (NH), 3,035 (Ar C–H), 1,651 (C=O), 1,564 (C=N), 1,502 (C=C), 1,120 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.31$ (s, 6H, 2 CH_3), 3.40 (s, 6H, 2 OCH_3), 5.21 (s, 1H, CH), 7.21–7.83 (m, 9H, Ar–H), 5.59 (s, 1H, pyridine-NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 50.69$ (ester CH_3), 19.42 (–CH_3), 29.49 (pyridine CH), 104.36, 116.66, 116.83, 117.52, 117.70, 118.92, 124.76, 126.32, 127.53, 129.04, 131.98, 139.86, 143.63, 148.61, 151.08 (Ar–C), 167.65 (ester C=O) ppm; LC–MS: $m/z = 480$ (M^++1), 481 (M^++2).

4-[3-(3,4-Difluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-diethanone (**4f**, $C_{26}H_{23}F_2N_3O_2$)
IR (KBr): $\bar{\nu} = 3{,}276$ (NH), 3,012 (Ar C–H), 1,656 (C=O), 1,568 (C=N), 1,530 (C=C), 1,021 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.34$ (s, 6H, 2 CH_3), 2.79 (s, 6H, 2 –CO–CH_3), 5.96 (s, 1H, CH), 7.62–7.97 (m, 9H, Ar–H), 8.34 (s, 1H, pyridine-NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 46.35$ (–CO–CH_3), 17.08 (–CH_3), 31.02 (pyridine CH), 100.68, 116.56, 123.16, 125.98, 127.98, 128.16, 129.54, 130.58, 131.68, 132.51, 134.96, 138.55, 141.78, 144.98, 147.78 (Ar–C), 176.18 (C=O) ppm; LC–MS: $m/z = 447$ (M^+).

*General procedure for the synthesis of 2,4,5-trisubstituted imidazoles **5a**, **5b***

A mixture of 3-(3,4-dihalophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2** (0.01 mol), benzil (0.01 mol), and ammonium acetate (0.05 mol) in 30 cm^3 acetic acid was refluxed for 6–7 h at 120 °C. After completion of the reaction, the reaction mixture was allowed to cool and filtered to remove any precipitate. The filtrate was added to the ice cold water and the precipitated product was collected by filtration. The crude product was recrystallized using ethanol-DMF mixture.

3-(3,4-Dichlorophenyl)-4-(4,5-diphenyl-1H-imidazol-2-yl)-1-phenyl-1H-pyrazole (**5a**, $C_{30}H_{20}Cl_2N_4$)
IR (KBr): $\bar{\nu} = 3{,}414$ (NH-str), 2,962 (Ar C–H), 1,654 (C=N), 1,562 (C=C), 802 (C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.19$–9.05 (m, 19H, Ar–H), 12.53 (s, 1H, imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 113.55$, 118.99, 127.53, 128.66, 129.16, 129.91, 130.23, 130.64, 131.09, 131.20, 131.21, 133.63, 139.39, 139.67, 147.67 (Ar–C) ppm; LC–MS: $m/z = 507$ (M^+), 509 (M^++2), 511 (M^++4).

3-(3,4-Difluorophenyl)-4-(4,5-diphenyl-1H-imidazol-2-yl)-1-phenyl-1H-pyrazole (**5b**, $C_{30}H_{20}F_2N_4$)
IR (KBr): $\bar{\nu} = 3{,}271$ (NH-str), 2,939 (Ar C–H), 1,602 (C=N), 1,508 (C=C), 1,087 (C–F) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.21$–9.05 (m, 19H, Ar–H), 12.12 (s, 1H, imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 111.55$, 117.22, 127.51, 128.66, 128.52, 129.18, 130.15, 130.78, 131.06, 131.89, 131.54, 133.28, 139.58, 139.62, 142.62 (Ar–C) ppm; LC–MS: $m/z = 474$ (M^++1).

Antibacterial activity

The newly synthesized pyrazoles were investigated for their antibacterial activity against *Staphylococcus aureus* MTCC-7,443, *Escherichia coli* MTCC-443, *Pseudomonas Aeruginosa* MTCC-424, and *Klebsiella pneumonia* MTCC-139 bacterial strains by the disc diffusion method [32, 33]. The discs 6.25 mm in diameter were punched from Whatman No.1 filter paper. Batches of 100 discs were dispensed to each screw-capped bottle and sterilized by dry heat at 140 °C for 1 h. The test compounds were prepared with different concentrations using dimethylformamide (DMF). One cm^3 containing 100 times the amount of chemical was added to each bottle, which contained 100 discs, which were then placed in triplicate in a nutrient agar medium separately seeded with fresh bacteria. The incubation was carried out at 37 °C for 24 h. Solvent and growth controls were kept, and the zones of inhibition and minimum inhibitory concentrations (MIC) were noted. The results were compared with the standard streptomycin.

Antifungal activity

Newly synthesized pyrazoles were screened for their antifungal activity against *Aspergillus niger* and *Aspergillus Flavus* in DMSO by serial plate dilution method [34, 35]. Sabouraud's agar media were prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in 100 cm^3 distilled water and the pH was adjusted to 5.7. Normal saline was used to make a suspension of the spores of the fungal strain for lawning. A loopful of a particular fungal strain was transferred to 3 cm^3 of saline to get a suspension of the corresponding species. Agar media of 20 cm^3 was poured into each Petri dish. An excess of the suspension was decanted, and the plates were dried by placing them in an incubator at 37 °C for 1 h. Using an agar punch, wells were made on these seeded agar plates, and 10–50 mg/cm^3 of the test compounds in DMSO was added into each well labeling. A control was also prepared for the plates in the same way using the solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 °C for 3–4 days. Antifungal activity was determined by measuring the inhibition zone. The results studies were compared with the standard itraconozole.

DPPH radical scavenging assay

The DPPH assay was based on the reported method [36]. Briefly, 1 mM solution of DPPH in ethanol was prepared, and this solution (1 cm^3) was added to sample solutions 1 mg/cm^3 of DMSO. The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect } (\%) = (A_0 - A_1)/A_0 \times 100,$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples or standards. Each sample was assayed at 1 mg/cm^3 and all experiments were carried out in triplicate.

Reducing power assay

The reducing powers of the synthesized compounds were determined according to the reported method [37]. Different concentrations of the samples (100–1,000 $\mu g/cm^3$) in 1 cm^3 DMSO were mixed with 2.5 cm^3 phosphate buffer (0.2 M, pH = 6.6) and 2.5 cm^3 potassium ferricyanide (1 % solution). The mixture was incubated at 50 °C for 20 min. after which 2.5 cm^3 10 % trichloroacetic acid was added to the mixture and centrifuged for 10 min. The upper layer of the solution (2.5 cm^3) was mixed with 2.5 cm^3 distilled water and 0.5 cm^3 $FeCl_3$ (0.1 %), and then the absorbance at 700 nm was measured using a spectrophotometer. The higher absorbance of the reaction mixture indicated greater reducing power. All experiments were carried out in triplicate and the reducing power assay was represented by effective concentration (mg/cm^3) equivalent to 0.5 absorbance glutathione.

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