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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and characterization of novel benzimidazole bearing pyrazoline derivatives as potential antimicrobial agents

N. C. Desai · D. D. Pandya · G. M. Kotadiya · Priyanka Desai

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Abstract A new series of compounds *N*-(4-(2-(3-(1*H*-benzo[*d*]imidazol-2-yl)-5-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamides (**5a–u**) were synthesized and structures of these compounds were elucidated by spectral (IR, ^{1}H NMR, ^{13}C NMR, and mass spectra) analysis. Antimicrobial activity was measured against *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282), and *Aspergillus clavatus* (MTCC 1323) by serial broth dilution method. Evaluation of antimicrobial activity revealed that compounds **5f**, **5i**, **5q**, and **5t** were the most active antibacterial, while compounds **5e**, **5g**, **5h**, **5j**, **5p**, **5r**, and **5u** were the most potent antifungal agents as compared to standard drugs and thus could be promising new lead molecules.

Keywords Benzimidazole · Pyrazoline · Antibacterial activity · Antifungal activity

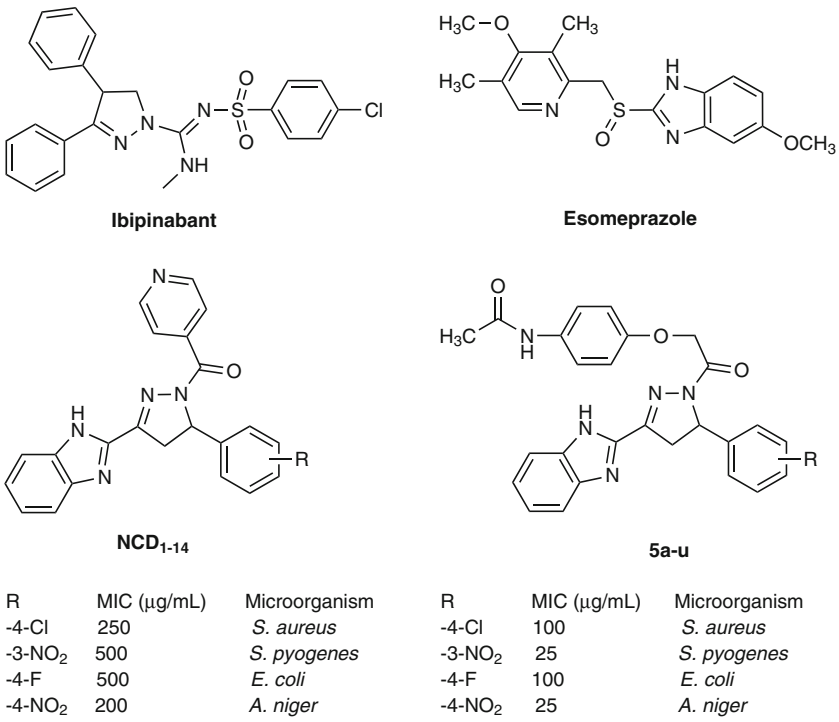
Introduction

Previously from our laboratory (Desai *et al*., 2012a), we have reported (3-(1*H*-benzo[*d*]imidazol-2-yl)-5-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanones (**NCD$_{1–14}$**) and these compounds were screened for their antibacterial and antifungal activity. In an effort to improve the antimicrobial activity through continued broad screen evaluation, we have been interested in the effect of structural variations in title compounds. Here, we have replaced isoniazide by hydrazide of paracetamol ester compound (**4**) at nitrogen of pyrazoline ring. There are no reports of antimicrobial activity of *N*-(4-(2-(3-(1*H*-benzo[*d*]imidazol-2-yl)-5-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamides (**5a–u**). In continuation to this, we have incorporated the synthesis of new compounds with an observation of increased antimicrobial activity. Structural relevance of title compounds (**5a–u**) with previously synthesized compounds (**NCD$_{1–14}$**) and structural similarity of target compounds (**5a–u**) with certain commercially available drugs containing benzimidazole and pyrazole nucleus are shown in Fig. 1.

Despite numerous attempts to search and develop new structural prototype as effective antimicrobials, benzimidazole derivatives are unique and exhibit a broad spectrum of biological activities such as antihistaminic, antipyretic, antiulcerative (Scott *et al*., 2002), antiallergic (Nakano *et al*., 2000), antimicrobial (Kus *et al*., 2009), anticancer (Thimmegowda *et al*., 2008), anti-inflammatory (Mader *et al*., 2008), antiviral (Vazquez *et al*., 2001), antiparasitic (Kazimierczuk *et al*., 2002), and protein kinase inhibitors (Bernatowicz *et al*., 2009). Moreover, benzimidazole derivatives are structural isosteres of naturally occurring nucleotides, which allow them to interact easily with the biophores (Starcevic *et al*., 2007). The azole group of heterocyclic compounds possessed significant pharmacokinetic property, lipophilicity that influence the ability of drug to reach the target by transmembrane diffusion and showed promising activity against resistant TB by inhibiting the biosynthesis of lipids (Andreani *et al*., 2001; Kini *et al*., 2009).

Pyrazoline derivatives are electron-rich nitrogen heterocycles which play an important role in diverse

N. C. Desai (✉) · D. D. Pandya · G. M. Kotadiya · P. Desai
Division of Medicinal Chemistry, Department of Chemistry, UGC NON-SAP & DST-FIST Sponsored Department, Maharaja Krishnakumarsinhji Bhavnagar University, Mahatma Gandhi Campus, Bhavnagar 364 002, India
e-mail: dnisheeth@rediffmail.com



R	MIC (μg/mL)	Microorganism
-4-Cl	250	*S. aureus*
-3-NO_2	500	*S. pyogenes*
-4-F	500	*E. coli*
-4-NO_2	200	*A. niger*

R	MIC (μg/mL)	Microorganism
-4-Cl	100	*S. aureus*
-3-NO_2	25	*S. pyogenes*
-4-F	100	*E. coli*
-4-NO_2	25	*A. niger*

Fig. 1 Structural relevance of title compounds (**5a–u**) with **$NCD_{1–14}$** and structural similarity of title compounds (**5a–u**) with commercially available drugs bearing benzimidazole and pyrazole nucleus

biological activities. Considerable attention has been focused on pyrazolines due to their interesting pharmacological activities. These compounds have been found to possess antifungal (Korgaokar *et al*., 1996), antidepressant (Prasad *et al*., 2005), anticonvulsant (Amnerkar and Bhusari, 2010), anti-inflammatory (Udupi *et al*., 1998), analgesic (Gursoy *et al*., 2000), antibacterial (Nauduri and Reddy, 1998), and antitumor (Brzozwskim *et al*., 2000) activity and also serve as human acyl-CoA: cholesterol acyl transferase inhibitors (Sook *et al*., 2004).

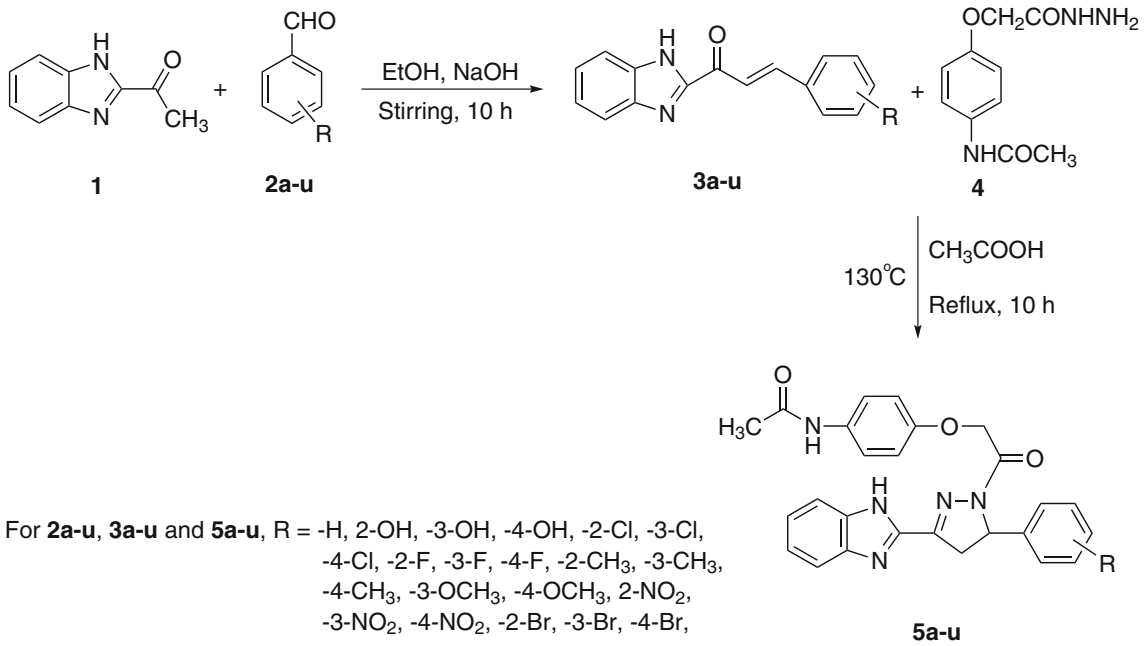
Prompted by above mentioned observations and in continuation of our search for new antimicrobial agents (Desai *et al*., 2012b, c), we have focused toward the synthesis of novel benzimidazole derivatives bearing pyrazoline motifs and bioevaluation of these derivatives by hybrid approach. In continuation to this, we have synthesized *N*-(4-(2-(3-(1*H*-benzo[*d*]imidazol-2-yl)-5-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide derivatives (**5a–u**) and screened for their antimicrobial activity. The synthesized compounds (**5a–u**) were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry techniques.

Results and discussion

Chemistry

Synthesis of intermediates and target compounds (**5a–u**) was accomplished according to the steps illustrated in Scheme 1. According to Scheme 1, the key chalcone derivatives 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(aryl)prop-2-en-1-ones (**3a–u**) were used as precursors for the synthesis of title compounds (**5a–u**). This was achieved through the Claisen–Schmidt condensation of equimolar amounts of different aldehyde derivatives (**2a–u**) and compound (**1**) by stirring the reactants in aqueous alcoholic solution containing sodium hydroxide at room temperature in accordance with the method described in the literature (Shaharyar *et al*., 2010). The cyclocondensation of chalcone derivatives (**3a–u**) with intermediate hydrazide (**4**) in acetic acid at 130 °C afforded title compounds (**5a–u**) in excellent yields. Here, compounds (**5a–u**) have pyrazoline nucleus and were generated through cyclization, migration of α, β unsaturated double bonds, and diminishing of carbonyl group.

Designed series of molecules (**5a–u**) were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry techniques before evaluating for in vitro antimicrobial activity. Two strong absorption bands appeared at 3,388–3,396 and 3,237–3,345 cm^{-1} in the IR spectra of compounds (**5a–u**) corresponding to secondary amine (N–H) group present in benzimidazole ring and –$NHCOCH_3$– group, respectively. Compounds (**5a–u**) showed two C–H stretching absorption bands at 3,036–3,045 and 2,858–2,866 cm^{-1} due to aromatic C–H and ethereal C–H (–OCH_2–), respectively. Two strong absorption bands were observed at 1,688–1,696 cm^{-1} and 1,658–1,665 cm^{-1} due to carbonyl groups attached to –$COCH_2$ and –$NHCOCH_3$, respectively. The characteristic signals in ^{1}H NMR of compounds (**5a–u**) were of three pyrazoline protons which displayed



Scheme 1 Synthetic route for the preparation of title compounds (**5a**–**u**)

doublet of doublet. Methine proton (C_5–H) of pyrazoline displayed a signal at $\delta = 6.01$–6.12 ppm as a doublet of doublet with coupling constants in range of 11.11–11.20 and 3.10–3.18 Hz. The two methylene protons (C_4–H) attached with asymmetric carbon (C_5–H) displayed two signals; a doublet of doublet at $\delta = 3.41$–3.52 ppm with coupling constants of 17.46–17.54 and 3.10–3.17 Hz and a doublet of doublet at $\delta = 3.90$–3.96 ppm with coupling constants of 17.48–17.54 and 11.10–11.18 Hz. In addition, two protons of methylene group appeared as a singlet at $\delta = 4.90$–4.97 ppm. Three methyl protons of $-NHCOCH_3$ group were observed as a singlet at $\delta = 2.06$–2.14 ppm. Secondary amine present in benzimidazole nucleus showed characteristic singlet at $\delta = 10.07$–10.20 ppm and was confirmed by the disappearance of peak when exchanged with D_2O. Furthermore, ^{13}C NMR confirmed the proposed structure by the appearance of signals at $\delta = 172.4$–172.9 and 168.2–168.8 ppm due to carbonyl carbon directly attached to pyrazoline nitrogen and carbonyl group present in $-NHCOCH_3$ group, respectively. Compounds (**5a**–**u**) showed signals at $\delta = 39.0$–39.9 and 67.0–67.4 ppm corresponding to carbon of methylene group present in pyrazoline nucleus and methylene group carbon linked with oxygen and carbonyl group, respectively. Methine carbon showed a chemical shift at $\delta = 60.1$–66.6 ppm. Moreover, the mass spectrum of (**5a**–**u**) showed a molecular ion peak corresponding to molecular formula (**5a**–**u**) along with of other fragment peaks, which supported the structure of compounds. The details of characterization data are given in "Experimental" section.

Antibacterial activity

All the newly synthesized compounds (**5a**–**u**) were initially evaluated for in vitro antibacterial activity against Gram-positive bacteria [*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442)] and Gram-negative bacteria [*Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688)] using conventional broth dilution method. The individual minimal inhibitory concentration (MIC, μg/mL) values of test compounds are listed in Table 1 along with MIC values of reference compound ampicillin. From antibacterial activity data (Table 1), it was observed that compounds **5f** (3-Cl), **5i** (3-F), **5q** (3-NO_2), and **5t** (3-Br) were the most active antibacterial compounds. Compounds **5h** (2-F), **5j** (4-F), and **5r** (4-NO_2) exhibited good activity against *E. coli*. When compounds having electron-withdrawing groups at 3rd position except fluorine group, i.e., **5f** (3-Cl), **5q** (3-NO_2), and **5t** (3-Br), displayed very good activity at MIC = 50 μg/mL against *E. coli*, but compound having fluorine at 3rd position, i.e., **5i** (3-F), revealed highest inhibition at MIC = 25 μg/mL against both Gram-negative bacterial strains as compared to standard ampicillin. Further, compounds **5g** (4-Cl), **5j** (4-F), and **5p** (2-NO_2) showed good activity, while compounds **5f** (3-Cl), **5q** (3-NO_2), and **5t** (3-Br) exhibited very good activity at MIC = 50 μg/mL against *P. aeruginosa* as compared to standard ampicillin (MIC = 100 μg/mL). In case of *S. aureus*, compounds **5e** (2-Cl), **5g** (4-Cl), **5h** (2-F), **5j** (4-F), **5p** (2-NO_2), and **5r** (4-NO_2) showed very good activity, while compounds **5f** (3-Cl) and **5t** (3-Br) displayed excellent

activity at MIC = 50 μg/mL. In addition, compounds **5i** (3-F) and **5q** (3-NO_2) exhibited highest inhibition at MIC = 25 μg/mL against *S. aureus* as compared to standard ampicillin (MIC = 250 μg/mL). Moreover, compounds **5e** (2-Cl), **5g** (4-Cl), **5h** (2-F), **5p** (2-NO_2), and **5r** (4-NO_2) revealed good activity, while compound **5j** (4-F) displayed very good activity against *S. pyogenes*. Furthermore, hydrogen of phenyl ring in **5a** when replaced by chloro, nitro, and bromo groups at 3rd position, i.e., **5f** (3-Cl), **5q** (3-NO_2), and **5t** (3-Br), increased the activity and compounds exhibited excellent activity against *S. pyogenes* at MIC = 25 μg/mL. In addition, compound **5i** (3-F) showed highest inhibition at MIC = 12.5 μg/mL against *S. pyogenes* as compared to standard ampicillin (MIC = 100 μg/mL).

Antifungal activity

Compounds (**5a**–**u**) were also evaluated for its in vitro antifungal activity against three fungal strains, *C. albicans*, *A. niger*, and *A. clavatus*. Compounds **5e** (2-Cl), **5g** (4-Cl), **5h** (2-F), **5j** (4-F), **5p** (2-NO_2), **5r** (4-NO_2), and **5u** (4-Br) were most potent against all the fungal strains (Table 2). Here, electron-withdrawing effects also played a major role for increasing the antifungal activity. A reverse trend was observed when substituents at *ortho* or *para* positions disclosed higher potency than *meta*-substituted derivatives. Therefore, compounds **5e** (2-Cl), **5g** (4-Cl), **5p** (2-NO_2), **5r** (4-NO_2), and **5u** (4-Br) exhibited very good activity, while compounds **5h** (2-F) and **5j** (4-F) displayed excellent activity at MIC = 50 μg/mL against *C. albicans* as compared to standard griseofulvin (MIC = 500 μg/mL). Moreover, compounds **5i** (3-F) and **5s** (2-Br) showed good activity against *A. niger*. In addition, compounds **5g** (4-Cl), **5h** (2-F), and **5p** (2-NO_2) showed very good activity against *A. niger*, while hydrogen in **5a** when replaced by fluorine, nitro, and bromine at 4th position, i.e., **5j** (4-F), **5r** (4-NO_2), and **5u** (4-Br), enhanced the activity and displayed excellent activity at MIC = 25 μg/mL against

Table 1 Results of antibacterial screening of compounds (**5a**–**u**)

Sr. No.	–R	Minimum inhibitory concentration (MIC) for bacteria (μg/mL) ± SD			
		Gram-negative		Gram-positive	
		E. coli MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenes* MTCC 442
5a	–H	500 ± 2.64*	1,000	500 ± 3.04*	500 ± 4.58*
5b	–2-OH	250 ± 2.65*	500 ± 1.85**	500 ± 1.53	500 ± 1.54**
5c	–3-OH	250 ± 1.48	250 ± 1.68*	250 ± 1.24*	250 ± 2.64*
5d	–4-OH	250 ± 1.62**	500 ± 1.85*	500 ± 3.64*	500 ± 1.68**
5e	–2-Cl	250 ± 4.65**	250 ± 1.48**	100 ± 1.90**	100 ± 1.73***
5f	–3-Cl	50 ± 1.45**	50 ± 1.04***	50 ± 1.46***	25 ± 2.43**
5g	–4-Cl	250 ± 1.45**	100 ± 1.63**	100 ± 1.64**	100 ± 2.76***
5h	–2-F	100 ± 2.02***	250 ± 1.54**	100 ± 1.86**	100 ± 2.49**
5i	–3-F	25 ± 1.64**	25 ± 1.84***	25 ± 1.58***	12.5 ± 1.00***
5j	–4-F	100 ± 1.00**	100 ± 2.62**	100 ± 2.82**	50 ± 2.83**
5k	–2-CH_3	500 ± 3.64	500 ± 1.54	500 ± 1.45	500 ± 2.68*
5l	–3-CH_3	500 ± 1.45**	250 ± 1.56*	250 ± 1.86*	250 ± 1.49*
5m	–4-CH_3	1,000	1,000	1,000	500 ± 2.43*
5n	–3-OCH_3	500 ± 1.68**	500 ± 2.54*	500 ± 1.46	250 ± 2.95*
5o	–4-OCH_3	1,000	1,000	1,000	500 ± 1.12
5p	–2-NO_2	250 ± 1.64**	100 ± 1.54**	100 ± 2.42**	100 ± 1.83**
5q	–3-NO_2	50 ± 1.02***	50 ± 1.56***	25 ± 1.45***	25 ± 1.68**
5r	–4-NO_2	100 ± 2.38**	250 ± 1.63**	100 ± 1.86**	100 ± 1.49*
5s	–2-Br	500 ± 2.01*	250 ± 2.54*	250 ± 1.96***	250 ± 2.43*
5t	–3-Br	50 ± 1.38**	50 ± 1.03***	50 ± 1.46**	25 ± 1.95***
5u	–4-Br	500 ± 1.08***	250 ± 1.54**	250 ± 3.64**	250 ± 1.12**
Ampicillin		100 ± 2.05	100 ± 1.00	250 ± 1.52*	100 ± 2.06*

All the values are presented as mean of six experiments ($n = 6$). All significant differences are considered from control value (0.00). 2 % DMSO is used as control and its antibacterial activity is nil or zero

±*SD* Standard deviation

*** $P < 0.001$ extremely significant; ** $P < 0.01$ moderately significant; * $P < 0.05$ significant

Table 2 Results of antifungal screening of compounds (**5a–u**)

Sr. No.	–R	Minimum inhibitory concentration (MIC) for fungi (μg/mL) ± SD		
		C. albicans MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
5a	–H	500 ± 2.12*	250 ± 2.67	500 ± 3.64*
5b	–2-OH	500 ± 1.71	500 ± 1.43**	250 ± 1.00
5c	–3-OH	1,000	500 ± 2.61	500 ± 2.64*
5d	–4-OH	500 ± 1.68*	500 ± 2.79*	500 ± 1.16
5e	–2-Cl	1001 ± .93**	50 ± 1.34***	25 ± 1.48***
5f	–3-Cl	250 ± 2.84**	500 ± 2.82**	100 ± 2.61**
5g	–4-Cl	100 ± 1.14**	50 ± 1.43**	50 ± 1.10**
5h	–2-F	50 ± 1.64**	50 ± 1.49**	12.5 ± 1.04**
5i	–3-F	250 ± 1.23*	100 ± 1.49***	100 ± 2.54*
5j	–4-F	501 ± .56**	25 ± 2.00**	25 ± 2.48
5k	–2-CH_3	500 ± 3.54*	500 ± 4.62*	250 ± 1.53
5l	–3-CH_3	1,000	1,000	500 ± 2.45**
5m	–4-CH_3	500 ± 2.63*	500 ± 2.49**	500 ± 2.21**
5n	–3-OCH_3	1,000	500 ± 1.96	500 ± 1.29*
5o	–4-OCH_3	500 ± 1.49**	250 ± 1.21*	1,000
5p	–2-NO_2	100 ± 2.84**	50 ± 1.43**	50 ± 1.48***
5q	–3-NO_2	250 ± 1.14**	500 ± 1.49**	250 ± 1.61**
5r	–4-NO_2	100 ± 1.64**	25 ± 1.49***	25 ± 1.10**
5s	–2-Br	250 ± 1.23**	100 ± 2.00**	50 ± 1.04**
5t	–3-Br	500 ± 1.56**	250 ± 2.62*	250 ± 2.54*
5u	–4-Br	100 ± 3.54*	25 ± 2.61*	50 ± 1.48**
Griseofulvin		500 ± 0.50	100 ± 1.10	100 ± 1.20

All the values are presented as mean of six experiments ($n = 6$). All significant differences are considered from control value (0.00). 2 % DMSO is used as control and its antifungal activity is nil or zero

±*SD* Standard deviation

*** $P < 0.001$ extremely significant; ** $P < 0.01$ moderately significant; * $P < 0.05$ significant

A. niger as compared to standard griseofulvin (MIC = 100 μg/mL). Furthermore, compounds **5g** (4-Cl), **5p** (2-NO_2), **5s** (2-Br), and **5u** (4-Br) exhibited very good activity as well as compounds **5e** (2-Cl), **5j** (4-F), and **5r** (4-NO_2) displayed excellent activity at MIC = 25 μg/mL against *A. clavatus*. Compound **5h** (2-F) revealed highest inhibition at MIC = 12.5 μg/mL against *A. clavatus* as compared to standard griseofulvin (MIC = 100 μg/mL).

Experimental

All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. Melting points were determined on an electro thermal melting point apparatus by open capillary method and were reported uncorrected. TLC on silica gel plates (Merck, 60, F_{254}) was used for reaction monitoring. Column chromatography on silica gel (Merck, 70–230 mesh and 230–400 mesh ASTH for flash chromatography) was applied when necessary to isolate and purify the reaction products. Yields refer to purified products and were not optimized. Elemental analysis (% C, H, N) was carried out by a Perkin-Elmer 2400 CHN analyzer. IR spectra of all compounds were recorded on a Perkin-Elmer FT-IR spectrophotometer in KBr, frequencies are reported in cm^{-1}. ^1H NMR spectra were run on Varian Gemini 300 MHz and ^{13}C NMR spectra on Varian Mercury-400 100 MHz in DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported as units (ppm) values. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer. Anhydrous reactions were carried out in oven-dried glassware in nitrogen atmosphere.

General synthetic procedure for the preparation of 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(aryl)-prop-2-en-1-ones (**3a–u**)

A mixture of compound 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanone (**1**) (0.01 mol) and different aromatic aldehydes (**2a–u**) (0.01 mol) was stirred in ethanolic sodium hydroxide for 10 h. The yellow product generated was filtered off, washed with water, and crystallized from ethanol (99 %).

*1-(1H-benzo[d]imidazol-2-yl)-3-phenylprop-2-en-1-one (**3a**)*

Yield: 76 %; m.p.: 176 °C; IR (KBr) ν_{max}/cm^{-1}: 3374 (N–H stretching, benzimidazole), 3031 (C–H stretching, aromatic ring), 2910 (C–H stretching, –CH=CH–), 1681 (CO

stretching), 1578 (C=N stretching), 1522 (C=C stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.81 (d, 1H, J = 14.4 Hz, –COCH=), 7.24 (d, 2H, J = 8.1 Hz, Ar–H), 7.35 (t, 1H, J = 7.8 Hz, Ar–H), 7.44 (t, 2H, J = 7.7 Hz, Ar–H), 7.58 (d, 2H, J = 8.2 Hz, Ar–H), 7.66 (d, 2H, J = 7.7 Hz, Ar–H), 7.78 (d, 1H, J = 15.6 Hz, =CH–Ar), 9.96 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 121.4 (1C, –CH= attached with C=O), 141.6 (1C, benzimidazole-C_2), 145.2 (1C, =CH–Ar), 187.2 (1C, C=O); LCMS (m/z): 249.09 [$(M)^+$, 78 %]; Anal. Calcd. for $C_{16}H_{12}N_2O$: C-77.40, H-4.87, N-11.28; Found: C-77.54, H-4.79, N-11.36 %.

General synthetic procedure for the preparation of *N*-(4-(2-(3-(1*H*-benzo[*d*]imidazol-2-yl)-5-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamides (**5a**–**u**)

A mixture of differently substituted benzimidazolyl chalcones (**3a**–**u**) (0.01 mol) and paracetamol hydrazide compound (**4**) (0.02 mol) was taken in 20 mL glacial acetic acid and refluxed at 130 °C for a period of 10 h. The mixture was concentrated under vacuum and diluted with ice-cold water. The separated solid is filtered, dried, and crystallized from chloroform.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoetho-xy)phenyl)acetamide (**5a**)*

Yield: 64 %; m.p.: 188–190 °C; IR (KBr) ν_{max}/cm^{-1}: 3390 (N–H stretching, benzimidazole), 3240 (N–H stretching, –$NHCOCH_3$), 3042 (C–H stretching, aromatic ring), 2860 (C–H stretching, –CH_2), 1690 (CO stretching, –$COCH_2$), 1662 (CO stretching, –$NHCOCH_3$), 1584 (C=N stretching), 1520 (C=C stretching), 1460 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.12 (s, 3H, NHC-OCH_3), 3.41 (dd, 1H, J = 17.52 Hz, 3.13 Hz, C_4–H pyrazoline), 3.91 (dd, 1H, J = 17.49 Hz, 11.11 Hz, C_4–H pyrazoline), 4.94 (s, 2H, –CH_2CO), 5.48 (s, 1H, $NHCOCH_3$), 6.03 (dd, 1H, J = 11.15, 3.12 Hz, C_5–H pyrazoline), 6.98 (d, 2H, J = 8.2 Hz, Ar–H), 7.18 (d, 2H, J = 8.2 Hz, Ar–H), 7.27 (t, 1H, J = 7.4 Hz, Ar–H), 7.36 (d, 2H, J = 7.6 Hz, Ar–H), 7.44 (t, 2H, J = 7.8 Hz, Ar–H), 7.52 (d, 2H, J = 8.0 Hz, Ar–H), 7.64 (d, 2H, J = 8.1 Hz, Ar–H), 10.12 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.1 (1C, CH_3 in –$NHCOCH_3$), 39.7 (1C, pyrazoline-CH_2), 66.3 (1C, pyrazoline-CH), 67.3 (1C, CH_2 attached with C=O), 151.6 (1C, benzimidazole-C_2), 168.7 (1C, C=O present in $NHCOCH_3$), 172.5 (1C, C=O attached with pyrazoline ring); LCMS (m/z): 453.16 [$(M)^+$, 81 %]; Anal. Calcd. for $C_{26}H_{23}N_5O_3$: C-68.86, H-5.11, N-15.44; Found: C-68.78, H-5.16, N-15.49 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5b**)*

Yield: 68 %; m.p.: 208 °C; IR (KBr) ν_{max}/cm^{-1}: 3432 (O–H stretching), 3392 (N–H stretching, benzimidazole), 3242 (N–H stretching, –$NHCOCH_3$), 3045 (C–H stretching, aromatic ring), 2856 (C–H stretching, –CH_2), 1690 (CO stretching, –$COCH_2$), 1660 (CO stretching, –$NHCOCH_3$), 1586 (C=N stretching), 1522 (C=C stretching), 1456 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.08 (s, 3H, $NHCOCH_3$), 3.44 (dd, 1H, J = 17.48 Hz, 3.10 Hz, C_4–H pyrazoline), 3.90 (dd, 1H, J = 17.51 Hz, 11.12 Hz, C_4–H pyrazoline), 4.96 (s, 2H, –CH_2CO), 5.52 (s, 1H, $NHCOCH_3$), 6.01 (dd, 1H, J = 11.15, 3.12 Hz, C_5–H pyrazoline), 6.90 (t, 2H, J = 7.8 Hz, Ar–H), 7.01 (d, 2H, J = 8.1 Hz, Ar–H), 7.11 (t, 1H, J = 7.6 Hz, Ar–H), 7.17 (d, 1H, J = 7.6 Hz, Ar–H), 7.26 (d, 2H, J = 8.0 Hz, Ar–H), 7.52 (d, 2H, J = 8.1 Hz, Ar–H), 7.66 (d, 2H, J = 8.2 Hz, Ar–H), 9.16 (s, 1H, OH D_2O exch.), 10.14 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.1 (1C, CH_3 in $NHCOCH_3$), 39.9 (1C, pyrazoline-CH_2), 60.2 (1C, pyrazoline-CH), 67.2 (1C, CH_2 attached with C=O), 151.4 (1C, benzimidazole-C_2), 156.2 (1C, C–OH), 168.8 (1C, C=O present in $NHCOCH_3$), 172.6 (1C, C=O attached with pyrazoline ring); LCMS (m/z): 469.13 [$(M)^+$, 79 %]; Anal. Calcd. for $C_{26}H_{23}N_5O_4$: C-66.51, H-4.94, N-14.92; Found: C-66.59, H-4.91, N-14.99 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5c**)*

Yield: 70 %; m.p.: 190 °C; IR (KBr) ν_{max}/cm^{-1}: 3428 (O–H stretching), 3392 (N–H stretching, benzimidazole), 3240 (N–H stretching, –$NHCOCH_3$), 3036 (C–H stretching, aromatic ring), 2858 (C–H stretching, –CH_2), 1694 (CO stretching, –$COCH_2$), 1662 (CO stretching, –$NHCOCH_3$), 1584 (C=N stretching), 1524 (C=C stretching), 1457 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.10 (s, 3H, $NHCOCH_3$), 3.45 (dd, 1H, J = 17.51 Hz, 3.12 Hz, C_4–H pyrazoline), 3.92 (dd, 1H, J = 17.51 Hz, 11.10 Hz, C_4–H pyrazoline), 4.94 (s, 2H, –CH_2CO), 5.54 (s, 1H, $NHCOCH_3$), 6.04 (dd, 1H, J = 11.16, 3.10 Hz, C_5–H pyrazoline), 6.81 (d, 1H, J = 7.8 Hz, Ar–H), 6.91 (d, 1H, J = 7.4 Hz, Ar–H), 7.01 (d, 2H, J = 8.1 Hz, Ar–H), 7.09 (s, 1H, J = 7.6 Hz, Ar–H), 7.18 (d, 2H, J = 8.1 Hz, Ar–H), 7.28 (t, 1H, J = 7.8 Hz, Ar–H), 7.54 (d, 2H, J = 8.0 Hz, Ar–H), 7.67 (d, 2H, J = 8.4 Hz, Ar–H), 9.20 (s, 1H, OH D_2O exch.), 10.11 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.2 (1C, CH_3 in $NHCOCH_3$), 39.6 (1C, pyrazoline-CH_2), 66.4 (1C, pyrazoline-CH), 67.4 (1C, CH_2 attached with C=O), 151.6 (1C,

benzimidazole-C_2), 156.6 (1C, C–OH), 168.6 (1C, C=O present in $NHCOCH_3$), 172.9 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 469.16 [$(M)^+$, 79 %]; Anal. Calcd. for $C_{26}H_{23}N_5O_4$: C-66.51, H-4.94, N-14.92; Found: C-66.60, H-4.90, N-14.97 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5d**)*

Yield: 70 %; m.p.: 190 °C; IR (KBr) ν_{max}/cm^{-1}: 3,434 (O–H stretching), 3,396 (N–H stretching, benzimidazole), 3,244 (N–H stretching, $–NHCOCH_3$), 3042 (C–H stretching, aromatic ring), 2860 (C–H stretching, $–CH_2$), 1694 (CO stretching, $–COCH_2$), 1664 (CO stretching, $–NHCOCH_3$), 1588 (C=N stretching), 1526 (C=C stretching), 1458 (C–H bending, $–CH_2$); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.12 (s, 3H, $NHCOCH_3$), 3.46 (dd, 1H, $J = 17.49$ Hz, 3.15 Hz, C_4–H pyrazoline), 3.90 (dd, 1H, $J = 17.50$ Hz, 11.14 Hz, C_4–H pyrazoline), 4.96 (s, 2H, $–CH_2CO$), 5.51 (s, 1H, $NHCOCH_3$), 6.06 (dd, 1H, $J = 11.18$, 3.12 Hz, C_5–H pyrazoline), 6.80 (d, 2H, $J = 7.6$ Hz, Ar–H), 6.97 (d, 2H, $J = 7.6$ Hz, Ar–H), 7.12 (d, 2H, $J = 7.6$ Hz, Ar–H), 7.22 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.54 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.68 (d, 2H, $J = 8.2$ Hz, Ar–H), 9.18 (s, 1H, OH D_2O exch.), 10.12 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.1 (1C, CH_3 in $NHCOCH_3$), 39.4 (1C, pyrazoline-CH_2), 66.6 (1C, pyrazoline-CH), 67.2 (1C, CH_2 attached with C=O), 151.4 (1C, benzimidazole-C_2), 155.7 (1C, C–OH), 168.8 (1C, C=O present in $NHCOCH_3$), 172.7 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 469.15 [$(M)^+$, 81 %]; Anal. Calcd. for $C_{26}H_{23}N_5O_4$: C-66.51, H-4.94, N-14.92; Found: C-66.60, H-4.91, N-14.99 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5e**)*

Yield: 72 %; m.p.: 222 °C; IR (KBr) ν_{max}/cm^{-1}: 3396 (N–H stretching, benzimidazole), 3244 (N–H stretching, $–NHCOCH_3$), 3044 (C–H stretching, aromatic ring), 2858 (C–H stretching, $–CH_2$), 1696 (CO stretching, $–COCH_2$), 1664 (CO stretching, $–NHCOCH_3$), 1586 (C=N stretching), 1522 (C=C stretching), 1454 (C–H bending, $–CH_2$), 754 (C–Cl stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.09 (s, 3H, $NHCOCH_3$), 3.45 (dd, 1H, $J = 17.51$ Hz, 3.16 Hz, C_4–H pyrazoline), 3.91 (dd, 1H, $J = 17.48$ Hz, 11.15 Hz, C_4–H pyrazoline), 4.92 (s, 2H, $–CH_2CO$), 5.54 (s, 1H, $NHCOCH_3$), 6.08 (dd, 1H, $J = 11.20$, 3.12 Hz, C_5–H pyrazoline), 6.98 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.17 (t, 1H, $J = 7.6$ Hz, Ar–H), 7.24 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.32 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.42 (t, 1H, $J = 7.6$ Hz, Ar–H), 7.52 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.66 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.75 (d, 1H, $J = 7.8$ Hz, Ar–H), 10.14 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.0 (1C, CH_3 in $NHCOCH_3$), 39.2 (1C, pyrazoline-CH_2), 61.2 (1C, pyrazoline-CH), 67.0 (1C, CH_2 attached with C=O), 132.3 (1C, C–Cl), 151.2 (1C, benzimidazole-C_2), 168.5 (1C, C=O present in $NHCOCH_3$), 172.6 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 487.16 [$(M)^+$, 79 %]; Anal. Calcd. for $C_{26}H_{22}ClN_5O_3$: C-64.00, H-4.54, N-14.35; Found: C-64.10, H-4.59, N-14.28 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5f**)*

Yield: 68 %; m.p.: 198 °C; IR (KBr) ν_{max}/cm^{-1}: 3392 (N–H stretching, benzimidazole), 3240 (N–H stretching, $–NHCOCH_3$), 3041 (C–H stretching, aromatic ring), 2860 (C–H stretching, $–CH_2$), 1690 (CO stretching, $–COCH_2$), 1662 (CO stretching, $–NHCOCH_3$), 1584 (C=N stretching), 1520 (C=C stretching), 1454 (C–H bending, $–CH_2$), 756 (C–Cl stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.10 (s, 3H, $NHCOCH_3$), 3.48 (dd, 1H, $J = 17.51$ Hz, 3.16 Hz, C_4–H pyrazoline), 3.92 (dd, 1H, $J = 17.52$ Hz, 11.18 Hz, C_4–H pyrazoline), 4.96 (s, 2H, $–CH_2CO$), 5.56 (s, 1H, $NHCOCH_3$), 6.14 (dd, 1H, $J = 11.21$, 3.14 Hz, C_5–H pyrazoline), 6.96 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.15 (d, 1H, $J = 7.6$ Hz, Ar–H), 7.23 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.32 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.44 (t, 1H, $J = 7.4$ Hz, Ar–H), 7.51 (s, 1H, $J = 7.6$ Hz, Ar–H), 7.61 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.69 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.18 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.1 (1C, CH_3 in $NHCOCH_3$), 39.3 (1C, pyrazoline-CH_2), 65.6 (1C, pyrazoline-CH), 67.2 (1C, CH_2 attached with C=O), 134.2 (1C, C–Cl), 151.3 (1C, benzimidazole-C_2), 168.4 (1C, C=O present in $NHCOCH_3$), 172.4 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 487.15 [$(M)^+$, 81 %]; Anal. Calcd. for $C_{26}H_{22}ClN_5O_3$: C-64.00, H-4.54, N-14.35; Found: C-64.09, H-4.58, N-14.29 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5g**)*

Yield: 68 %; m.p.: 201 °C; IR (KBr) ν_{max}/cm^{-1}: 3396 (N–H stretching, benzimidazole), 3244 (N–H stretching, $–NHCOCH_3$), 3044 (C–H stretching, aromatic ring), 2864 (C–H stretching, $–CH_2$), 1694 (CO stretching, $–COCH_2$), 1664 (CO stretching, $–NHCOCH_3$), 1584 (C=N stretching), 1524 (C=C stretching), 1458 (C–H bending, $–CH_2$), 760

(C–Cl stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.14 (s, 3H, $NHCOCH_3$), 3.51 (dd, 1H, $J = 17.50$ Hz, 3.16 Hz, C_4–H pyrazoline), 3.91 (dd, 1H, $J = 17.48$ Hz, 11.16 Hz, C_4–H pyrazoline), 4.94 (s, 2H, $–CH_2CO$), 5.54 (s, 1H, $NHCOCH_3$), 6.10 (dd, 1H, $J = 11.24$, 3.14 Hz, C_5–H pyrazoline), 6.97 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.18 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.41 (d, 2H, $J = 7.6$ Hz, Ar–H), 7.52 (d, 2H, $J = 7.6$ Hz, Ar–H), 7.61 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.70 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.20 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.2 (1C, CH_3 in $NHCOCH_3$), 39.2 (1C, pyrazoline-CH_2), 66.2 (1C, pyrazoline-CH), 67.1 (1C, CH_2 attached with C=O), 132.2 (1C, C–Cl), 151.5 (1C, benzimidazole-C_2), 168.6 (1C, C=O present in $NHCOCH_3$), 172.6 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 487.16 [$(M)^+$, 79 %]; Anal. Calcd. for $C_{26}H_{22}ClN_5O_3$: C-64.00, H-4.54, N-14.35; Found: C-64.11, H-4.60, N-14.27 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(2-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5h**)*

Yield: 62 %; m.p.: 188 °C; IR (KBr) ν_{max}/cm^{-1}: 3398 (N–H stretching, benzimidazole), 3245 (N–H stretching, $–NHCOCH_3$), 3045 (C–H stretching, aromatic ring), 2866 (C–H stretching, $–CH_2$), 1696 (CO stretching, $–COCH_2$), 1664 (CO stretching, $–NHCOCH_3$), 1586 (C=N stretching), 1525 (C=C stretching), 1460 (C–H bending, $–CH_2$), 1121 (C–F stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.14 (s, 3H, $NHCOCH_3$), 3.48 (dd, 1H, $J = 17.54$ Hz, 3.14 Hz, C_4–H pyrazoline), 3.94 (dd, 1H, $J = 17.49$ Hz, 11.16 Hz, C_4–H pyrazoline), 4.94 (s, 2H, $–CH_2CO$), 5.57 (s, 1H, $NHCOCH_3$), 6.12 (dd, 1H, $J = 11.19$, 3.14 Hz, C_5–H pyrazoline), 7.00 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.16 (t, 1H, $J = 7.6$ Hz, Ar–H), 7.24 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.33 (d, 1H, $J = 7.6$ Hz, Ar–H), 7.49 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.57 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.63 (t, 1H, $J = 7.8$ Hz, Ar–H), 7.71 (d, 2H, $J = 8.2$ Hz, Ar–H), 10.18 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.0 (1C, CH_3 in $NHCOCH_3$), 39.1 (1C, pyrazoline-CH_2), 59.4 (1C, pyrazoline-CH), 67.0 (1C, CH_2 attached with C=O), 151.6 (1C, benzimidazole-C_2), 159.4 (1C, C–F), 168.4 (1C, C=O present in $NHCOCH_3$), 172.5 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 471.18 [$(M)^+$, 82 %]; Anal. Calcd. for $C_{26}H_{22}FN_5O_3$: C-66.23, H-4.70, N-14.85; Found: C-66.29, H-4.76, N-14.89 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(3-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5i**)*

Yield: 60 %; m.p.: 198 °C; IR (KBr) ν_{max}/cm^{-1}: 3395 (N–H stretching, benzimidazole), 3243 (N–H stretching, $–NHCOCH_3$), 3041 (C–H stretching, aromatic ring), 2862 (C–H stretching, $–CH_2$), 1693 (CO stretching, $–COCH_2$), 1662 (CO stretching, $–NHCOCH_3$), 1582 (C=N stretching), 1522 (C=C stretching), 1458 (C–H bending, $–CH_2$), 1117 (C–F stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.12 (s, 3H, $NHCOCH_3$), 3.47 (dd, 1H, $J = 17.52$ Hz, 3.12 Hz, C_4–H pyrazoline), 3.92 (dd, 1H, $J = 17.51$ Hz, 11.17 Hz, C_4–H pyrazoline), 4.93 (s, 2H, $–CH_2CO$), 5.56 (s, 1H, $NHCOCH_3$), 6.11 (dd, 1H, $J = 11.19$, 3.14 Hz, C_5–H pyrazoline), 6.84 (s, 1H, Ar–H), 6.98 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.06 (d, 1H, $J = 7.6$ Hz, Ar–H), 7.11 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.18 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.41 (t, 1H, $J = 7.6$ Hz, Ar–H), 7.52 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.67 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.16 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.2 (1C, CH_3 in $NHCOCH_3$), 39.0 (1C, pyrazoline-CH_2), 66.1 (1C, pyrazoline-CH), 67.2 (1C, CH_2 attached with C=O), 151.4 (1C, benzimidazole-C_2), 162.7 (1C, C–F), 168.2 (1C, C=O present in $NHCOCH_3$), 172.6 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 471.14 [$(M)^+$, 80 %]; Anal. Calcd. for $C_{26}H_{22}FN_5O_3$: C-66.23, H-4.70, N-14.85; Found: C-66.30, H-4.77, N-14.90 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5j**)*

Yield: 60 %; m.p.: 170 °C; IR (KBr) ν_{max}/cm^{-1}: 3396 (N–H stretching, benzimidazole), 3245 (N–H stretching, $–NHCOCH_3$), 3042 (C–H stretching, aromatic ring), 2862 (C–H stretching, $–CH_2$), 1694 (CO stretching, $–COCH_2$), 1664 (CO stretching, $–NHCOCH_3$), 1584 (C=N stretching), 1524 (C=C stretching), 1458 (C–H bending, $–CH_2$), 1120 (C–F stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.11 (s, 3H, $NHCOCH_3$), 3.48 (dd, 1H, $J = 17.48$ Hz, 3.14 Hz, C_4–H pyrazoline), 3.96 (dd, 1H, $J = 17.54$ Hz, 11.15 Hz, C_4–H pyrazoline), 4.94 (s, 2H, $–CH_2CO$), 5.55 (s, 1H, $NHCOCH_3$), 6.12 (dd, 1H, $J = 11.20$, 3.15 Hz, C_5–H pyrazoline), 6.97 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.16 (d, 2H, $J = 7.6$ Hz, Ar–H), 7.23 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.32 (d, 2H, $J = 7.4$ Hz, Ar–H), 7.54 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.66 (d, 2H, $J = 8.3$ Hz, Ar–H), 10.12 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.1 (1C, CH_3 in $NHCOCH_3$), 39.2 (1C, pyrazoline-CH_2), 66.0 (1C, pyrazoline-CH), 67.0 (1C, CH_2 attached with C=O), 151.6 (1C, benzimidazole-C_2), 160.8 (1C, C–F), 168.4 (1C, C=O present in $NHCOCH_3$), 172.5 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 471.14 [$(M)^+$, 82 %]; Anal. Calcd. for $C_{26}H_{22}FN_5O_3$: C-66.23, H-4.70, N-14.85; Found: C-66.30, H-4.78, N-14.81 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-o-tolyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxo-ethoxy)phenyl) acetamide (**5k**)*

Yield: 64 %; m.p.: 211 °C; IR (KBr) ν_{max}/cm^{-1}: 3390 (N–H stretching, benzimidazole), 3238 (N–H stretching, –$NHCOCH_3$), 3040 (C–H stretching, aromatic ring), 2931 (C–H stretching, CH_3), 2858 (C–H stretching, –CH_2), 1688 (CO stretching, –$COCH_2$), 1660 (CO stretching, –$NHCOCH_3$), 1581 (C=N stretching), 1521 (C=C stretching), 1452 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.06 (s, 3H, $NHCOCH_3$), 2.36 (s, 3H, CH_3), 3.44 (dd, 1H, $J = 17.46$ Hz, 3.12 Hz, C_4–H pyrazoline), 3.91 (dd, 1H, $J = 17.51$ Hz, 11.12 Hz, C_4–H pyrazoline), 4.90 (s, 2H, –CH_2CO), 5.51 (s, 1H, $NHCOCH_3$), 6.04 (dd, 1H, $J = 11.16$, 3.14 Hz, C_5–H pyrazoline), 6.91 (d, 1H, $J = 7.6$ Hz, Ar–H), 7.00 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.10 (t, 1H, $J = 7.6$ Hz, Ar–H), 7.17 (t, 1H, $J = 7.4$ Hz, Ar–H), 7.24 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.44 (d, 1H, $J = 7.7$ Hz, Ar–H), 7.52 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.65 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.08 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 19.2 (1C, CH_3), 24.3 (1C, CH_3 in $NHCOCH_3$), 39.4 (1C, pyrazoline-CH_2), 63.8 (1C, pyrazoline-CH), 67.2 (1C, CH_2 attached with C=O), 134.6 (1C, C–CH_3), 151.7 (1C, benzimidazole-C_2), 168.6 (1C, C=O present in $NHCOCH_3$), 172.6 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 467.18 [$(M)^+$, 79 %]; Anal. Calcd. for $C_{27}H_{25}N_5O_3$: C-69.36, H-5.39, N-14.98; Found: C-69.45, H-5.44, N-14.93 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-m-tolyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxo-ethoxy)phenyl) acetamide (**5l**)*

Yield: 60 %; m.p.: 232 °C; IR (KBr) ν_{max}/cm^{-1}: 3388 (N–H stretching, benzimidazole), 3237 (N–H stretching, –$NHCOCH_3$), 3038 (C–H stretching, aromatic ring), 2930 (C–H stretching, CH_3), 2858 (C–H stretching, –CH_2), 1690 (CO stretching, –$COCH_2$), 1658 (CO stretching, –$NHCOCH_3$), 1580 (C=N stretching), 1521 (C=C stretching), 1454 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.07 (s, 3H, $NHCOCH_3$), 2.37 (s, 3H, CH_3), 3.44 (dd, 1H, $J = 17.46$ Hz, 3.12 Hz, C_4–H pyrazoline), 3.92 (dd, 1H, $J = 17.50$ Hz, 11.13 Hz, C_4–H pyrazoline), 4.91 (s, 2H, –CH_2CO), 5.52 (s, 1H, $NHCOCH_3$), 6.06 (dd, 1H, $J = 11.15$, 3.14 Hz, C_5–H pyrazoline), 6.98 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.07 (d, 1H, $J = 7.6$ Hz, Ar–H), 7.15 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.21 (s, 1H, Ar–H), 7.27 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.44 (t, 1H, $J = 7.6$ Hz, Ar–H), 7.51 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.67 (d, 2H, $J = 8.3$ Hz, Ar–H), 10.07 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 21.7 (1C, CH_3), 24.4 (1C, CH_3 in $NHCOCH_3$), 39.5 (1C, pyrazoline-CH_2), 66.5 (1C, pyrazoline-CH), 67.2 (1C, CH_2 attached with C=O), 138.3 (1C, C–CH_3), 151.6 (1C, benzimidazole-C_2), 168.4 (1C, C=O present in $NHCOCH_3$), 172.6 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 467.17 [$(M)^+$, 83 %]; Anal. Calcd. for $C_{27}H_{25}N_5O_3$: C-69.36, H-5.39, N-14.98; Found: C-69.45, H-5.43, N-14.92 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxo-ethoxy)phenyl) acetamide (**5m**)*

Yield: 61 %; m.p.: 209 °C; IR (KBr) ν_{max}/cm^{-1}: 3391 (N–H stretching, benzimidazole), 3240 (N–H stretching, –$NHCOCH_3$), 3040 (C–H stretching, aromatic ring), 2934 (C–H stretching, CH_3), 2861 (C–H stretching, –CH_2), 1692 (CO stretching, –$COCH_2$), 1661 (CO stretching, –$NHCOCH_3$), 1581 (C=N stretching), 1523 (C=C stretching), 1457 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.06 (s, 3H, $NHCOCH_3$), 2.32 (s, 3H, CH_3), 3.43 (dd, 1H, $J = 17.47$ Hz, 3.14 Hz, C_4–H pyrazoline), 3.90 (dd, 1H, $J = 17.50$ Hz, 11.14 Hz, C_4–H pyrazoline), 4.91 (s, 2H, –CH_2CO), 5.51 (s, 1H, $NHCOCH_3$), 6.07 (dd, 1H, $J = 11.12$, 3.13 Hz, C_5–H pyrazoline), 6.96 (d, 2H, $J = 7.9$ Hz, Ar–H), 7.09 (d, 2H, $J = 7.6$ Hz, Ar–H), 7.16 (d, 2H, $J = 7.4$ Hz, Ar–H), 7.24 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.52 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.64 (d, 2H, $J = 8.2$ Hz, Ar–H), 10.06 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 21.3 (1C, CH_3), 24.2 (1C, CH_3 in $NHCOCH_3$), 39.4 (1C, pyrazoline-CH_2), 66.2 (1C, pyrazoline-CH), 67.1 (1C, CH_2 attached with C=O), 136.4 (1C, C–CH_3), 151.4 (1C, benzimidazole-C_2), 168.5 (1C, C=O present in $NHCOCH_3$), 172.7 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 467.19 [$(M)^+$, 80 %]; Anal. Calcd. for $C_{27}H_{25}N_5O_3$: C-69.36, H-5.39, N-14.98; Found: C-69.44, H-5.44, N-14.91 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5n**)*

Yield: 64 %; m.p.: 241 °C; IR (KBr) ν_{max}/cm^{-1}: 3388 (N–H stretching, benzimidazole), 3238 (N–H stretching, –$NHCOCH_3$), 3038 (C–H stretching, aromatic ring), 2861 (C–H stretching, –CH_2), 2812 (C–H stretching, OCH_3), 1688 (CO stretching, –$COCH_2$), 1660 (CO stretching, –$NHCOCH_3$), 1580 (C=N stretching), 1521 (C=C stretching), 1458 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.03 (s, 3H, $NHCOCH_3$), 3.42 (dd, 1H, $J = 17.47$ Hz, 3.11 Hz, C_4–H pyrazoline), 3.81 (s, 3H, OCH_3), 3.92 (dd, 1H, $J = 17.49$ Hz, 11.12 Hz, C_4–H pyrazoline), 4.91 (s, 2H, –CH_2CO), 5.50 (s, 1H, –$NHCOCH_3$), 6.04 (dd, 1H, $J = 11.15$, 3.15 Hz, C_5–H pyrazoline), 6.82 (d, 1H, $J = 7.5$ Hz, Ar–H), 6.91 (d, 1H,

$J = 7.3$ Hz, Ar–H), 7.00 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.09 (s, 1H, Ar–H), 7.21 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.31 (t, 1H, $J = 7.4$ Hz, Ar–H), 7.51 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.64 (d, 2H, $J = 8.3$ Hz, Ar–H), 10.07 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.1 (1C, CH_3 in $NHCOCH_3$), 39.5 (1C, pyrazoline-CH_2), 55.7 (1C, OCH_3), 66.4 (1C, pyrazoline-CH), 67.3 (1C, CH_2 attached with C=O), 151.5 (1C, benzimidazole-C_2), 160.4 (1C, C–OCH_3), 168.4 (1C, C=O present in $NHCOCH_3$), 172.6 (1C, C=O attached with pyrazoline ring); LCMS (*m*/*z*): 483.19 [$(M)^+$, 84 %]; Anal. Calcd. for $C_{27}H_{25}N_5O_4$: C-67.07, H-5.21, N-14.48; Found: C-67.00, H-5.26, N-14.54 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5o**)*

Yield: 66 %; m.p.: 223 °C; IR (KBr) ν_{max}/cm^{-1}: 3390 (N–H stretching, benzimidazole), 3240 (N–H stretching, –NHC-OCH_3), 3039 (C–H stretching, aromatic ring), 2862 (C–H stretching, –CH_2), 2815 (C–H stretching, OCH_3), 1690 (CO stretching, –$COCH_2$), 1662 (CO stretching, –$NHCOCH_3$), 1581 (C=N stretching), 1522 (C=C stretching), 1459 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.04 (s, 3H, $NHCOCH_3$), 3.43 (dd, 1H, $J = 17.47$ Hz, 3.11 Hz, C_4–H pyrazoline), 3.84 (s, 3H, OCH_3), 3.93 (dd, 1H, $J = 17.48$ Hz, 11.11 Hz, C_4–H pyrazoline), 4.90 (s, 2H, –CH_2CO), 5.52 (s, 1H, $NHCOCH_3$), 6.03 (dd, 1H, $J = 11.14$, 3.14 Hz, C_5–H pyrazoline), 6.92 (d, 2H, $J = 7.4$ Hz, Ar–H), 6.99 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.16 (d, 2H, $J = 7.3$ Hz, Ar–H), 7.23 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.50 (d, 2H, $J = 7.9$ Hz, Ar–H), 7.64 (d, 2H, $J = 8.2$ Hz, Ar–H), 10.06 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.2 (1C, CH_3 in $NHCOCH_3$), 39.6 (1C, pyrazoline-CH_2), 55.8 (1C, OCH_3), 66.2 (1C, pyrazoline-CH), 67.1 (1C, CH_2 attached with C=O), 151.4 (1C, benzimidazole-C_2), 158.7 (1C, C–OCH_3), 168.6 (1C, C=O present in $NHCOCH_3$), 172.6 (1C, C=O attached with pyrazoline ring); LCMS (*m*/*z*): 483.17 [$(M)^+$, 81 %]; Anal. Calcd. for $C_{27}H_{25}N_5O_4$: C-67.07, H-5.21, N-14.48; Found: C-67.01, H-5.27, N-14.55 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(2-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5p**)*

Yield: 70 %; m.p.: 201 °C; IR (KBr) ν_{max}/cm^{-1}: 3394 (N–H stretching, benzimidazole), 3242 (N–H stretching, –$NHCOCH_3$), 3044 (C–H stretching, aromatic ring), 2864 (C–H stretching, –CH_2), 1690 (CO stretching, –$COCH_2$), 1664 (CO stretching, –$NHCOCH_3$), 1584 (C=N stretching), 1524 (C=C stretching), 1492 (N=O stretching, NO_2), 1461 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.11 (s, 3H, $NHCOCH_3$), 3.48 (dd, 1H, $J = 17.51$ Hz, 3.16 Hz, C_4-H pyrazoline), 3.96 (dd, 1H, $J = 17.50$ Hz, 11.14 Hz, C_4–H pyrazoline), 4.95 (s, 2H, –CH_2CO), 5.58 (s, 1H, $NHCOCH_3$), 6.15 (dd, 1H, $J = 11.17$, 3.16 Hz, C_5–H pyrazoline), 6.99 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.23 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.52 (d, 2H, $J = 7.9$ Hz, Ar–H), 7.59 (t, 1H, $J = 7.4$ Hz, Ar–H), 7.64 (d, 1H, $J = 7.3$ Hz, Ar–H), 7. 71 (d, 2H, $J = 8.3$ Hz, Ar–H), 7.81 (t, 1H, $J = 7.4$ Hz, Ar–H), 8.05 (d, 1H, $J = 7.6$ Hz, Ar–H), 10.14 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.0 (1C, CH_3 in $NHCOCH_3$), 38.5 (1C, pyrazoline-CH_2), 61.5 (1C, pyrazoline-CH), 67.0 (1C, CH_2 attached with C=O), 147.3 (1C, C–NO_2), 151.5 (1C, benzimidazole-C_2), 168.4 (1C, C=O present in $NHCOCH_3$), 172.5 (1C, C=O attached with pyrazoline ring); LCMS (*m*/*z*): 498.17 [$(M)^+$, 81 %]; Anal. Calcd. for $C_{26}H_{22}N_6O_5$: C-62.64, H-4.45, N-16.86; Found: C-62.71, H-4.49, N-16.80 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5q**)*

Yield: 72 %; m.p.: 191 °C; IR (KBr) ν_{max}/cm^{-1}: 3392 (N–H stretching, benzimidazole), 3242 (N–H stretching, –NHC-OCH_3), 3042 (C–H stretching, aromatic ring), 2862 (C–H stretching, –CH_2), 1692 (CO stretching, –$COCH_2$), 1662 (CO stretching, –$NHCOCH_3$), 1584 (C=N stretching), 1524 (C=C stretching), 1491 (N=O stretching, NO_2), 1457 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.12 (s, 3H, $NHCOCH_3$), 3.49 (dd, 1H, $J = 17.52$ Hz, 3.17 Hz, C_4–H pyrazoline), 3.96 (dd, 1H, $J = 17.52$ Hz, 11.15 Hz, C_4–H pyrazoline), 4.97 (s, 2H, –CH_2CO), 5.60 (s, 1H, $NHCOCH_3$), 6.14 (dd, 1H, $J = 11.17$, 3.16 Hz, C_5–H pyrazoline), 7.01 (d, 2H, $J = 8.01$ Hz, Ar–H), 7.24 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.54 (d, 2H, $J = 8.0$ Hz, Ar–H), 7. 69 (d, 2H, $J = 8.3$ Hz, Ar–H), 7.76 (t, 1H, $J = 7.4$ Hz, Ar–H), 7.82 (d, 1H, $J = 7.6$ Hz, Ar–H), 8.01 (d, 1H, $J = 7.5$ Hz, Ar–H), 8.18 (s, 1H, Ar–H), 10.16 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.2 (1C, CH_3 in $NHCOCH_3$), 39.5 (1C, pyrazoline-CH_2), 65.2 (1C, pyrazoline-CH), 67.2 (1C, CH_2 attached with C=O), 147.8 (1C, C–NO_2), 151.6 (1C, benzimidazole-C_2), 168.6 (1C, C=O present in $NHCOCH_3$), 172.7 (1C, C=O attached with pyrazoline ring); LCMS (*m*/*z*): 498.19 [$(M)^+$, 78 %]; Anal. Calcd. for $C_{26}H_{22}N_6O_5$: C-62.64, H-4.45, N-16.86; Found: C-62.72, H-4.50, N-16.80 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5r**)*

Yield: 68 %; m.p.: 213 °C; IR (KBr) ν_{max}/cm^{-1}: 3394 (N–H stretching, benzimidazole), 3244 (N–H stretching,

–$NHCOCH_3$), 3044 (C–H stretching, aromatic ring), 2863 (C–H stretching, –CH_2), 1692 (CO stretching, –$COCH_2$), 1664 (CO stretching, –$NHCOCH_3$), 1586 (C=N stretching), 1524 (C=C stretching), 1494 (N=O stretching, NO_2), 1460 (C–H bending, –CH_2); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.13 (s, 3H, $NHCOCH_3$), 3.47 (dd, 1H, $J = 17.51$ Hz, 3.16 Hz, C_4–H pyrazoline), 3.96 (dd, 1H, $J = 17.51$ Hz, 11.16 Hz, C_4–H pyrazoline), 4.96 (s, 2H, –CH_2CO), 5.59 (s, 1H, $NHCOCH_3$), 6.15 (dd, 1H, $J = 11.15$, 3.18 Hz, C_5–H pyrazoline), 7.00 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.22 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.52 (d, 2H, $J = 7.9$ Hz, Ar–H), 7. 61 (d, 2H, $J = 7.6$ Hz, Ar–H), 7.71 (d, 2H, $J = 8.2$ Hz, Ar–H), 8.22 (d, 2H, $J = 7.6$ Hz, Ar–H), 10.15 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.1 (1C, CH_3 in $NHCOCH_3$), 39.4 (1C, pyrazoline-CH_2), 66.2 (1C, pyrazoline-CH), 67.1 (1C, CH_2 attached with C=O), 145.8 (1C, C–NO_2), 151.4 (1C, benzimidazole-C_2), 168.4 (1C, C=O present in $NHCOCH_3$), 172.5 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 498.19 [$(M)^+$, 82 %]; Anal. Calcd. for $C_{26}H_{22}N_6O_5$: C-62.64, H-4.45, N-16.86; Found: C-62.72, H-4.51, N-16.81 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5s**)*

Yield: 74 %; m.p.: 198 °C; IR (KBr) ν_{max}/cm^{-1}: 3392 (N–H stretching, benzimidazole), 3242 (N–H stretching, –$NHCOCH_3$), 3041 (C–H stretching, aromatic ring), 2862 (C–H stretching, –CH_2), 1691 (CO stretching, –$COCH_2$), 1662 (CO stretching, –$NHCOCH_3$), 1584 (C=N stretching), 1524 (C=C stretching), 1462 (C–H bending, –CH_2), 541 (C–Br stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.10 (s, 3H, $NHCOCH_3$), 3.45 (dd, 1H, $J = 17.49$ Hz, 3.15 Hz, C_4–H pyrazoline), 3.94 (dd, 1H, $J = 17.49$ Hz, 11.13 Hz, C_4–H pyrazoline), 4.96 (s, 2H, –CH_2CO), 5.57 (s, 1H, $NHCOCH_3$), 6.12 (dd, 1H, $J = 11.16$, 3.15 Hz, C_5–H pyrazoline), 6.99 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.14 (t, 1H, $J = 7.4$ Hz, Ar–H), 7.21 (d, 1H, $J = 7.6$ Hz, Ar–H), 7.26 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.35 (t, 1H, $J = 7.5$ Hz, Ar–H), 7.49 (d, 2H, $J = 7.9$ Hz, Ar–H), 7.58 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.66 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.09 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.0 (1C, CH_3 in $NHCOCH_3$), 38.8 (1C, pyrazoline-CH_2), 62.4 (1C, pyrazoline-CH), 67.0 (1C, CH_2 attached with C=O), 121.8 (1C, C–Br), 151.2 (1C, benzimidazole-C_2), 168.2 (1C, C=O present in $NHCOCH_3$), 172.4 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 531.09 [$(M)^+$, 79 %]; Anal. Calcd. for $C_{26}H_{22}BrN_5O_3$: C-58.66, H-4.17, N-13.15; Found: C-58.60, H-4.21, N-13.20 %.

N-(4-(2-(3-(1*H*-benzo[*d*]imidazol-2-yl)-5-(3-bromophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5t**)

Yield: 72 %; m.p.: 218 °C; IR (KBr) ν_{max}/cm^{-1}: 3390 (N–H stretching, benzimidazole), 3241 (N–H stretching, –$NHCOCH_3$), 3040 (C–H stretching, aromatic ring), 2861 (C–H stretching, –CH_2), 1692 (CO stretching, –$COCH_2$), 1662 (CO stretching, –$NHCOCH_3$), 1585 (C=N stretching), 1522 (C=C stretching), 1461 (C–H bending, –CH_2), 544 (C–Br stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.08 (s, 3H, $NHCOCH_3$), 3.44 (dd, 1H, $J = 17.48$ Hz, 3.15 Hz, C_4–H pyrazoline), 3.94 (dd, 1H, $J = 17.49$ Hz, 11.13 Hz, C_4–H pyrazoline), 4.95 (s, 2H, –CH_2CO), 5.54 (s, 1H, $NHCOCH_3$), 6.10 (dd, 1H, $J = 11.14$, 3.14 Hz, C_5–H pyrazoline), 6.97 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.18 (d, 2H, $J = 8.2$ Hz, Ar–H), 7.27 (d, 1H, $J = 7.6$ Hz, Ar–H), 7.34 (t, 1H, $J = 7.5$ Hz, Ar–H), 7.41 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.48 (s, 1H, Ar–H), 7.54 (d, 2H, $J = 7.9$ Hz, Ar–H), 7.66 (d, 2H, $J = 8.3$ Hz, Ar–H), 10.07 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.0 (1C, CH_3 in $NHCOCH_3$), 39.6 (1C, pyrazoline-CH_2), 65.4 (1C, pyrazoline-CH), 67.0 (1C, CH_2 attached with C=O), 122.7 (1C, C–Br), 151.3 (1C, benzimidazole-C_2), 168.4 (1C, C=O present in $NHCOCH_3$), 172.5 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 531.09 [$(M)^+$, 80 %]; Anal. Calcd. for $C_{26}H_{22}BrN_5O_3$: C-58.66, H-4.17, N-13.15; Found: C-58.59, H-4.21, N-13.19 %.

*N-(4-(2-(3-(1H-benzo[d]imidazol-2-yl)-5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethoxy)phenyl)acetamide (**5u**)*

Yield: 72 %; m.p.: 209 °C; IR (KBr) ν_{max}/cm^{-1}: 3394 (N–H stretching, benzimidazole), 3245 (N–H stretching, –$NHCOCH_3$), 3043 (C–H stretching, aromatic ring), 2864 (C–H stretching, –CH_2), 1694 (CO stretching, –$COCH_2$), 1665 (CO stretching, –$NHCOCH_3$), 1586 (C=N stretching), 1524 (C=C stretching), 1463 (C–H bending, –CH_2), 547 (C–Br stretching); ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.09 (s, 3H, $NHCOCH_3$), 3.45 (dd, 1H, $J = 17.49$ Hz, 3.15 Hz, C_4–H pyrazoline), 3.95 (dd, 1H, $J = 17.50$ Hz, 11.14 Hz, C_4–H pyrazoline), 4.96 (s, 2H, –CH_2CO), 5.54 (s, 1H, $NHCOCH_3$), 6.11 (dd, 1H, $J = 11.15$, 3.15 Hz, C_5–H pyrazoline), 6.98 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.14 (d, 2H, $J = 7.7$ Hz, Ar–H), 7.22 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.51 (d, 2H, $J = 7.9$ Hz, Ar–H), 7.67 (d, 2H, $J = 8.3$ Hz, Ar–H), 7.94 (d, 2H, $J = 7.6$ Hz, Ar–H), 10.07 (s, 1H, –NH D_2O exch.); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.1 (1C, CH_3 in $NHCOCH_3$), 39.5 (1C, pyrazoline-CH_2), 66.3 (1C, pyrazoline-CH), 67.1 (1C, CH_2 attached with C=O), 121.2 (1C,

C–Br), 151.2 (1C, benzimidazole-C_2), 168.3 (1C, C=O present in $NHCOCH_3$), 172.4 (1C, C=O attached with pyrazoline ring); LCMS (*m/z*): 531.11 [$(M)^+$, 81 %]; Anal. Calcd. for $C_{26}H_{22}BrN_5O_3$: C-58.66, H-4.17, N-13.15; Found: C-58.59, H-4.22, N-13.21 %.

Antibacterial bioassay

Antibacterial studies of newly synthesized compounds (**5a**–**u**) were carried out against the representative panel of Gram-positive [*S. aureus* (MTCC-96), *S. pyogenes* (MTCC-442)] and Gram-negative [*E. coli* (MTCC-443), *P. aeruginosa* (MTCC-1688)] bacteria. All MTCC cultures were collected from the Institute of Microbial Technology, Chandigarh. The activity of compounds was determined as per the National Committee for Clinical Laboratory Standards (NCCLS) protocol using Mueller–Hinton Broth (Becton–Dickinson, USA) (Desai *et al*., 2012d, e). Primary screening was done first for antibacterial activity in six sets against *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. pyogenes* at different concentrations of 1,000, 500, and 250 μg/mL. The compounds found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, and 12.5 μg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. Inoculum size for test strain was adjusted to 10^6 CFU/mL (Colony Forming Unit per milliliter) by comparing the turbidity (turbidimetric method). Mueller–Hinton Broth was used as a nutrient medium to grow and dilute the compound suspension for test organisms. 2 % DMSO was used as a diluent/vehicle to obtain the desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. Synthesized compounds were diluted to 1,000 μg/mL concentration as stock solution. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over quarter of a plate of medium suitable for the growth of test organisms. The culture tubes were then incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. 10 μg/mL suspensions were further inoculated on an appropriate media and growth was noted after 24 and 48 h. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC), i.e., the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Solvent had no influence on strain growth. The result of this was greatly affected by the size of inoculum. The test mixture contained 10^6 CFU/mL organisms. DMSO and sterilized distilled water were used as negative control while ampicillin antibiotic (1 U strength) was used as positive control. Standard drug used in the present study was 'ampicillin' for evaluating antibacterial activity which showed 100, 100, 250, and 100 μg/mL MIC against *E. coli, P. aeruginosa, S. aureus*, and *S. pyogenes*, respectively.

Antifungal bioassay

The newly prepared compounds (**5a**–**u**) were screened for their antifungal activity as primary screening in six sets against *C. albicans*, *A. niger*, and *A. clavatus* at various concentrations of 1,000, 500, and 250 μg/mL. The primary active compounds were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, and 12.5 μg/mL concentrations for secondary screening to test in a second set of dilution against all fungi. The fungal activity of each compound was compared with griseofulvin as a standard drug, which showed 500, 100, and 100 μg/mL MIC against *C. albicans*, *A. niger*, and *A. clavatus*, respectively. For fungal growth, in the present protocol, we have used Sabourauds dextrose broth at 28 °C in aerobic condition for 48 h. DMSO and sterilized distilled water were used as negative control while griseofulvin (1 U strength) was used as positive control.

The cytotoxic potential of compounds **5e**–**5j**, **5p**–**5r**, **5t**, and **5u** was also determined in human cancer cell lines such as A549, HL-60, and HepG2 according to protocols (Skehan *et al*., 1990). All the tested compounds did not show significant cytotoxic activity but showed selectivity, in that they possessed potent antibacterial and antifungal activity without the cytotoxicity in mammalian cells (Ryu *et al*., 2003).

Statistical analysis

Standard deviation was expressed in terms of ±SD. On the basis of calculated values by using one-way ANOVA followed by independent two sample *t* test, it was observed that differences below 0.001 level were considered statistically significant. Compounds (**5a**–**u**) were screened for their antibacterial and antifungal activities in six sets (*n*) against bacteria and fungi used in the present protocol.

Structure–activity relationship

The results of the antimicrobial screening of compounds (**5a**–**u**) demonstrated that the substitution pattern of the hybrid benzimidazole and pyrazoline derivatives was carefully selected to impart different electronic environments to the molecules. As seen from activity data, antibacterial activity was considerably affected by substitution pattern on the phenyl ring and the incorporation of electron-withdrawing groups was responsible for enhancing the activity against most of the test microorganisms. The role of electron-withdrawing group in improving antimicrobial activity is very well supported by previous studies (Sharma

et al., 2004, 2009). From antimicrobial activity data, some analogs of this series were found to have even more potency than the reference drugs while some of them have comparable potency. Compounds **5f**, **5i**, **5q**, and **5t** with electron-withdrawing substituents (F, Cl, NO_2, and Br) in the aromatic ring were most active against all test bacterial strains, while compounds **5e**, **5g**, **5h**, **5j**, **5p**, **5r**, and **5u** were most potent against all the fungal strains. Moreover, newly synthesized compounds had higher potency against Gram-positive bacteria as compared to Gram-negative bacterial strains. In addition, antibacterial activity showed electron-withdrawing substituents at *meta* position which displayed higher activity than substituents at *ortho* or *para* positions. On the other hand, antifungal activity displayed conflicting results wherein compounds having electron-withdrawing substituents (Cl, F, NO_2, and Br) present at 2nd or 4th positions revealed higher inhibition than compounds having substitution at third position. Clearly, *meta* position was a favorable site for high antibacterial, while *ortho* or *para* positions had high antifungal activity indicating that structural requirements are different for binding of drug to bacterial targets.

Conclusion

To conclude, our attempts at exploring benzimidazole-based pyrazoline derivatives had unexpectedly led to the identification of a novel chemotype with substantial antimicrobial activity. Among the newly synthesized compounds (**5a**–**u**), analogs **5f** (3-Cl), **5i** (3-F), **5q** (3-NO_2), and **5t** (3-Br) showed highest inhibition against nearly all the tested bacteria, while analogs **5e** (2-Cl), **5g** (4-Cl), **5h** (2-F), **5j** (4-F), **5p** (2-NO_2), **5r** (4-NO_2), and **5u** (4-Br) were most active against all the fungal strains. Results of antimicrobial activity clearly demonstrated that the presence of electron-withdrawing groups/atoms on phenyl ring was essential for enhancing antimicrobial activity. On the basis of structure activity relationship, electron-withdrawing substituents (F, Cl, NO_2, and Br) at *meta* position were beneficial for antibacterial activity and substituents at 2nd or 4th positions were crucial for antifungal activity. From the activity data, compound **5i** (3-F) showed highest antibacterial inhibition whereas compound **5 h** (2-F) displayed maximum antifungal inhibition. In addition, the promising activity of these title compounds could also be attributed to the incorporation of heterocyclic scaffolds viz., benzimidazole and pyrazoline. Thus, suggesting that the compounds from the present series with electron-withdrawing fluoro, chloro, nitro, and bromo groups on phenyl ring can serve as important gateways for the design and development of new antimicrobial agents with potent activity and minimal toxicity.

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