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Short communication

Synthesis, characterization and antimicrobial studies of some new pyrazole incorporated imidazole derivatives

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ABSTRACT

In the present study two series of novel imidazole derivatives containing substituted pyrazole moiety (**3a**–**d** and **5a**–**j**) were synthesized. The first series were synthesized by the reaction of 3-aryl-1*H*-pyrazole-4-carbaldehyde thiosemicarbazones (**2a**–**d**) with DMAD and the second series by the reaction of 3-aryl-1*H*-pyrazole-4-carbaldehydes (**1a**–**e**) with 1,2-diketones (**4a,b**) in the presence of ammonium acetate. Structures of newly synthesized compounds were characterized by spectral studies. New compounds were screened for antifungal and antibacterial activities. Among the synthesized compounds, compound **3c** was found to be potent antimicrobial agent. The acute oral toxicity study for the compound **3c** was carried out and the experimental studies revealed that compound **3c** is safe up to 3000 mg/kg and no death of animals were recorded.

1. Introduction

Imidazole and its derivatives are an important class of heterocycles. 2,4,5-Triaryl-1*H*-imidazole compounds have gained the remarkable importance due to their widespread biological activities and their use in synthetic chemistry. Imidazole derivatives possess a broad spectrum of pharmacological activities such as anti-inflammatory [1], analgesic, anti-convulsant [2], antitubercular [3], antimicrobial [4], anticancer and anti-Parkinson [5] activities. Imidazole and its derivatives are of great significance due to their important roles in biological systems, particularly in enzymes, as proton donors and/or acceptors, coordination system ligands and the base of charge–transfer processes. The imidazole nucleus appears in a number of naturally occurring products like the amino acids histidine and purines, which comprise many of the most important bases in nucleic acids.

The synthesis of pyrazoles remains of great interest due to the wide applications of such heterocycles in the pharmaceutical and agrochemical industry. Pyrazole derivatives have showed significant biological activities, such as anti-microbial [6], analgesic [7], anti-inflammatory [8] and anticancer [9] activities. This gave a great impetus to the search for potential pharmacologically active drugs carrying pyrazole substituents.

Although we have newer less toxic antimicrobial agents that are available for clinical use, their clinical efficacy in some invasive fungal infections, is not optimal [10]. In recent years, the widespread use of antimicrobial agents has resulted in the development of resistance to these drugs by pathogenic microorganisms, causing an increase in morbidity and mortality [11]. Thus, intense efforts in antimicrobial drug discovery are still needed to develop more promising and effective antifungal agents for use in the clinical arena [10]. Selected azole drugs have supplied many effective antifungal agents, which are currently in clinical use. Ketoconazole and Omeprazole are well-known drugs contains imidazole ring system.

Prompted by these observations and in continuation of our research on biologically important heterocycles [12–15], we hereby report the synthesis, characterization and antimicrobial studies of some new substituted imidazoles carrying pyrazole moiety.

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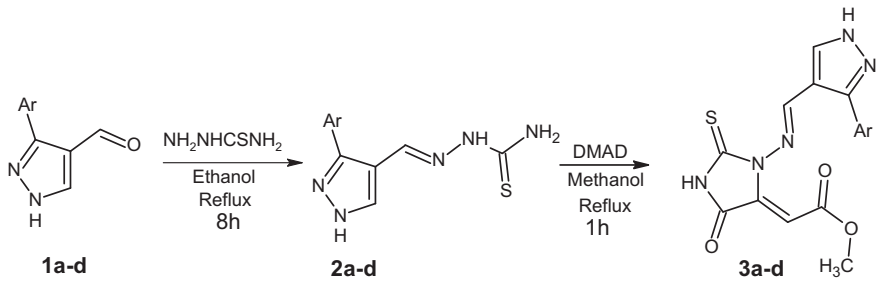


Scheme 1. Synthetic route for the compounds **3a**–**d**. Where Ar = 2,4-dichlorophenyl, 4-SCH_3–C_6H_4, 2,5-dichlorothiophene, 4-CH_3–C_6H_4.

2. Results and discussion

2.1. Chemistry

3-Substituted-1*H*-pyrazole-4-carbaldehydes (**1a**–**e**) were synthesized by the Vilsmayer Haack reaction of semicarbazones [16]. The targeted imidazoles (**3a**–**d**) were obtained in good yield by refluxing substituted thiosemicarbazones (**2a**–**d**) with dimethylacetylenedicarboxylate (DMAD) in methanol for 1 h [17]. The starting material **2a**–**d** in turn were synthesized by refluxing equimolar amount of 3-aryl-1-*H*-pyrazole-4-carbaldehyde with thiosemicarbazide in the presence of anhydrous sodium acetate in ethanol [17]. 2,4,5-Trisubstituted imidazoles (**5a**–**j**) were obtained in excellent yields by refluxing 3-substituted-1*H*-pyrazole-4-carbaldehydes (**1a**–**e**) with 1,2-diketones (**4a,b**) and ammonium acetate in acetic acid for 6–7 h via Debus reaction [18]. The reaction pathway has been summarized in Scheme 1 and Scheme 2. Newly synthesized compounds (**3a**–**d** and **5a**–**j**) were characterized by IR, NMR, mass spectral and C, H, N elemental analyses.

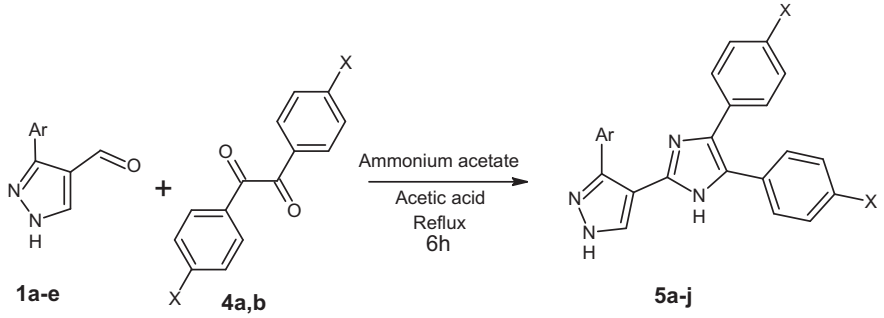
Formation of methyl(2*Z*)-[3-({(*E*)-[3-(substituted)-1*H*-pyrazol-4-yl]methylidene}amino)-5-oxo-2-thioxoimidazolidin-4-ylidene] ethanoate (**3a**–**d**) and 4-(4,5-aryl-1*H*-imidazol-2-yl)-3-[substituted]-1*H*-pyrazole (**5a**–**j**) were confirmed by recording their IR, ^{1}H NMR, ^{13}C NMR and mass spectra. For first series, IR spectrum of compound **3a** showed absorption at 3242 cm^{-1} which is due to the NH stretching. Bands at 1707, 1645 cm^{-1} are due to C=O of ester and cyclic amide respectively. Similarly, bands at 1607 cm^{-1} and 1106 cm^{-1} are due to C=N and C=S groups. The C–O stretching frequency of ester appeared at 1238 cm^{-1} and 1195 cm^{-1} further confirms the structure. The ^{1}H NMR spectrum of **3a** showed a singlet at δ 3.80 is due to OCH_3 protons. A singlet at δ 6.58 is due to C=CH. Aromatic protons appeared as multiplet at δ 7.41–7.57. Pyrazole-5H appeared as a singlet at δ 7.96. Similarly a singlet appeared at δ 8.28 is due to –N=CH protons. Two singlets at δ 12.64 and δ 13.50 are due to pyrazole-NH and imidazole-NH respectively further confirms the structure. The mass spectrum of **3a** showed molecular ion peak at m/z = 423.9 (M^+), which is in agreement with the molecular formula $C_{16}H_{11}Cl_2N_5O_3S$. For second series, IR spectrum of compound **5a** showed absorption at 3135 cm^{-1} which is due to the NH stretching. Band at 1663 cm^{-1} is due to C=N. Formation of 2,4,5-trisubstituted imidazoles further confirmed by the absence of CH stretching of aldehydic group (2700 cm^{-1}) in the IR spectrum of the final compounds. The ^{1}H NMR spectrum of **5a** showed a singlet at δ 2.53 is due to SCH_3 protons. Similarly multiplets at δ 7.32–7.99 are due to aromatic protons. Pyrazole-5H appeared as a singlet at δ 8.10. Two singlets at δ 12.40 and δ 13.30 are due to pyrazole-NH and imidazole-NH respectively further confirmed the structure of the molecule. The mass spectrum of **5a** showed molecular ion peak at m/z = 409.2 (M + 1), which is in agreement with the molecular formula $C_{25}H_{20}N_4S$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and the characterization is provided in Table 1.

2.2. Antimicrobial studies

2.2.1. Antibacterial studies

The *in vitro* antibacterial activity of newly synthesized compounds **3a**–**d** and **5a**–**j** were determined by well plate method [19,20]. In this work, *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis, Salmonella typhimorium. Clostridium profingens and Pseudomonas aeruginosa* were used to investigate the activity. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentrations of 1 and 0.5 mg/mL.

The antibacterial screening revealed that some of the tested compounds showed good inhibition against various tested microbial strains. The result indicated that among the tested compounds, **3c** showed excellent activity against *P. aeruginosa* at concentrations of 1 and 0.5 mg/mL compared to standard drug streptomycin. **3c** Showed similar activity as that of standard, against *C. profingens*, at 1 and 0.5 mg/mL concentrations. The remaining compounds showed moderately good activity against all of the six tested bacterial



Scheme 2. Synthetic route for the compounds **5a**–**j**. Where Ar = 2,4-dichlorophenyl, 4-SCH_3–C_6H_4, 2,5-dichlorothiophene, 4-CH_3–C_6H_4, Biphenyl X = H, Br.

Table 1
Characterization data of the compounds **3a**–**d** and **5a**–**j**.

Compounds	Ar	X	Molecular Formula (Mol. wt.)	Yield (%)	M.p.(°C)
3a	2,4-Dichlorophenyl	–	$C_{16}H_{11}Cl_2N_5O_3S$ (424.2)	81	282–284
3b	2,5-Dichlorothiophene	–	$C_{14}H_9Cl_2N_5O_3S_2$ (430.2)	86	280–282
3c	4-SCH_3–C_6H_4	–	$C_{17}H_{15}N_5O_3S_2$ (401.4)	84	230–232
3d	4-CH_3–C_6H_4	–	$C_{17}H_{15}N_5O_3S$ (369.3)	80	286–288
5a	4-SCH_3–C_6H_4	H	$C_{25}H_{20}N_4S$ (408.5)	73	214–216
5b	2,4-Dichlorophenyl	H	$C_{24}H_{16}Cl_2N_4$ (431.3)	72	210–212
5c	Biphenyl	H	$C_{30}H_{22}N_4$ (438.5)	75	140–142
5d	4-CH_3–C_6H_4	H	$C_{25}H_{20}N_4$ (376.4)	68	180–182
5e	2,5-Dichlorothiophene	H	$C_{22}H_{14}Cl_2N_4S$ (437.3)	70	188–190
5f	4-SCH_3–C_6H_4	Br	$C_{25}H_{18}Br_2N_4S$ (566.3)	77	190–192
5g	2,4-Dichlorophenyl	Br	$C_{24}H_{14}Br_2Cl_2N_4$ (589.1)	73	168–170
5h	Biphenyl	Br	$C_{30}H_{20}Br_2N_4$ (596.3)	75	158–160
5i	4-CH_3–C_6H_4	Br	$C_{25}H_{18}Br_2N_4$ (534.2)	72	194–196
5j	2,5-Dichlorothiophene	Br	$C_{22}H_{12}Br_2Cl_2N_4S$ (595.1)	78	140–142

Table 2
Antibacterial activity of the compounds **3a**–**b** and **5a**–**j**.

Compound name	*Staphylococcus aureus*		*Bacillus subtillis*		*Escherichia coli*		*Clostridium profingens*		*Salmonella typhimorium*		*Pseudomonas aureginosa*	
Concn (μg/ml)	1000	500	1000	500	1000	500	1000	500	1000	500	1000	500
3a	6 ± 0.02	5 ± 0.01	8 ± 0.02	7 ± 0.02	8 ± 0.01	7 ± 0.02	7 ± 0.03	6 ± 0.03	4 ± 0.02	3 ± 0.01	7 ± 0.01	6 ± 0.03
3b	7 ± 0.03	6 ± 0.02	8 ± 0.01	7 ± 0.01	9 ± 0.01	8 ± 0.03	12 ± 0.01	11 ± 0.02	10 ± 0.01	9 ± 0.01	9 ± 0.03	8 ± 0.02
3c	6 ± 0.02	5 ± 0.01	10 ± 0.02	9 ± 0.02	12 ± 0.02	9 ± 0.02	16 ± 0.01	15 ± 0.02	12 ± 0.01	11 ± 0.02	16 ± 0.02	15 ± 0.02
3d	4 ± 0.01	3 ± 0.02	9 ± 0.01	8 ± 0.01	5 ± 0.01	4 ± 0.01	6 ± 0.01	5 ± 0.01	6 ± 0.01	5 ± 0.02	4 ± 0.01	3 ± 0.01
5a	4 ± 0.01	3 ± 0.02	5 ± 0.02	4 ± 0.01	7 ± 0.01	6 ± 0.01	4 ± 0.02	3 ± 0.01	4 ± 0.01	3 ± 0.03	5 ± 0.02	4 ± 0.01
5b	6 ± 0.02	5 ± 0.02	6 ± 0.01	5 ± 0.01	10 ± 0.03	9 ± 0.02	6 ± 0.02	5 ± 0.02	3 ± 0.02	2 ± 0.01	4 ± 0.01	3 ± 0.02
5c	2 ± 0.01	1 ± 0.02	3 ± 0.02	2 ± 0.01	3 ± 0.01	2 ± 0.02	2 ± 0.02	1 ± 0.01	4 ± 0.02	3 ± 0.02	4 ± 0.02	3 ± 0.01
5d	7 ± 0.02	6 ± 0.02	8 ± 0.01	7 ± 0.02	14 ± 0.02	8 ± 0.01	6 ± 0.01	5 ± 0.02	6 ± 0.02	5 ± 0.01	6 ± 0.01	4 ± 0.01
5e	6 ± 0.02	5 ± 0.02	4 ± 0.02	3 ± 0.01	6 ± 0.01	5 ± 0.02	8 ± 0.01	7 ± 0.02	7 ± 0.02	6 ± 0.01	6 ± 0.03	5 ± 0.02
5f	3 ± 0.02	2 ± 0.02	5 ± 0.01	4 ± 0.02	3 ± 0.02	2 ± 0.02	4 ± 0.02	3 ± 0.02	4 ± 0.01	3 ± 0.02	3 ± 0.01	2 ± 0.02
5g	4 ± 0.02	3 ± 0.01	5 ± 0.02	4 ± 0.03	5 ± 0.02	4 ± 0.02	3 ± 0.01	2 ± 0.01	4 ± 0.02	3 ± 0.02	5 ± 0.03	4 ± 0.02
5h	2 ± 0.01	1 ± 0.02	4 ± 0.01	3 ± 0.02	3 ± 0.02	2 ± 0.02	2 ± 0.02	1 ± 0.02	3 ± 0.02	2 ± 0.01	2 ± 0.02	1 ± 0.02
5i	3 ± 0.02	2 ± 0.01	4 ± 0.02	3 ± 0.02	4 ± 0.01	3 ± 0.02	3 ± 0.01	2 ± 0.01	4 ± 0.02	3 ± 0.02	2 ± 0.01	1 ± 0.02
5j	3 ± 0.02	2 ± 0.02	2 ± 0.01	1 ± 0.03	4 ± 0.01	3 ± 0.01	5 ± 0.02	4 ± 0.02	2 ± 0.01	1 ± 0.03	4 ± 0.03	3 ± 0.02
Streptomycin (Std.)	15 ± 0.02	10 ± 0.01	21 ± 0.02	11 ± 0.02	16 ± 0.02	10 ± 0.01	17 ± 0.02	16 ± 0.01	18 ± 0.02	17 ± 0.01	13 ± 0.02	9 ± 0.01

strains compared to standard, streptomycin. Results of antibacterial studies have been presented in Table 2.

2.2.2. Antifungal studies

Newly synthesized compounds **3a**–**d** and **5a**–**j** were also screened for their antifungal activity against *Aspergillus flavus, Aspergillus niger*, *Candida albicans, Microsporum gypseum*, and *Trichophyton rubrum*. The compounds were dissolved in DMSO and antimicrobial activity was determined by well plate method [21,22] at concentration of 1 and 0.5 mg/mL.

Among the tested compounds, the compound **3c** has emerged as active against *T. rubrum* compared with standard, fluconazole. Whereas the other compounds showed less activity against all the tested microorganisms compared to standard. It can be concluded that none of the prepared compounds were superior to standard against various tested microbial strains, but the antifungal activities

Table 3
Antifungal activity of the compounds **3a**–**b** and **5a**–**j**.

Compound name	*Candida albicans*		*Microsporum gypseum*		*Aspergillus flavus*		*Aspergillus niger*		*Trichophyton rubrum*	
Concn (μg/ml)	1000	500	1000	500	1000	500	1000	500	1000	500
3a	7 ± 0.03	6 ± 0.01	7 ± 0.02	6 ± 0.01	12 ± 0.01	11 ± 0.01	6 ± 0.03	5 ± 0.03	10 ± 0.01	9 ± 0.02
3b	6 ± 0.02	5 ± 0.01	6 ± 0.01	5 ± 0.01	10 ± 0.02	9 ± 0.01	6 ± 0.02	5 ± 0.01	13 ± 0.02	12 ± 0.01
3c	5 ± 0.03	4 ± 0.02	5 ± 0.01	4 ± 0.01	7 ± 0.01	6 ± 0.03	10 ± 0.02	9 ± 0.03	16 ± 0.02	15 ± 0.01
3d	4 ± 0.01	3 ± 0.01	4 ± 0.02	3 ± 0.01	6 ± 0.02	5 ± 0.01	10 ± 0.02	9 ± 0.01	10 ± 0.02	9 ± 0.01
5a	10 ± 0.01	9 ± 0.01	6 ± 0.01	5 ± 0.01	5 ± 0.01	4 ± 0.02	10 ± 0.02	9 ± 0.01	12 ± 0.01	11 ± 0.02
5b	12 ± 0.02	11 ± 0.01	4 ± 0.02	3 ± 0.01	8 ± 0.02	7 ± 0.03	9 ± 0.01	7 ± 0.01	10 ± 0.02	8 ± 0.02
5c	9 ± 0.01	8 ± 0.01	5 ± 0.01	4 ± 0.02	9 ± 0.01	7 ± 0.02	10 ± 0.01	9 ± 0.02	9 ± 0.03	8 ± 0.02
5d	10 ± 0.03	9 ± 0.02	3 ± 0.02	2 ± 0.01	10 ± 0.02	9 ± 0.01	10 ± 0.01	9 ± 0.02	12 ± 0.01	11 ± 0.02
5e	10 ± 0.02	9 ± 0.01	5 ± 0.03	4 ± 0.02	10 ± 0.01	9 ± 0.01	10 ± 0.02	9 ± 0.01	10 ± 0.01	9 ± 0.02
5f	12 ± 0.02	11 ± 0.01	4 ± 0.02	3 ± 0.01	13 ± 0.02	12 ± 0.01	6 ± 0.02	5 ± 0.03	8 ± 0.03	7 ± 0.02
5g	9 ± 0.01	8 ± 0.01	4 ± 0.01	3 ± 0.03	5 ± 0.02	4 ± 0.02	5 ± 0.03	4 ± 0.01	10 ± 0.01	9 ± 0.01
5h	3 ± 0.02	2 ± 0.01	2 ± 0.01	1 ± 0.02	4 ± 0.01	3 ± 0.01	3 ± 0.01	2 ± 0.01	4 ± 0.02	3 ± 0.02
5i	4 ± 0.01	3 ± 0.02	2 ± 0.01	1 ± 0.01	3 ± 0.01	2 ± 0.02	3 ± 0.02	2 ± 0.01	3 ± 0.01	2 ± 0.01
5j	6 ± 0.01	5 ± 0.01	3 ± 0.03	2 ± 0.02	4 ± 0.01	3 ± 0.03	4 ± 0.01	3 ± 0.02	6 ± 0.01	5 ± 0.01
Flucanazole (Std.)	22 ± 0.03	21 ± 0.01	15 ± 0.03	14 ± 0.02	22 ± 0.01	21 ± 0.02	20 ± 0.01	18 ± 0.02	24 ± 0.01	22 ± 0.01

of some of the compounds are comparable to those of standard. Results of antifungal studies have been presented in Table 3.

2.2.3. Acute toxicity and behavioral studies

The acute oral toxicity study for the test compound **3c** was carried out by following the OECD guidelines. Swiss albino female mice weighing 25–30 g were used for the evaluation. Each group consisting of 6 female mice (overnight fasted) was kept in the colony cage at 25 ± 2 °C with 55% relative humidity and 12 h light/dark cycle was maintained. A Different dose from 1000 to 3000 mg/kg was selected and administered orally as a single dose as fine suspension prepared in double distilled water using Tween 80. The acute toxic symptoms and the behavioral changes produced by the test compound were observed continuously for 4th h, 8th h, 12th h and 24th h onset of toxic symptoms and behavioral changes were also recorded [23,24]. The experimental studies revealed that the synthesized compound **3c** is safe up to 3000 mg/kg and no death of animals was recorded. Further, no significant behavioral changes were observed in experimental animals.

3. Conclusion

Two series of novel substituted imidazole derivatives were synthesized in reasonably good yields. They were characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry, IR studies and elemental analyses. All the newly synthesized compounds were screened for antimicrobial activity by well plate method. Among the screened samples, compound **3c** has showed excellent anti-microbial activity at 1 and 0.5 mg/mL concentrations against tested microbial strains as compared to the standard drug.

As regards the relationships between the structure of the heterocyclic scaffold and the detected antibacterial properties, it showed varied biological activity. Among the tested compounds, compound **3c** showed excellent activity against *P. aeruginosa* at concentrations of 1 and 0.5 mg/mL compared to standard drug streptomycin. **3c** Showed similar activity as that of standard, against *C. profingens*, at 1 and 0.5 mg/mL concentrations. Compound **3c** has thioanisyl moiety, which is accounted for the enhanced antibacterial activity. However from the second series, compounds showed moderate antimicrobial activity. Imidazole and pyrazole nucleus which is present in both the series are responsible for the biological activity. However the presence of other substituents is responsible for the varied biological activity of the compounds.

The acute oral toxicity study for the compound **3c** was carried out and the experimental studies revealed that compound **3c** is safe up to 3000 mg/kg and no death of animals were recorded.

4. Experimental

4.1. Chemistry

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a JASCO FT/IR-4100 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz) using TMS as internal standard. Chemical shift values are given in δ (ppm) scales. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254) obtained from Merck. Commercial grade solvents and reagents were used without further purification.

*4.2. General procedure for the synthesis of methyl(2Z)-[3-({(E)-[3-aryl-1H-pyrazol-4-yl]methylidene}amino)-5-oxo-2-thioxoimidazolidin-4-ylidene]ethanoate (**3a**–**d**)*

An equimolar mixture of 3-aryl-1*H*-pyrazole-4-carbaldehyde thiosemicarbazone **2a**–**d** (0.01 mol) and dimethylacetylenedicarboxylate (DMAD) (0.01 mol) in methanol (20 mL) were refluxed for 1 h. After completion of the reaction, the reaction mixture was allowed to cool to the room temperature. The solid thus separated was collected by filtration and recrystallized using ethanol-DMF mixture.

4.2.1. Characterization of synthesized compounds

*4.2.1.1. (Z)-Methyl 2-(3-((E)-(3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl)methyleneamino)-5-oxo-2-thioxoimidazolidin-4-ylidene)acetate (**3a**).* IR (KBr, ν_{max} cm^{-1}): 3242 (N–H-str), 3068, 2951 (C–H-str), 1707 (C=O ester), 1645 (C=O cyclic amide), 1607 (C=N), 1106 (C=S), 1238, 1195 (C-O ester); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 6.58 (s, 1H, C=CH), 7.41–7.57 (m, 3H, Ar–H), 7.96 (s, 1H, pyrazole-5H), 8.28 (s, 1H, N=CH), 12.64 (s, 1H, pyrazole-NH), 13.50 (s, 1H, imidazole-NH); MS: m/z = 423.9 (M^+), 425.9 (M + 2), 427.9 (M + 4); Anal. calcd. for $C_{16}H_{11}Cl_2N_5O_3S$: C, 45.30; H, 2.61; N, 16.71; Found: C, 45.23; H, 2.57; N, 16.67%.

*4.2.1.2. (Z)-Methyl 2-(3-((E)-(3-(2,5-dichlorothiophen-3-yl)-1H-pyrazol-4-yl)methyleneamino)-5-oxo-2-thioxoimidazolidin-4-ylidene) acetate (**3b**).* IR (KBr, ν_{max} cm^{-1}): 3213 (N–H-str), 3051, 2953 (C–H-str), 1713 (C=O ester), 1640 (C=O cyclic amide), 1602 (C=N), 1023 (C=S), 1247, 1197 (C–O ester); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 6.62 (s, 1H, C=CH), 7.23 (s, 1H, Ar–H), 8.35 (s, 1H, pyrazole-5H), 8.38 (s, 1H, N=CH), 12.71 (s, 1H, pyrazole-NH), 13.60 (s, 1H, imidazole-NH); ^{13}C NMR: δ 165.77, 165.65, 151.60, 142.92, 131.66, 128.72, 125.06, 114.89, 114.13, 52.38; MS: m/z = 430.0 (M^+), 432.0 (M + 2). 434.0 (M + 4); Anal. calcd. for $C_{14}H_9Cl_2N_5O_3S_2$: C, 39.08; H, 2.11; N, 16.28; Found: C, 39.03; H, 2.06; N, 16.26%.

*4.2.1.3. (Z)-Methyl 2-(3-((E)-(3-(4-(methylthio)phenyl)-1H-pyrazol-4-yl)methyleneamino)-5-oxo-2-thioxoimidazolidin-4-ylidene)acetate (**3c**).* IR (KBr, ν_{max} cm^{-1}): 3121 (N–H-str), 3033, 2950 (C–H-str), 1711 (C=O ester), 1636 (C=O cyclic amide), 1598 (C=N), 1096 (C=S), 1240, 1188 (C–O ester); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.55 (s, 3H, SCH_3), 3.79 (s, 3H, OCH_3), 6.65 (s, 1H, C=CH), 7.40–7.77 (m, 4H, Ar–H), 8.48 (s, 1H, pyrazole-5H), 8.64 (s, 1H, N=CH), 12.75 (s, 1H, pyrazole-NH), 13.48 (s, 1H, imidazole-NH); MS: m/z = 402.0 (M + 1); Anal. calcd. for $C_{17}H_{15}N_5O_3S_2$: C, 50.86; H, 3.77; N, 17.44; Found: C, 50.79; H, 3.71; N, 17.41%.

*4.2.1.4. (Z)-Methyl2-(5-oxo-2-thioxo-3-((E)-(3-p-tolyl-1H-pyrazol-4-yl)methyleneamino) imidazolidin-4-ylidene)acetate (**3d**).* IR (KBr, ν_{max} cm^{-1}): 3241 (N–H-str), 3029, 2950 (C–H-str), 1706 (C=O ester), 1641 (C=O cyclic amide), 1611 (C=N), 1098 (C=S), 1240, 1195 (C–O ester); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 6.65 (s, 1H, C=CH), 7.35–7.65 (m, 4H, Ar–H), 7.96 (s, 1H, pyrazole-5H), 8.42 (s, 1H, N=CH), 12.72 (s, 1H, pyrazole-NH), 13.41 (s, 1H, imidazole-NH); MS: m/z = 370.1 (M + 1); Anal. calcd. for $C_{17}H_{15}N_5O_3S$: C, 55.27; H, 4.09; N, 18.96; Found: C, 55.21; H, 4.05; N, 18.93%.

*4.3. General procedure for the synthesis of new derivatives of 2,4,5-trisubstituted imidazoles (**5a**–**i**)*

A mixture of 3-aryl-1*H*-pyrazole-4-carbaldehyde **1a**–**d** (0.01 mol), 1,2-diketone **4a,b** (0.01 mol) and ammonium acetate (0.05 mol) in acetic acid (50 mL) were 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. 300 mL of ice-water was added to the filtrate and the precipitated product was collected by filtration. The crude product was recrystallized using ethanol-DMF mixture.

4.3.1. Characterization of synthesized compounds

*4.3.1.1. 4-(4,5-Diphenyl-1H-imidazol-2-yl)-3-[4-(methylsulfanyl)phenyl]-1H-pyrazole (**5a**).* IR (KBr, ν_{max} cm^{-1}): 3120 (N–H-str), 3058, 2920 (C–H-str), 1663 (C=N), 1602 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.53 (s, 3H, SCH_3), 7.32–7.99 (m, 14H, Ar–H), 8.10 (s, 1H, pyrazole-5H), 12.40 (s, 1H, pyrazole-NH), 13.30 (s, 1H, imidazole-NH); MS: m/z = 409.2 (M + 1); Anal. calcd. for $C_{25}H_{20}N_4S$: C, 73.50; H, 4.93; N, 13.71; Found: C, 73.41; H, 4.88; N, 13.69%.

*4.3.1.2. 3-(2,4-Dichlorophenyl)-4-(4,5-diphenyl-1H-imidazol-2-yl)-1H-pyrazole (**5b**).* IR (KBr, ν_{max} cm^{-1}): 3135 (N–H-str), 3062, 2922 (C–H-str), 1668 (C=N), 1598 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14–7.92 (m, 13H, Ar–H), 8.27 (s, 1H, pyrazole-5H), 12.22 (s, 1H, pyrazole-NH), 13.24 (s, 1H, imidazole-NH); ^{13}C NMR: δ 194.77, 184.39, 171.94, 140.16, 135.4, 134.5, 133.95, 133.27, 132.22, 131.38, 129.55, 129.46, 128.51, 128.05, 126.55, 120.97, 111.56; MS: m/z = 431.2 (M^+), 433.1 (M + 2), 435.1 (M + 4); Anal. calcd. for $C_{24}H_{16}Cl_2N_4$: C, 66.83; H, 3.74; N, 12.99; Found: C, 66.76; H, 3.68; N, 12.95%.

*4.3.1.3. 3-(Biphenyl-4-yl)-4-(4,5-diphenyl-1H-imidazol-2-yl)-1H-pyrazole (**5c**).* IR (KBr, ν_{max} cm^{-1}): 3129 (N–H-str), 3055, 2922 (C–H-str), 1669 (C=N), 1599 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.34–8.14 (m, 19H, Ar–H), 8.17 (s, 1H, pyrazole-5H), 12.39 (s, 1H, pyrazole-NH), 13.19 (s, 1H, imidazole-NH); ^{13}C NMR: δ 140.52, 139.63, 139.46, 135.45, 129.52, 129.44, 128.89, 128.47, 127.45, 126.85, 126.54, 126.17, 109.74; MS: m/z = 439.3 (M + 1); Anal. calcd. for $C_{30}H_{22}N_4$: C, 82.17; H, 5.06; N, 12.78; Found: C, 82.06; H, 5.01; N, 12.75%.

*4.3.1.4. 4-(4,5-Diphenyl-1H-imidazol-2-yl)-3-(4-methylphenyl)-1H-pyrazole (**5d**).* IR (KBr, ν_{max} cm^{-1}): 3130 (N–H-str), 3054, 2919 (C–H-str), 1654 (C=N), 1604 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.07 (s, 3H, CH_3), 7.16–7.91 (m, 14H, Ar–H), 7.92 (s, 1H, pyrazole-5H), 12.28 (s, 1H, pyrazole-NH), 13.10 (s, 1H, imidazole-NH); ^{13}C NMR: δ 184.70, 140.62, 137.21, 129.53, 129.44, 128.54, 128.11, 127.91, 127.03, 109.39, 20.98; MS: m/z = 377.2 (M + 1); Anal. calcd. for $C_{25}H_{20}N_4$: C, 79.76; H, 5.35; N, 14.88; Found: C, 79.66; H, 5.28; N, 14.55%.

*4.3.1.5. 3-(2,5-Dichlorothiophen-3-yl)-4-(4,5-diphenyl-1H-imidazol-2-yl)-1H-pyrazole (**5e**).* IR (KBr, ν_{max} cm^{-1}): 3135 (N–H-str), 3049, 2924 (C–H-str), 1676 (C=N), 1604 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.16–7.64 (m, 11H, Ar–H), 8.24 (s, 1H, pyrazole-5H), 12.31 (s, 1H, pyrazole-NH), 13.34 (s, 1H, imidazole-NH); ^{13}C NMR: δ 194.79, 171.95, 139.85, 135.98, 135.50, 132.23, 131.22, 130.18, 129.56, 129.47, 128.62, 128.15, 127.61, 126.57, 123.25, 111.34; MS: m/z = 437.1 (M^+), 439.1 (M + 2), 441.1 (M + 4); Anal. calcd. for $C_{22}H_{14}Cl_2N_4S$: C, 60.42; H, 3.23; N, 12.81; Found: C, 60.39; H, 3.17; N, 12.76%.

*4.3.1.6. 4-[4,5-Bis(4-bromophenyl)-1H-imidazol-2-yl]-3-[4-(methylsulfanyl)phenyl]-1H-pyrazole (**5f**).* IR (KBr, ν_{max} cm^{-1}): 3290 (N–H-str), 3050, 2960 (C–H-str), 1658 (C=N), 1573 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.49 (s, 3H, SCH_3), 7.30–7.95 (m, 12H, Ar–H), 8.19 (s, 1H, pyrazole-5H), 12.45 (s, 1H, pyrazole-NH), 13.19 (s, 1H, imidazole-NH); MS: m/z = 567.0 (M + 1), 568.0 (M + 2); Anal. calcd. for $C_{25}H_{18}Br_2N_4S$: C, 53.02; H, 3.20; N, 9.89; Found: C, 52.95; H, 3.16; N, 9.84%.

*4.3.1.7. 4-[4,5-bis(4-bromophenyl)-1H-imidazol-2-yl]-3-(2,4-dichlorophenyl)-1H-pyrazole (**5g**).* IR (KBr, ν_{max} cm^{-1}): 3387 (N–H-str), 3068, 2961 (C–H-str), 1617 (C=N), 1485 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.25–7.68 (m, 11H, Ar–H), 8.25 (s, 1H, pyrazole-5H), 12.37 (s, 1H, pyrazole-NH), 13.28 (s, 1H, imidazole-NH); MS: m/z = 589.0 (M^+), 591.0 (M + 2), 593.0 (M + 4); Anal. calcd. for $C_{24}H_{14}Br_2Cl_2N_4$: C, 48.93; H, 2.40; N, 9.51; Found: C, 48.88; H, 2.36; N, 9.49%.

*4.3.1.8. 3-(biphenyl-4-yl)-4-(4,5-bis(4-bromophenyl)-1H-imidazol-2-yl)-1H-pyrazole (**5h**).* IR (KBr, ν_{max} cm^{-1}): 3148 (N–H-str), 3020, 2923 (C–H-str), 1655 (C=N), 1599 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28–7.91 (m, 17H, Ar–H), 8.08 (s, 1H, pyrazole-5H), 12.36 (s, 1H, pyrazole-NH), 13.21 (s, 1H, imidazole-NH); MS: m/z = 596.9 (M + 1), 597.9 (M + 2); Anal. calcd. for $C_{30}H_{20}Br_2N_4$: C, 60.42; H, 3.38; N, 9.40; Found: C, 60.38; H, 3.33; N, 9.36%.

*4.3.1.9. 4-[4,5-bis(4-bromophenyl)-1H-imidazol-2-yl]-3-(4-methylphenyl)-1H-pyrazole (**5i**).* IR (KBr, ν_{max} cm^{-1}): 3135 (N–H-str), 3040, 2916 (C–H-str), 1608 (C=N), 1552 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.33 (s, 3H, CH_3), 7.22–7.88 (m, 12H, Ar–H), 8.05 (s, 1H, pyrazole-5H), 12.42 (s, 1H, pyrazole-NH), 13.11 (s, 1H, imidazole-NH); ^{13}C NMR: δ 171.97, 141.29, 137.37, 131.48, 129.51, 128.62, 127.93, 120.34, 109.10, 21.02; MS: m/z = 535.1 (M + 1), 536.1 (M + 2); Anal. calcd. for $C_{25}H_{18}Br_2N_4$: C, 56.20; H, 3.40; N, 10.49; Found: C, 56.15; H, 3.34; N, 10.45%.

*4.3.1.10. 4-[4,5-bis(4-bromophenyl)-1H-imidazol-2-yl]-3-(2,5-dichlorothiophen-3-yl)-1H-pyrazole (**5j**).* IR (KBr, ν_{max} cm^{-1}): 3123 (N–H-str), 3010, 2922 (C–H-str), 1615 (C=N), 1575 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.37–7.85 (m, 12H, Ar–H), 8.27 (s, 1H, pyrazole-5H), 12.41 (s, 1H, pyrazole-NH), 13.36 (s, 1H, imidazole-NH); ^{13}C NMR: δ 206.46, 192.85, 140.50, 132.56, 131.73, 131.59, 131.20, 130.03, 128.63, 125.95, 120.84, 119.49, 111.07; MS: m/z = 594.9 (M^+), 596.9 (M + 2), 598.9 (M + 4); Anal. calcd. for $C_{22}H_{12}Br_2Cl_2N_4S$: C, 44.40; H, 2.03; N, 9.41; Found: C, 44.35; H, 2.01; N, 9.38%.

4.4. Antibacterial studies

The antibacterial activity of newly synthesized compounds **3a**–**d** and **5a**–**j** were determined by well plate method in Mueller-Hinton Agar. The *in vitro* antibacterial activity was carried out against 24 h old cultures of bacterial strains. In this work, *E. coli* (ATTC-25922), *S. aureus, B. subtilis, S. typhimorium. profingens, and P. aeruginosa* (ATCC-27853) were used to investigate the activity. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 1 and 0.5 mg/mL. Twenty milliliters of sterilized agar media was poured into each pre-sterilized Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for an hour. About 60 μl of 24 h old culture suspension were poured and neatly swabbed with the pre-sterilized cotton swabs. Six millimeter diameter well were then punched carefully using a sterile cork borer and 30 μl of test solutions of different concentrations were added into each labeled well. The plates were incubated for 24 h at 37 °C. The inhibition zone that appeared after 24 h, around the well in each plate were measured as zone of inhibition in mm. Experiments were triplicates and standard deviation was calculated.

4.5. Antifungal studies

Antifungal studies of newly synthesized compounds **3a**–**d** and **5a**–**j** were carried out against *A. flavus, A. niger, C. albicans, M. gypseum, T. rubrum*. Sabourands agar media was prepared by dissolving peptone (10 g), D-glucose (40 g) and agar (20 g) in distilled water (1000 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was

decanted and plates were dried by placing in incubator at 37 °C for 1 h. Using sterile cork borer punched carefully, wells were made on these seeded agar plates different concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 25 °C for 72 h. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with fluconazole as standard. Zones of inhibition were determined for compounds **3a**–**3d** and **5a**–**5j**.

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