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Facile NMI-MsCl mediated synthesis of novel pyrazole derivatives bearing heteroaryl amides as potent antimicrobial agents

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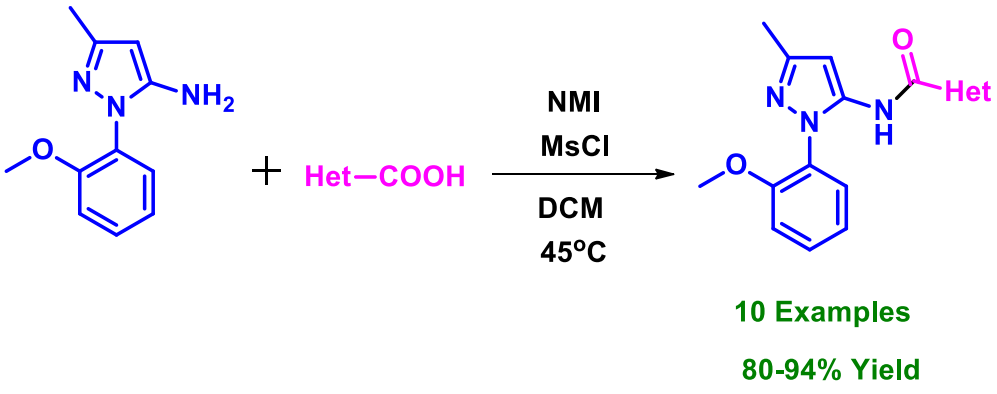
Abstract

We herein report the convenient synthesis of a series of novel pyrazole derivatives linked to heteroaryl groups via amide functionality.NMI-MsCl mediated amide bond formation reaction has been successfully employed for the synthesis of novel pharmacologically relevant pyrazole derivatives. The antimicrobial potential of the newly synthesized compoundswere evaluated at the later stage.



Keywords: Pyrazole, mesyl chloride, *N*-methylimidazole, antimicrobial

Introduction

The nitrogen, and oxygen containing heterocyclic compounds are of considerable interest nowadays owing to its various applications in drug-discovery, agrochemicals, and material science.[1-3]Among the various heterocyclic compounds discovered so far, pyrazole, and its derivatives have a dominant space in the area of drug discovery owing to their varied applications in pharmaceutical industries, and academics.[4-6] The molecules derived from pyrazoles are utilized as agrochemicals for the extensive protection of crops in the form of insecticides, fungicides, and herbicides.[7] Pyrazole based architectures are known to possess a significant role in the area of medicinal chemistry for designing, synthesizing, and developing novel biologically active molecules leading to drugs.[8,9] The presence of pyrazole nucleus in many FDA approved marketed drugs further underlines its significance (Fig 1). In addition to this, many pyrazole based molecules are under different phases of clinical trials, and could appear as possible drugs in near future.[10]Among the diverse pyrazole based architectures, 5-aminopyrazole is an important scaffold because of its synthetic versatility, and varied applications in drug-discovery.[11] 5-Aminopyrazoles are included in many bioactive compounds that target Aurora kinases as well as polo-like (PLK), and cyclin-dependent (CDK) kinases. They also targets adenosine A1 receptor, neuropeptide Y receptor 5 (NPY5), alpha-7 nicotinic acetylcholine receptors (a7nAChR), and corticotrophin-releasing factor-1 (CRF-1) receptor.[12-14] In addition to this, 5-aminopyrazoles are useful building blocks for the synthesis of several fused nitrogen heterocycles of potential biological importance.[15]

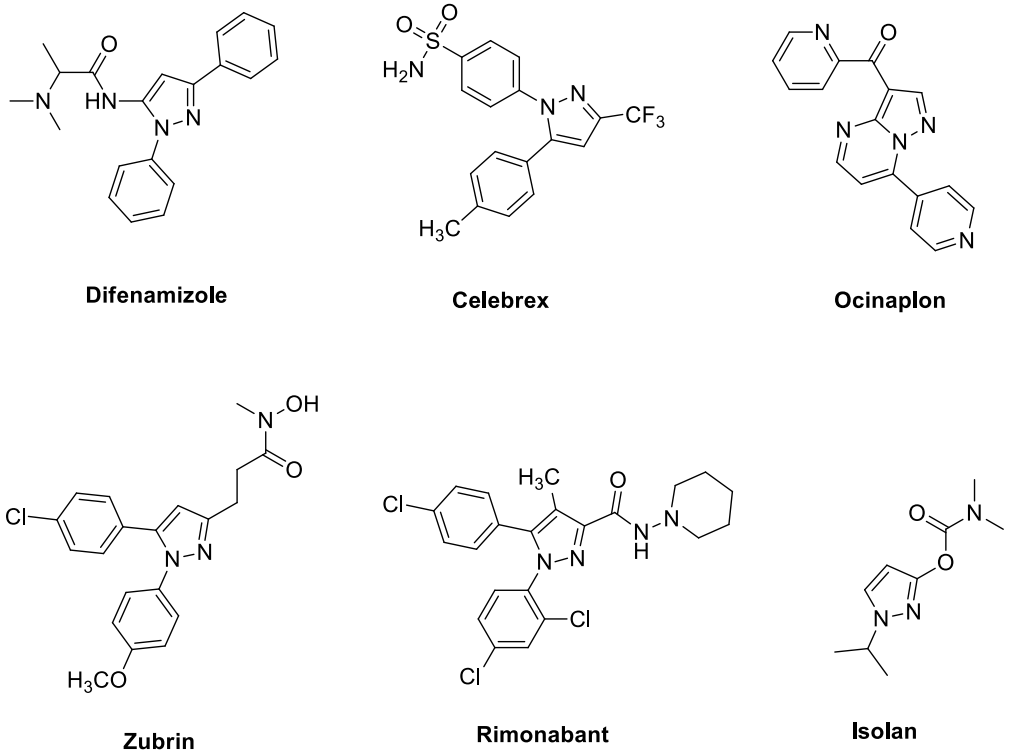


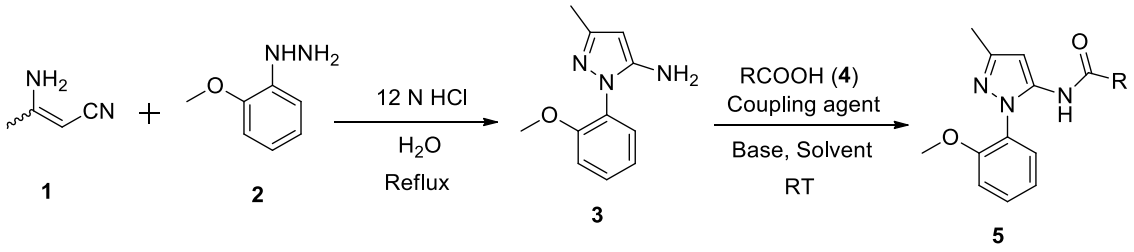
Figure 1. Some marketed drugs containing pyrazole moiety.

The role of amides, and thioamides as efficient linkers in the modern area of drug-discovery is well reported in literature.[16]The amides attached to various heterocyclic groups are hypothesized to be acting as

linkers facilitating the enhancement of the pharmacological activities by suitable binding to the active site of the protein.In view of these aforementioned observations concerning amides, pyrazoles, and 5-aminopyrazoles, it was rationalized that the synthesis of pyrazoles linked with different heteroaryl functionalities via amide bond will result in novel molecules of improved pharmacological potential. Accordingly, it was planned to design a convenient methodology for the facile synthesis of amide functionality at C-5 of pyrazole moiety from corresponding amine. The synthesis of *N*-aryl-5-aminopyrazoles by the reaction of phenylhydrazine, and 3-aminocrotononitrile has already been reported in the literature.[17]In this communication, we herein report the N-methylimidazole (NMI), and methanesulfonylchloride (MsCl) mediated facile synthesis of a series of novel substituted pyrazole derivatives linked to heteroaryl groups via amide functionality.

Results and Discussion

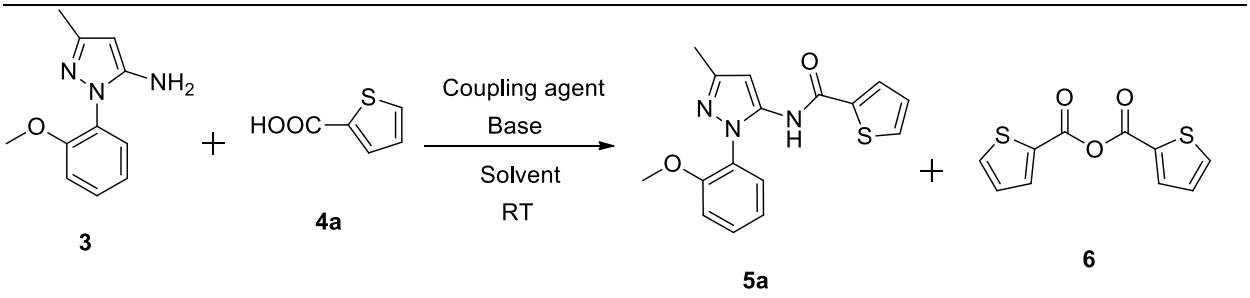
The synthetic methodology adopted by us has been depicted in Scheme 1. The reaction of 2-methoxyphenyl hydrazine **2** with 3-aminocrotononitrile **1** in presence of 12 N HCl, and water as solvent at reflux conditions procured the 1-(2-methoxyphenyl)-3-methyl-1*H*-pyrazol-5-amine precursor **3** at 90% yield.[17] This key amino precursor was then subjected to classical peptide coupling reaction conditions with various heteroaryl acids **4a-j** for synthesizing an array of pharmacologically relevant pyrazole linked with amides **5a-j**.



Scheme 1. Synthesis of amino precursor, and its coupling with various heteroaryl acids.

The key amino precursor **3,** and thiophene-2-carboxylic acid **4a** was selected for our initial control experiments in view of identifying the optimum reaction reactions for obtaining the maximum yield of the desired products (Table 1). Our first screening experiment was carried out by employing EDC·HCl as the coupling reagent, and DIPEA as a base in DCM solvent for 3 hours at room temperature (Table 1, entry 1). The reaction got completed within the specific time as monitored by TLC. However, the desired amide product **5a** was not obtained as a major product. Instead, we obtained the anhydride **6** that was derived from the acid, as the major product. This obtained result enlightened us the need for a detailed optimization study to improve the yield of the desired product **5a** by minimizing the formation of anhydride **6.**

Table 1. Optimization of reaction conditions[a]



Entry	Coupling Reagent	Base	Solvent	Yield[b] (%)	
				5a	**6**
1	EDC·HCl	DIPEA	DCM	30	60
2	EDC·HCl	DIPEA	DMF	20	65
3	EDC·HCl	TEA	DCM	25	60
4	HBTU	DIPEA	DMF	20	65
5	HBTU	TEA	DMF	33	55
6	HBTU	DIPEA	DCM	35	60
7	T3P	DIPEA	DCM	30	65
8	HATU	DIPEA	DCM	35	55
9	HCTU	DIPEA	DCM	38	55
10	MsCl	DIPEA	DCM	55	35
11	MsCl	NMI	DCM	80	10
12[c]	MsCl	NMI	DCM	90	Trace
13[c]	TsCl	NMI	DCM	70	25
14[c]	TfCl	NMI	DCM	75	23
15[c]	MsCl	NMI	DMF	75	20
16[c]	MsCl	NMI	ACN	80	15

[a]Reaction conditions: Amino precursor **3** (1 mmol), Thiophene-2-carboxylic acid **4a** (1.2 mmol), Base (2 mmol), Coupling reagent (1 mmol), Solvent (3 mL), 3h, RT.

[b]Isolated yield.

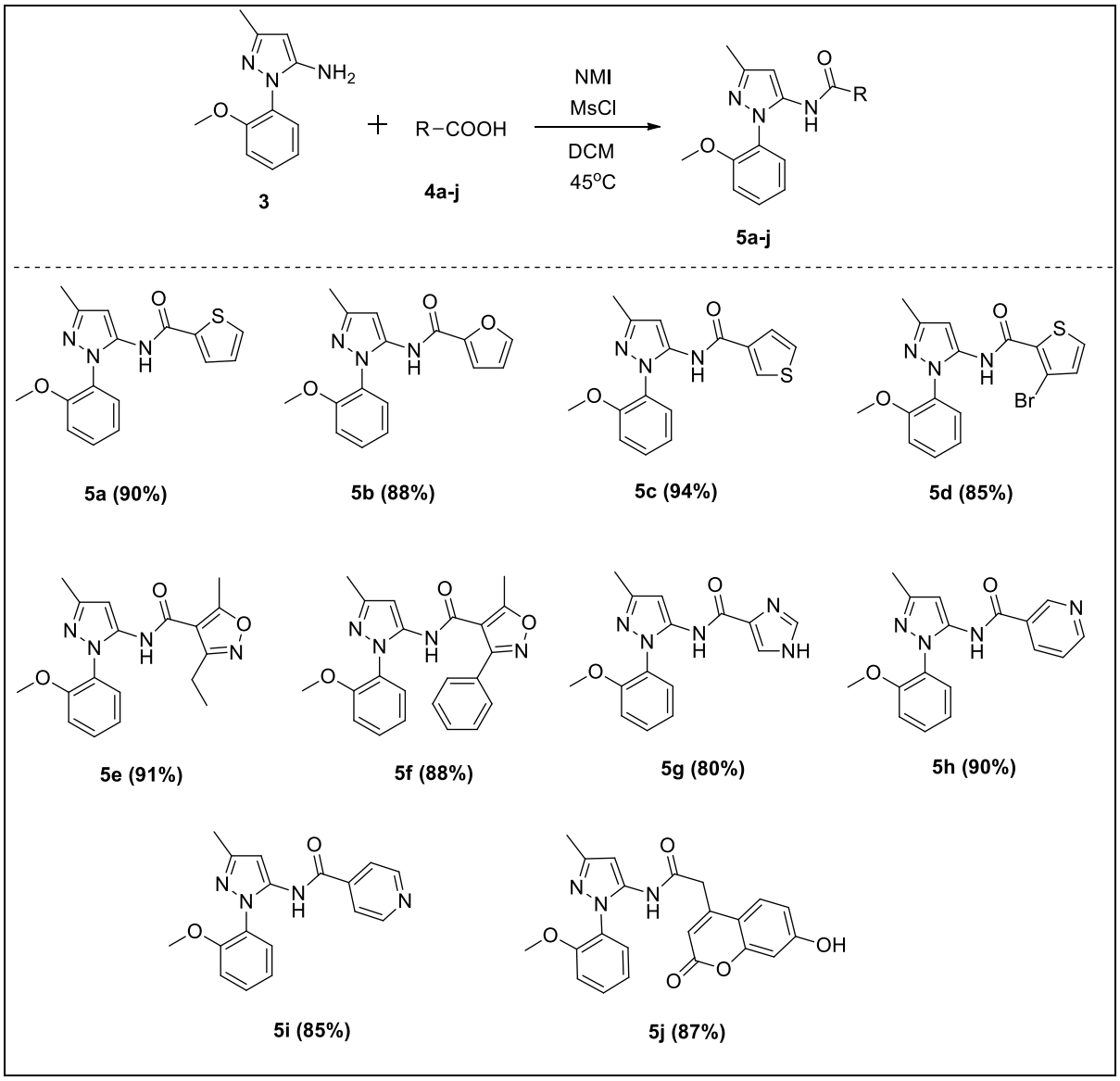
[c]Reaction carried at 45 ℃.

In view of the above obtained result, we decided to screen different coupling reagents, bases, and solvents for our optimization studies (Table 1). However, the traditional coupling reagents like EDC·HCl, HATU, HBTU, HCTU, and T3P in different solvents, and bases afforded the anhydride **6** as the major product (Table 1, entries 1-9). In all these screening studies, a reasonable amount of the desired amide product **5a** was obtained. To our delight, we got the required product **5a** in 55% yield when methanesulfonyl chloride (MsCl) was employed as the coupling reagent (Table 1, entry 10). This observation prompted us to screen different bases by fixing MsCl as the coupling reagent (Table 1, entries 11,12). Accordingly, the desired product was obtained in 80% yield when *N*-methylimidazole (NMI) was used as a base, and DCM as a solvent (Table 6.1, entry 11). We found that the usage of NMI as a base significantly minimized the generation of anhydride **6**. Finally, the expected product **5a** was obtained in 90% isolated yield when the reaction was carried out at 45 °C (Table 6.1, entry 12). The utilization of other sulfonyl halides like toslyl chloride (TsCl), and trifluoromethanesulfonyl chloride (TfCl) as a coupling reagent also decreased the formation of unwanted side-product, anhydride **6** (Table 6.1, entries 13,14). However, the desired product was procured in lesser yield when compared to MsCl. Among the various solvents screened, DCM was found to be essential for the formation of the desired amide product **5a** in high yield (Table 6.1).

After identifying the optimum reaction conditions for the synthesis of amide product **5a**, our next attention was to evaluate the generality of this developed protocol. Keeping this in mind, a series of heteroaryl acids **4a-j** were treated with the key amino precursor **3** in our optimized reaction conditions for synthesizing a variety of pyrazoles linked with amides **5a-j** (Table 2). To our delight, all the acids reacted well enough to generate the desired amides in good to excellent yields (80-94%). The different acids containing heterocyclic groups like furan, thiophene, imidazole, coumarin, isoxazole, and pyridine were tolerant in this reaction conditions to procure the corresponding amides in high yields (Table 2). However, the acid having imidazole heterocyclic group rendered the desired amide **5g** in slightly lower yield (80%). It is noteworthy that thiophene-3-carboxylic acid yielded the desired amide **5c** in 94% yield.

All the newly synthesized amides **5a-j** were then subjected to evaluate their potential as antimicrobial agents. The in vitro antibacterial activity studies of the newly synthesized pyrazole based molecules **5a-j** were carried out against three bacterial strains, namely, *Escherichia coli*, *Klebsiella pneumoniae,* and *Staphylococcus aureus*.The antifungal activity studies of **5a-j** have been carried out against three different fungal strains, *Candida albicans*, *Aspergillusflavus,* and *Rhizopus sp*.These studies were carried out by serial plate dilution method,[18,19] and ciprofloxacin, and Amphotericin Bwas employed as the reference standard for antibacterial, and antifungal studies respectively. The minimum inhibitory concentration values (MIC) of all the newly synthesized pyrazole derivatives **5a-j** were evaluated, and our results are summarized in Table 3.

Table 2. Scope of various acids in the synthesis of amides[a,b]



[a]Reaction conditions: Amino precursor **3** (1 mmol), Acid **4a-j** (1.2 mmol), NMI (2 mmol), MsCl (1 mmol), DCM (3 mL), 3h, 45 °C.
[b]Isolated yield.

Table 3. Antimicrobial activity data of the synthesized compounds **5a-j**

Entry	Minimum inhibitory concentration (MIC in µg/mL)[a]					
	Bacterial strains			Fungal strains		
	S. aureus	*E. coli*	*K. pneumoniae*	*C. albicans*	*A. flavus*	*Rhizopus sp*
Control	00	00	00	00	00	00
5a	64	64	64	8	4	8
5b	128	128	128	>256	>256	>256
5c	64	32	64	8	4	8
5d	256	256	>256	4	4	4
5e	32	32	16	>256	>256	>256
5f	32	16	32	>256	>256	>256
5g	8	8	8	64	64	64
5h	>256	>256	>256	8	8	8
5i	>256	>256	>256	16	8	16
5j	8	8	8	64	32	64
Ciprofloxacin	8	4	16	-	-	-
Amphotericin B	-	-	-	8	8	16

[a]MIC values were evaluated at concentration ranging between 4-256 µg/mL. The figures in the table show the MIC values in µg/mL; MIC (µg/mL) = minimum inhibitory concentration, i.e., lowest concentration to completely inhibit bacterial growth.

From our studies, we found that the compounds **5e**,**5f**, **5g,** and **5j**were promising antibacterial agents when compared with the standard. The most active compoundsagainst all the bacterial strains tested in our investigation were found to be **5g,** and **5j**.The compounds**5a**,**5c**,**5d**,**5h,** and **5i** exhibited promising antifungal acticvity with respect to the standard drug. However, the compound **5d** was found to be the best antifungal agent when compared to the other synthesized molecules. All the other tested molecules exhibited either moderate or lower activity profile, and hence can be considered as inactive.

Conclusions

The NMI-MsCl mediated convenient synthesis of novel pyrazole derivatives containing amides **5a-j** have been efficiently carried out in good to excellent yields. The developed methodology paved the way for the synthesis of pyrazoles linked to diverse heteroaryl groups via amide functionality. The newly synthesized molecules were evealuated for their antimicrobial potential employing ciprofloxacin, and amphotericin B as the reference standard for antibacterial, and antifungal activity respectively.The compounds **5g,** and **5j**were found to be promising antibacterial agents whereas the compound **5d** was identified as the best antifungal agent when compared to respective standards. The detailed studies on the mode of action of these compounds, and the synthesis of more specialized derivatives are currently in process in our laboratory.

Experimental Section

General. All solvents, and reagents were purchased from commercial suppliers, and used without any further purification. Analytical TLC was performed on pre-coated aluminum sheets of silica (60 F254 nm), and visualized by short-wave UV light at λ 254. Melting points were noted on an EZ-Melt automated apparatus. ^{1}H, and ^{13}C NMR spectra were recorded at 400 or 600 MHz, and 100 or 150 MHz respectively. Chemical shifts were reported in parts per million (ppm), and coupling constants in Hertz (Hz). Tetramethylsilane (TMS) (δ0.00 ppm) or residual solvent peakin DMSO-d_6 (δ2.50 ppm), and $CDCl_3$ (δ 7.26 ppm) served as internal standard for recording.[20] Molecular weights of new compounds were measured by GCMS-QP2010 Ultra gas chromatograph operating at an ionization potential of 70 eV (EI). Microanalyses were performed on PerkinElmer Series II CHNS/O 2400 elemental analyzer. Melting points were determined using a Stuart SMP 3 apparatus.

Procedure for the synthesis of amino precursor 3. 3-Aminocrotononitrile(**1,** 10 mmol)was added to a suspension of 2-methoxyphenylhydrazine **2** (10 mmol) in 12N HCl-H_2O (12 mL, 1:3), and the resulting mixture was refluxed for 12 hours. After the specified time, the reaction mixture was cooled, and neutralized with 2.5M sodium hydroxide solution. The suspension was extracted with CH_2Cl_2 (3x30 mL). The combined organic phases were washed with brine (20 mL), dried over sodium sulphate, and the solvent was distilled off under reduced pressure. The obtained residue oil was triturated with hexane to obtain a solid which was recrystallized in ethanol to obtain the entitled 1-(2-methoxyphenyl)-3-methyl-1*H*-pyrazol-5-amine precursor **3** as colourless oil in 90% yield.[171]H NMR (600 MHz, DMSO-d_6): δ_H 2.27 (3H,s, CH_3), 3.83 (3H, s, OCH_3), 5.70 (1H,s, CH aromatic), 6.86 (2H, bs, NH_2), 7.12-7.15 (1H,m, CH aromatic), 7.31 (1H, dd, *J*0.6, 8.4 Hz, CH aromatic), 7.47 (1H, dd, *J*1.8, 7.8 Hz, CH aromatic), 7.59-7.62 (1H, m, CH aromatic).^{13}C NMR (150 MHz, DMSO-d_6): δ_C 11.6 (CH_3), 56.5 (OCH_3), 90.7(C aromatic), 113.8(C aromatic), 121.3(C aromatic), 121.4(C aromatic), 129.9(C aromatic), 133.1(C aromatic), 147.6(C aromatic), 152.2(C aromatic), 155.5(C aromatic). MS (EI): *m*/*z* (%)203 (95) $[M]^+$, 93 (100).Anal. Calcd for $C_{11}H_{13}N_3O$: C, 65.01; H, 6.45; N, 20.68%. Found: C, 65.04; H, 6.75; N, 20.44%.

General procedure for the synthesis of final compounds 5a-j. To a solution of amino precursor **3** (1 mmol, 1 equiv.) in DCM (10 vol) taken in a round bottomed flask, NMI (2 mmol, 2 equiv.), and MsCl (1 mmol, 1 equiv.) was added at 0 ℃. The reaction mixture was then warmed to room temperature, and stirred for 20 minutes. After 20 minutes, acids **4a-j** (1.2 mmol, 1.2 equiv.) was added at 0 ℃, and the reaction mixture was then brought to room temperature. After that, the reaction mixture was heated at 45 ℃ for about 3 hours. The completion of the reaction was monitored by TLC. After reaction completion, the reaction mixture was diluted with ice water, and the aqueous layer was extracted thrice with DCM. The combined organic layers were

washed with brine, and dried over anhydrous sodium sulfate, and distilled under reduced pressure to get crude residue. The crude mixture was purified by column chromatography using hexane, and ethyl acetate as eluent to obtain the titled pyrazole derivatives **5a-j** in varying yields.

***N*-(1-(2-Methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)thiophene-2-carboxamide (5a).** Pale yellow solid (282 mg, 90%). mp 164-166 ℃.^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.36 (3H, s, CH_3), 3.98 (3H, s, OCH_3), 6.56 (1H, s, CH aromatic), 7.09 (1H, t, *J* 4 Hz, CH aromatic), 7.15 (2H, d, *J* 6 Hz, 2CH aromatic), 7.42 (1H, t, *J* 8 Hz, CH aromatic), 7.47 (1H, d, *J* 3.2 Hz, CH aromatic), 7.51-7.53 (2H, m, 2CH aromatic), 8.55 (1H, s, NH).^{13}C NMR (100 MHz, $CDCl_3$): δ_C 14.2 (CH_3), 57.1 (OCH_3), 98.6(C aromatic), 113.2(C aromatic), 122.7(C aromatic), 127.9(C aromatic), 128.1(C aromatic), 129.2(2C aromatic), 129.8(C aromatic), 131.0(C aromatic), 137.4(C aromatic), 138.1(C aromatic), 150.5(C aromatic), 151.7(C aromatic), 158.4(CO amide).MS (EI): *m/z* (%)313 (8) $[M]^+$, 111 (100).Anal. Calcd for $C_{16}H_{15}N_3O_2S$: C, 61.32; H, 4.82; N, 13.41%. Found: C, 61.16; H, 4.52; N, 13.71%.

***N*-(1-(2-Methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)furan-2-carboxamide (5b).** Off white solid (260 mg, 88%). mp 156-158 ℃.^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.35 (3H, s, CH_3), 3.96 (3H, s, OCH_3), 6.55 (1H, s, CH aromatic), 7.11 (1H, t, *J* 4.4 Hz, CH aromatic), 7.16 (2H, d, *J* 6.4 Hz, 2CH aromatic), 7.44 (1H, t, *J* 8.4 Hz, CH aromatic), 7.48 (1H, d, *J* 4 Hz, CH aromatic), 7.50-7.53 (2H, m, 2CH aromatic), 8.57 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C14.1(CH_3), 57.1(OCH_3), 98.8(C aromatic), 113.6(C aromatic), 122.9(C aromatic), 127.9(C aromatic), 128.2(C aromatic), 128.9(C aromatic), 129.4(C aromatic), 130.0(C aromatic), 131.3(C aromatic), 137.7(C aromatic), 138.3(C aromatic), 150.7(C aromatic), 151.9(C aromatic), 158.3(CO amide).MS (EI): *m/z* (%)297 (12) $[M]^+$, 95 (100).Anal. Calcd for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13%. Found: C, 64.65; H, 5.39; N, 13.86%.

***N*-(1-(2-Methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)thiophene-3-carboxamide (5c).** Off white solid (294 mg, 94%). mp 166-168 ℃.^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.37 (3H, s, CH_3), 3.95 (3H, s, OCH_3), 6.54 (1H, s, CH aromatic), 6.59 (1H, s, CH aromatic), 7.06 (2H, d, *J* 6.8 Hz, 2CH aromatic), 7.47 (1H, t, *J* 8 Hz, CH aromatic), 7.51 (1H, d, *J*6.4 Hz, CH aromatic), 7.56-7.57 (2H, m, 2CH aromatic), 8.59 (1H, s, NH).^{13}C NMR (100 MHz, $CDCl_3$): δ_C 14.1 (CH_3), 57.0 (OCH_3), 98.7(C aromatic), 113.4(C aromatic), 122.6(C aromatic), 127.8(C aromatic), 128.0(C aromatic), 129.1(C aromatic), 129.4(C aromatic), 129.9(C aromatic), 131.1(C aromatic), 137.6(C aromatic), 138.0(C aromatic), 150.6(C aromatic), 151.5(C aromatic), 158.5(CO amide).MS (EI): *m/z* (%)313 (14) $[M]^+$, 111 (100).Anal. Calcd for $C_{16}H_{15}N_3O_2S$: C, 61.32; H, 4.82; N, 13.41%. Found: C, 61.57; H, 5.08; N, 13.05%.

3-Bromo-*N*-(1-(2-methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)thiophene-2-carboxamide (5d). White solid (332 mg, 85%). mp 177-179 ℃.^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.36 (3H, s, CH_3), 3.96 (3H, s, OCH_3), 6.56 (1H, s, CH aromatic), 6.96 (2H, d, *J* 6 Hz, 2CH aromatic), 7.43-7.46 (2H, m, 2CH aromatic), 7.51-7.53 (2H, m, 2CH aromatic), 8.57 (1H, s, NH).^{13}C NMR (100 MHz, $CDCl_3$): δ_C 14.2 (CH_3), 57.1 (OCH_3), 98.8(C aromatic), 113.4(C aromatic), 122.7(C aromatic), 127.8(C aromatic), 128.1(C aromatic), 129.1(C aromatic), 129.4(C aromatic), 131.2(C aromatic), 137.5(C aromatic), 138.1(C aromatic), 145.2(C aromatic), 150.7(C aromatic), 151.6(C aromatic), 158.4(CO amide).MS (EI): *m/z* (%)391 (20) $[M]^+$, 111 (100).Anal. Calcd for $C_{16}H_{14}BrN_3O_2S$: C, 48.99; H, 3.60; N, 10.71%. Found: C, 49.27; H, 3.64; N, 11.07%.

3-Ethyl-*N*-(1-(2-methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)-5-methylisoxazole-4-carboxamide (5e). White solid (309 mg, 91%). mp 159-161 ℃.^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.15 (3H, t, *J* 7.2 Hz, CH_3), 2.34 (3H, s, CH_3), 2.49 (3H, s, CH_3), 2.64 (2H, q, *J* 7.6 Hz, CH_2), 3.85 (3H, s, OCH_3), 6.53 (1H, s, CH aromatic), 7.09-7.16 (2H, m, 2CH aromatic), 7.42-7.48 (2H, m, 2CH aromatic), 7.76 (1H, s, NH).^{13}C NMR (100 MHz, $CDCl_3$): δ_C 11.9 (CH_3), 12.9 (CH_3), 14.2 (CH_3), 19.7 (CH_2), 57.4 (OCH_3), 98.6(C aromatic), 111.1(C aromatic), 113.5(C aromatic), 122.8(C aromatic), 127.5(C aromatic), 129.7(C aromatic), 130.6(C aromatic), 137.1(C aromatic), 150.3(C aromatic), 152.9(C aromatic), 158.6(C aromatic), 162.6(CO amide), 172.5(C aromatic).MS (EI): *m/z* (%)340 (22) $[M]^+$, 138 (100).Anal. Calcd for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46%. Found: C, 63.85; H, 6.21; N, 16.22%.

***N*-(1-(2-Methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)-5-methyl-3-phenylisoxazole-4-carboxamide (5f).** Off white solid (341 mg, 88%). mp 172-174 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.25 (3H, s, CH_3), 2.64 (3H, s, CH_3), 3.38 (3H, s, OCH_3), 6.52 (1H, s, CH aromatic), 6.64 (1H, d, *J* 8 Hz, CH aromatic), 6.93 (1H, t, *J* 8 Hz, CH aromatic), 7.13-7.25 (5H, m, 5CH aromatic), 7.39 (2H, d, *J* 7.2 Hz, 2CH aromatic), 7.54 (1H, s, NH).^{13}C NMR (100 MHz, $CDCl_3$): δ_C 13.1 (CH_3), 14.1 (CH_3), 56.3 (OCH_3), 98.4(C aromatic), 110.8(C aromatic), 112.9(C aromatic), 122.0(C aromatic), 126.9(C aromatic), 127.2(C aromatic), 128.6(C aromatic), 128.9(C aromatic), 129.1(C aromatic), 130.1(C aromatic), 131.0(C aromatic), 137.1(C aromatic), 150.1(C aromatic), 152.3(C aromatic), 158.4(C aromatic), 159.7(CO amide), 175.7(C aromatic).MS (EI): *m*/*z* (%)388 (10) $[M]^+$, 144 (100).Anal. Calcd for $C_{22}H_{20}N_4O_3$: C, 68.03; H, 5.19; N, 14.42%. Found: C, 67.72; H, 5.01; N, 14.75%.

***N*-(1-(2-Methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)-1*H*-imidazole-4-carboxamide (5g).** Light yellow solid (238 mg, 80%). mp 156-158 °C.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.35 (3H, s, CH_3), 3.98 (3H, s, OCH_3), 6.54 (1H, s, CH aromatic), 6.99 (2H, d, *J* 7.2 Hz, 2CH aromatic), 7.44-7.46 (2H, m, 2CH aromatic), 7.50-7.53 (2H, m, 2CH aromatic), 8.54 (1H, s, NH), 9.27 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C14.2 (CH_3), 57.3 (OCH_3), 98.8(C aromatic), 111.3(C aromatic), 113.6(C aromatic), 122.9(C aromatic), 127.7(C aromatic), 129.9(C aromatic), 131.4(C aromatic), 135.4(C aromatic), 137.2(C aromatic), 150.5(C aromatic), 152.8(C aromatic), 158.7(C aromatic), 161.1(CO amide).MS (EI): *m*/*z* (%)297 (18) $[M]^+$, 136 (100).Anal. Calcd for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.56%. Found: C, 60.99; H, 5.32; N, 23.45%.

***N*-(1-(2-Methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)nicotinamide (5h).** Off white solid (277 mg, 90%). mp 160-162 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.36 (3H, s, CH_3), 3.94 (3H, s, OCH_3), 6.57 (1H, s, CH aromatic), 7.09 (2H, d, *J* 7.6 Hz, 2CH aromatic), 7.21 (1H, s, CH aromatic), 7.49 (2H, d, *J* 7.2 Hz, 2CH aromatic), 7.55 (1H, d, *J* 7.6 Hz, CH aromatic), 7.60-7.62 (2H, m, 2CH aromatic), 8.61 (1H, s, NH).^{13}C NMR (100 MHz, $CDCl_3$): δ_C 14.2 (CH_3), 57.1 (OCH_3), 98.9(C aromatic), 113.7(C aromatic), 122.9(C aromatic), 127.7(C aromatic), 128.3(C aromatic), 128.9(C aromatic), 129.2(C aromatic), 130.1(C aromatic), 131.2(C aromatic), 137.9(C aromatic), 138.4(C aromatic), 150.8(C aromatic), 151.8(C aromatic), 155.2(C aromatic), 158.4(CO amide).MS (EI): *m*/*z* (%)308 (26) $[M]^+$, 106 (100).Anal. Calcd for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17%. Found: C, 66.50; H, 5.56; N, 17.84%.

***N*-(1-(2-Methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)isonicotinamide (5i).** White solid (262 mg, 85%). mp 162-164 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.39 (3H, s, CH_3), 3.88 (3H, s, OCH_3), 6.55 (1H, s, CH aromatic), 7.11 (2H, d, *J* 7.2 Hz, 2CH aromatic), 7.52 (2H, d, *J* 6.8 Hz, 2CH aromatic), 7.57 (2H, d, *J* 7.2 Hz, 2CH aromatic), 7.63-7.65 (2H, m, 2CH aromatic), 8.55 (1H, s, NH).^{13}C NMR (100 MHz, $CDCl_3$): δ_C 14.1 (CH_3), 57.0 (OCH_3), 99.1(C aromatic), 113.6(C aromatic), 122.8(C aromatic), 127.8(C aromatic), 128.4(C aromatic), 128.9(C aromatic), 129.3(C aromatic), 130.3(C aromatic), 131.1(C aromatic), 138.0(C aromatic), 138.4(C aromatic), 150.7(C aromatic), 151.9(C aromatic), 155.1(C aromatic), 158.2(CO amide).MS (EI): *m*/*z* (%)308 (20) $[M]^+$, 106 (100).Anal. Calcd for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17%. Found: C, 66.36; H, 5.55; N, 18.04%.

2-(7-Hydroxy-2-oxo-2*H*-chromen-4-yl)-*N*-(1-(2-methoxyphenyl)-3-methyl-1*H*-pyrazol-5-yl)acetamide (5j). White solid (352 mg, 87%). mp 260-263 °C.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.15 (3H, s, CH_3), 3.70 (3H, s, CH_3), 3.79 (2H, s, CH_2), 6.11 (1H, s, CH aromatic), 6.24 (1H, s, CH aromatic), 6.72-6.75 (2H, m, 2CH aromatic), 7.00 (1H, t, *J* 7.6 Hz, CH aromatic), 7.15 (1H, d, *J* 8.4 Hz, CH aromatic), 7.25 (1H, d, *J* 7.6 Hz, CH aromatic), 7.42-7.44 (2H, m, 2CH aromatic), 9.82 (1H, s, NH), 10.54 (1H, s, OH).^{13}C NMR (100 MHz, DMSO-d_6): δ_C 13.7 (CH_3), 38.6 (CH_2), 55.6 (OCH_3), 98.4(C aromatic), 102.3(C aromatic), 111.2(C aromatic), 111.6(C aromatic), 112.4(C aromatic), 112.9(C aromatic), 120.3(C aromatic), 126.5(C aromatic), 126.7(C aromatic), 128.9(C aromatic), 130.1(C aromatic), 137.6(C aromatic), 147.5(C aromatic), 150.6(C aromatic), 154.1(C aromatic), 154.9(C aromatic), 160.1(C aromatic), 161.2(CO lactone), 166.0(CO amide).MS (EI): *m*/*z* (%)405 (58) $[M]^+$, 186 (100).Anal. Calcd for $C_{22}H_{19}N_3O_5$: C, 65.18; H, 4.72; N, 10.37%. Found: C, 65.34; H, 4.49; N, 10.57%.

Procedure for determination of antibacterial activity. The newly synthesized compounds **5a-j** were screened for their antibacterial activity against *S. aureus* (ATCC-25923), *E. coli* (ATCC-25922), and *K. pneumoniae* (ATCC-BBA-1705) bacterial strains by serial plate dilution method.[18] Serial dilutions of the drug in Muellere Hinton broth were taken in tubes, and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated, and incubated for 16-18 h. at 37 ℃. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth. A number of antimicrobial discs are placed on the agar for the sole purpose of producing zone of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted, and plates were dried by placing in an incubator at 37 ℃ for one hour. Using an agar punch, wells were made on these seeded agar plates, and minimum inhibitory concentration of the test compounds in dimethyl sulfoxide (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 37 ℃ for 3-4 days. Antibacterial activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with ciprofloxacin as standard. MIC (µg/mL), and zone of inhibition (mm) was determined for all the synthesized compounds.

Procedure forantifungal studies. The synthesized compounds **5a-j** was screened for their antifungal activity against *A. flavus*, *Rhizopus sp,* and *C. albicans* in DMSO by serial plate dilution method.[19] Sabouraud agar media was prepared by dissolving peptone (1 g), D-Glucose (4 g), and agar (2 g) in distilled water (100 mL), and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal stain 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 ℃ for 1 h. Using a punch, wells were made on these seeded agar plates, and minimum inhibitory 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 37 ℃ for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with Amphotericin B as standard. MIC (µg/mL), and zone of inhibition (mm) were determined for all the synthesized compounds.

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