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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Simulation results source for the identification of biological active compounds: synthesis, antimicrobial evaluation and SARs of three in one heterocyclic motifs

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Abstract

For comprehensive studies on drugs primarily in the form of biomimetic systems, electronic parameters are becoming essential tools in elucidating the structures of the investigated compounds. In this study we present the synthesis, characterization, and evaluation of biological potency of **4**(**a**–**g**), **5**(**a**–**g**), and **7**(**a**–**g**) by conducting structure–activity relationship (SAR) studies. Further conducting density functional theory (DFT) simulation studies for entitled compounds **4** (**a**–**g**), **5**(**a**–**g**), and **7**(**a**–**g**) allowed us to fully study the effect of the changes of electronic and molecular structures on their biological activity by demonstrating the role of frontier molecular orbitals, in particular LUMO. The electron withdrawing nitro group substituted compounds **5d** and **7d** have higher activity than all other active compounds. Thus, the results strongly suggest that the SARs are in good agreement with simulation studies.

Keywords SARs · Antimicrobial activity · DFT studies · LUMO energy · Three in one heterocycles · Claisen–Schmidt reaction

Introduction

Scientific accomplishments have been conducted from the past decades toward the determination of a series of antimicrobial and antioxidant biological drugs. In case of drugs, identification of prominent active drug from less active drugs is still a demanding action. For this, electronic parameters are useful tools for the classification of a high biological active motif from less active one (Helal et al. 2015; Reddy et al. 2016). Noteworthy, benzimidazoles are resourceful structures in many molecular drug design of antibacterials, antivirals, fungicides, and antimutagens (Davidse 1985; Spasov et al. 1999). Several benzimidazole-based molecules have shown potential anticancer activities (Gu et al. 2017; Kim et al. 2015). By virtue of having this property, bendamustine (Kath et al. 2001) and veliparib (Wagner 2015) are drugs (Fig. 1) approved for cancer treatment. In recent years substituted benzimidazole derivatives have been used to analyze their therapeutic potential (Khan et al. 2016; Reddy et al. 2015; Sharma et al. 2016). Likewise, benzimidazole derivatives have been optimized for development of antiparasitic agents like albendazole, mebendazole, and thiabendazole (Bansal and Silakari 2012).

However, pyrazole is also noted to be a motif which is also an important core for many drugs, for example, celecoxib is the one which is treated as a safe anti-inflammatory and analgesic agent (Dannahardt et al. 2000). Some other

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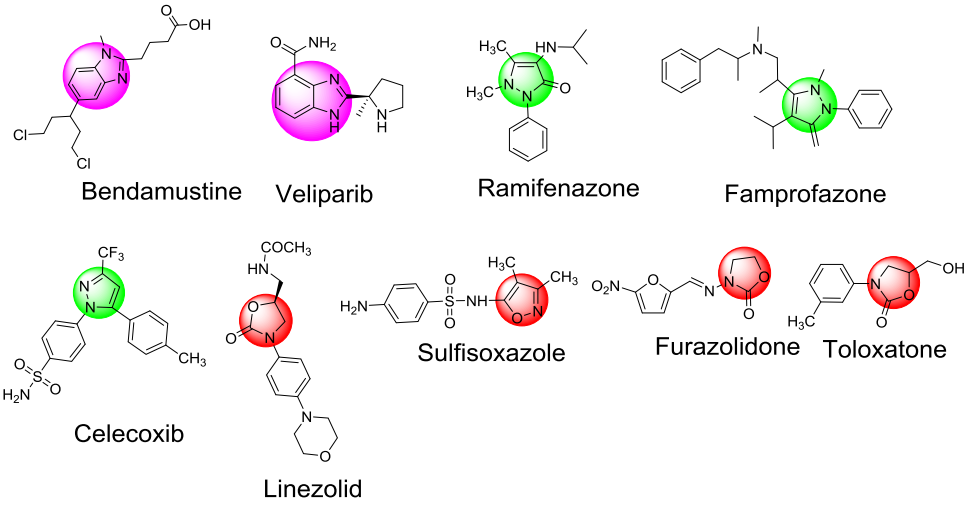


Fig. 1 Drugs containing benzimidazole, pyrazole, and oxazole units

examples of pyrazole derivatives as NSAIDs are mefobutazone, ramifenazone, and famprofazone (Reynold 1993; Amir and Kumar 2005; Gursoy et al. 2000) (Fig. 1). As one of the most effective core in several drugs, the oxazole rings are gifted with various bioactivities such as hypoglycemic (Conti et al. 1998), analgesic (Mishra et al. 1998), anti-inflammatory (Ko et al. 1998), and antibacterial. In previous studies, drugs containing oxazole rings exhibited potent anti-proliferative activity against human prostate cancer and human epidermoid carcinoma (Mohamed and EI-Sherbiny 2007; Dallemagne et al. 2003). The oxazole nucleus is also ubiquitous in natural products such as pyrronazol (Jansen et al. 2014), ulapualides (Rosener and Scheuer 1986), diazonamides (Li et al. 2001), and rhizopodin (Hagelueken et al. 2008). In addition, the oxazole derivatives have exhibited a high potential as efficient luminophores for liquid and fluorescent probes and markers in biological or supramolecular systems (Verrier et al. 2011). These are privileged scaffolds for the generation of target drugs (Fig. 1).

Because of the nitrogen and oxygen atoms of oxazole core are willing to bind with a variety of enzymes and receptors in biotic systems via diverse noncovalent interactions, and thus display versatile biological activities.

Encouraged by the above reports, herein we put the heterocyles in the synthesis of benzimidazole-containing pyrazolyl oxazoles (Scheme 1). This will make heterocyclic applications become increasingly popular obviously because of their tendency to exhibit excellent biological pharmacological activities. In our present study, we have correlated the bioactivity studies with appropriate electronic parameters.

Materials and methods

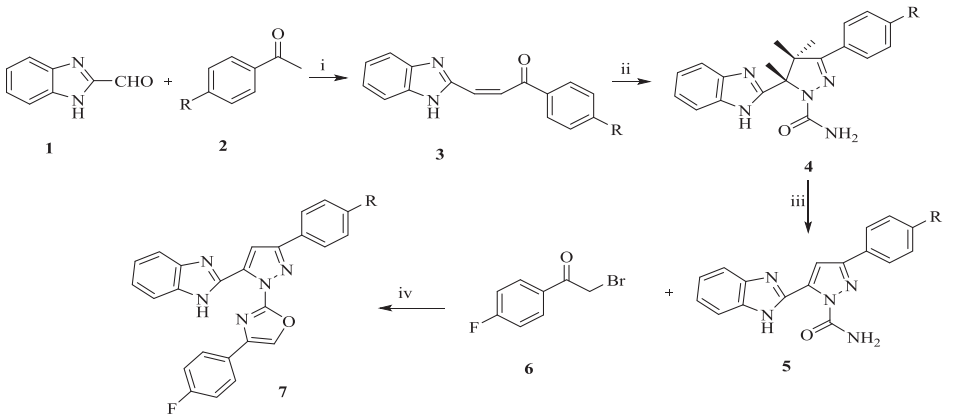
A new class of bis and tris heterocycles—4,5-dihydro-5-(1*H*-benzimidazol-2-yl)-3-aryl-pyrazole-1-carboxamide (**4**), 5-(1*H*-benzimidazol-2-yl)-3-aryl-1*H*-pyrazole-1-carboxamide (**5**), and 2-(5-(1*H*-benzimidazol-2-yl)-3-aryl-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)oxazole (**7**) were synthesized from (*E*)-3-(1*H*-benzimidazol-2-yl)-1-aryl-prop-2-en-1-one (**3**) (Scheme 1). In fact, the compound **3** was prepared by the Claisen–Schmidt reaction of benzimidazole-2-carboxaldehyde (**1**) and aryl ketones (**2**) in the presence of NaOH in methanol. The compound **4** was prepared by the reaction of **3** with semicarbazide in the presence of NaOH in ethanol. **4** with chloranil in xylene produced the aromatized product **5**. Moreover, exploitation of compound **5** furnished compound **7**. Thus, the reaction of **5** with p-fluorophenacyl bromide (**6**) and palladium acetate as the catalyst resulted in 2-(5-(1*H*-benzimidazol-2-yl)-3-aryl-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)oxazole (**7**). By using density functional theory (DFT) method, electronic parameters such as molecular electrostatic potential (MEP), dipole moment, HOMO (highest occupied molecular orbital), LUMO (lowest unoccupied molecular orbitals), and heat of formation (HF) were calculated for all the reported compounds. These calculations were calculated from all electronic energies of the cationic (E^+), anionic (E^-), and neutral (E^0) representations. For DFT calculations executed on GAMESS-US (Noro et al. 2012) line-up package, for iodine atom, SPK-DZP (Sapporo double zeta potential) (Gordon and Schmidt 2005) basis set was applied, and 6–31 + G(p) basis set, and B3LYP hybrid functional were applied for atoms like Cl, C, S, O, H, N, and F. In relation to entire system energy, the compound structure was completely relaxed.

Results and discussion

Chemistry

The ^{1}H NMR spectrum of **4a** exhibited an AMX splitting pattern for pyrazoline ring protons. The three doublets of doublets appearing at δ 4.25, 4.04, and 3.61 ppm were assigned to H_A, H_M, and H_X, respectively. The coupling

Scheme 1 Benzimidazolyl pyrazoles and benzimidazolyl pyrazolyl oxazoles



i) MeOH / NaOH, ii) $NH_2CONHNH_2$ / NaOH / EtOH, iii) Chloranil / Xylene, iv) $PdCl_2$, $CuBr_2$, $K_2S_2O_8$, $NaHCO_3$, DCE

R	a	b	c	d	e	f	g
	H	Me	OMe	NO_2	F	Cl	Br

constant values $J_{AM} = 12.4$, $J_{MX} = 10.4$, and $J_{AX} = 6.2$ Hz indicated that H_A and H_M are cis, H_A and H_x are trans, and H_M and H_X are geminal. In addition, two broad singlets were observed at δ 10.25 and 8.60 ppm due to NH and NH_2, respectively, which disappeared on deuteration. Oxidation of the ^{1}H NMR spectrum of **5a** showed a singlet at δ 6.42 and two broad singlets at 8.69 and 10.19 ppm due to C_4–H, NH_2, and NH, respectively. The signals due to highly acidic protons disappeared when D_2O was added. The ^{1}H NMR spectrum of **7a** displayed a singlet at δ 6.88 and a broad singlet at 10.13 ppm due to C_4–H and NH. Another singlet corresponding to C_5–H was observed at downfield region and merged with aromatic protons. The structures of all the compounds were further confirmed by IR, ^{13}C NMR, mass spectra, and elemental analyses.

Biological assays

Antimicrobial activity The in vitro antimicrobial studies were carried out by agar well diffusion method against test organisms (Chung et al. 1990; Azoro 2002). Nutrient broth (NB) plates were swabbed with 24 h old broth culture (100 µL) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each petriplate. The compounds were dissolved in DMSO of 5 mg/mL and from this 2.5, 5, 10, and 20 µL (12.5, 25, 50, and 100 µg/mL) were added into the wells by using sterile pipettes. Simultaneously the standard antibiotics, chloramphenicol for antibacterial activity, and ketoconazole for antifungal activity (as positive control) were tested against the pathogens. The samples were dissolved in DMSO, which showed no zone of inhibition acts as negative control. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. After appropriate incubation, the diameter of zone of inhibition of each well was measured. Triplicates were maintained and the average values were calculated for eventual antimicrobial activity.

Broth dilution test is used to determine minimum inhibitory concentration (MIC) of the above-mentioned samples (Janovska et al. 2003; Bishnu et al. 2009). Freshly prepared nutrient broth was used as diluents. The 24 h old culture of the test bacteria *S. aureus, B. subtilis, P. aeruginosa*, and *K. pneumoniae* and the test fungi *A. niger* and *P. chrysogenum* were diluted 100-folds in nutrient broth (100 µL bacterial cultures in 10 mL NB). The stock solution of the synthesized compounds was prepared in dimethyl sulfoxide (DMSO) by dissolving 5 mg of the compound in 1 mL of DMSO. Increasing concentrations of the test samples (1.25, 2.5, 5, 10, 20, and 40 µL of stock solution contains 6.25, 12.5, 25, 50, 100, and 200 µg of the compounds) were added to the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The tubes were examined for visible turbidity and using NB as control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC. To determine the minimum bactericidal concentration (MBC) (National Committee for Clinical Laboratory Standards 1993) and minimum fungicidal concentration (MFC) (National Committee for Clinical Laboratory Standards 1992) for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient broth (for bacteria) and potato dextrose agar (PDA) (for fungi) by streaking. Plates inoculated with bacteria and fungi were incubated at 37 °C for 24 h and at 28 °C for 48 h, respectively. After incubation, the lowest concentration was noted as MBC (for bacteria) or MFC (for fungi) at which no visible growth was observed.

Biological evaluation

Antimicrobial activity

The compounds **4**, **5**, and **7** were dissolved in DMSO at different concentrations of 12.5, 25, 50, and 100 µg/mL. Bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, and fungi *Aspergillus niger* and *Penicillium chrysogenum* were obtained from Department of Microbiology, S.V University, Tirupati, India.

Antibacterial activity

The compounds **4**, **5**, and **7** were screened for antibacterial activity at four different concentrations 12.5, 25, 50, and 100 µg/mL against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria), *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Gram-negative bacteria) bacterial strains and chloramphenicol was used as standard drug. The results of antibacterial activity shown in Table 1 specified that Gram-negative bacteria were more vulnerable toward the tested compounds than Gram-positive ones. It was observed that aromatized bis and tris heterocyclic compounds (**5** and **7**) showed slightly higher activity than the non-aromatized bis heterocyclic system (**4**). The compounds having electron-withdrawing groups (**5d**, **5e**, **5f**, **5g**, **7d**, **7e**, **7f**, and **7g**) were more active than those having donating groups (OCH_3 and CH_3). Interestingly, the compounds having NO_2 and F attached motifs **5d**, **5e**, **7d**, and **7e** exhibited prominent activity than the other active compounds. In fact, the compounds **5d** and **7d** were showed higher activity than all the tested compounds. In addition, **7d** has more antibacterial activity against *Pseudomonas aeruginosa* when compared with the standard drug chloramphenicol (Table 1 and Fig. 2). This may be due to the presence of more electronegative nitro group on the aromatic ring. Among bis heterocyclic compounds aromatized derivatives **5**(**a**–**g**) were more effective. On the other hand, except **4d**, the other non-aromatized **4**(**a**–**g**) compounds were inactive. This may be due to the greater electron-withdrawing capacity of the aromatized compounds.

Antifungal activity

All the tested compounds inhibited the spore germination against tested fungi. In general, most of the compounds showed slightly higher antifungal activity toward *Aspergillus niger* than *Penicillium chrysogenum*. Among all the active compounds, **7d** displayed greater inhibitory activity and its value was high at *Penicillium chrysogenum* when compared with the standard drug ketoconazole (Table 2 and Fig. 3). Moreover, compounds **5d**, **5e**, and **7e** exhibited excellent activity, while the other active compounds showed good to moderate activity.

MIC, MBC, and MFC of the compounds 5d, 7d, and 7e

The MIC, MBC, and MFC values of the compounds tested are listed in Table 3. MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. The MBC/MFC is the lowest concentration of antibiotic required to kill a bacterium/fungus. The MBC/MFC involves an additional set of steps performed once the MIC is determined. The antimicrobials are usually regarded as bactericidal/fungicidal if the MBC/MFC is not greater than four times the MIC (French 2006). The compound **7d** exhibited low MIC values when compared with **5d** and **7e**. In addition, MBC value in **7d** is 2× MIC in case of *P. aeruginosa* and MFC value is 2× MIC in case of *P. chrysogenum*. However, the other compounds showed bactericidal and fungicidal effects greater than 2× MIC. The structure–antimicrobial activity relationship of the synthesized compounds revealed that tris heterocyclic compounds have greater activity than the corresponding bis heterocycles. Among tris heterocyclic systems the nitro substituted **7d** displayed excellent antibacterial activity against *P. aeruginosa* with an inhibition zone of 34 mm at 100 µg/mL and MIC and MBC of 6.25 and 12.5 µg/mL, respectively. The compound **5d** also displayed strong antifungal activity against *P. chrysogenum* with an inhibition zone of 41 mm at 100 µg/mL and MIC and MFC of 12.5 and 25 µg/mL, respectively. Moreover, it was observed that the compounds having nitro substituent on aromatic ring enhanced the activity when compared with electron-donating compounds.

Electrostatic results

Electronic data of all the existed compounds were listed in Table 4 included the HOMO, LUMO energy, ionization energy (*I*), and electron affinity (*A*). The HOMO and LUMO energy stated in the sense of *A* and *I* are $I = -E_{\mathrm{HOMO}}$ $\mathrm{A} = -E_{\mathrm{LUMO}}$ by the Koopmans's statement. According to this theorem, HOMO and LUMO energies were related to *A* and *I*. Generally, the electron-donating and electron-withdrawing nature affects the bioactivity; based on this principle, the pharmacological assay is also influenced by HOMO and LUMO factors (Karelson et al. 1996; Katritzky et al. 2008; Mu et al. 2015) because HOMO has electron-releasing property while LUMO has electron-capturing property. Considering this, we chose prominent biologically active compounds such as **5d**, **7d**, **5e**, and **7e** as replicas to correlate the electronic information, particularly

Table 1 The in vitro antibacterial activity of compounds **4(a–g)**, **5(a–g)**, and **7(a–g)** oxazole

Compound	Zone of inhibition (mm)															
	Gram-positive bacteria								Gram-negative bacteria							
	Staphylococcus aureus				*Bacillus subtilis*				*Pseudomonas aeruginosa*				*Klebsiella pneumoniae*			
	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
4a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4d	09 ± 1	10 ± 1	11 ± 1	14 ± 2	–	–	13 ± 3	15 ± 1	11 ± 3	13 ± 3	15 ± 1	17 ± 3	10 ± 1	12 ± 2	14 ± 2	16 ± 2
4e	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4f	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4g	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
‘5a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5b	12 ± 2	14 ± 1	16 ± 3	19 ± 2	13 ± 3	14 ± 1	15 ± 1	16 ± 2	15 ± 3	17 ± 3	19 ± 2	21 ± 2	16 ± 2	18 ± 3	20 ± 2	22 ± 3
5c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5d	31 ± 2	32 ± 3	34 ± 1	35 ± 3	33 ± 2	34 ± 3	36 ± 2	38 ± 1	28 ± 3	30 ± 2	31 ± 1	32 ± 1	37 ± 3	38 ± 2	42 ± 1	43 ± 3
5e	29 ± 2	30 ± 3	32 ± 1	34 ± 1	30 ± 2	32 ± 1	34 ± 3	35 ± 2	23 ± 3	25 ± 2	26 ± 3	28 ± 2	30 ± 1	33 ± 3	34 ± 1	36 ± 2
5f	26 ± 3	28 ± 3	30 ± 1	32 ± 3	29 ± 2	31 ± 3	33 ± 1	35 ± 3	21 ± 1	23 ± 2	25 ± 3	27 ± 1	36 ± 2	37 ± 3	38 ± 2	39 ± 2
5g	21 ± 3	22 ± 2	23 ± 1	25 ± 2	24 ± 1	26 ± 3	27 ± 2	28 ± 1	16 ± 3	17 ± 1	18 ± 3	20 ± 1	29 ± 2	30 ± 3	31 ± 2	33 ± 3
7a	14 ± 3	16 ± 1	18 ± 3	22 ± 1	15 ± 2	16 ± 2	17 ± 3	19 ± 3	17 ± 1	19 ± 2	21 ± 1	23 ± 2	18 ± 1	20 ± 1	22 ± 2	24 ± 1
7b	13 ± 2	15 ± 1	17 ± 3	20 ± 2	14 ± 3	15 ± 1	16 ± 1	18 ± 2	16 ± 3	18 ± 3	20 ± 2	22 ± 2	17 ± 2	19 ± 3	21 ± 2	23 ± 3
7c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7d	32 ± 1	33 ± 3	35 ± 2	36 ± 3	34 ± 1	35 ± 3	36 ± 1	38 ± 2	31 ± 3	32 ± 1	33 ± 3	36 ± 2	38 ± 1	40 ± 3	43 ± 1	44 ± 3
7e	30 ± 2	32 ± 3	34 ± 1	35 ± 3	33 ± 2	34 ± 1	35 ± 3	37 ± 2	26 ± 3	27 ± 2	28 ± 3	30 ± 2	38 ± 1	39 ± 3	40 ± 1	41 ± 2
7f	28 ± 3	30 ± 3	32 ± 2	34 ± 2	31 ± 3	33 ± 2	35 ± 3	37 ± 1	23 ± 2	25 ± 3	27 ± 1	29 ± 2	36 ± 3	39 ± 2	40 ± 3	41 ± 1
7g	24 ± 1	26 ± 2	29 ± 3	31 ± 1	27 ± 2	28 ± 3	30 ± 2	32 ± 1	19 ± 2	21 ± 3	23 ± 1	25 ± 2	33 ± 1	35 ± 2	37 ± 2	39 ± 1
Chloram- phenicol	32 ± 1	34 ± 2	37 ± 2	39 ± 1	34 ± 3	36 ± 2	38 ± 1	42 ± 1	27 ± 2	29 ± 2	31 ± 2	34 ± 1	40 ± 2	42 ± 2	44 ± 1	46 ± 2
Control (DMSO)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Values were the means of three replicates ±SD

(–) No activity

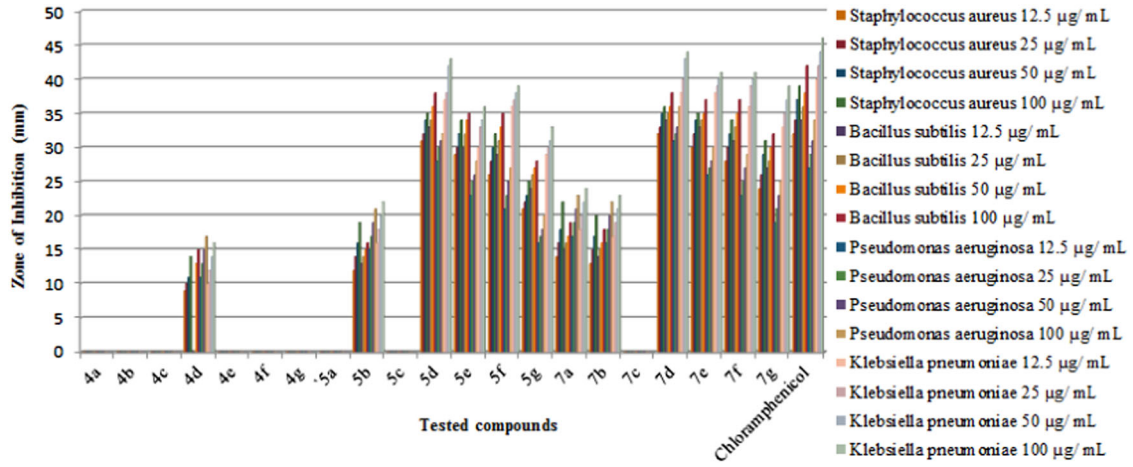


Fig. 2 The in vitro antibacterial activity of compounds **4**(**a**–**g**), **5**(**a**–**g**), and **7**(**a**–**g**)

Table 2 The in vitro antifungal activity of compounds **4**(**a**–**g**), **5**(**a**–**g**), and **7**(**a**–**g**)

Compound	Zone of inhibition (mm)							
	Aspergillus niger				*Penicillium chrysogenum*			
	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
4a	–	–	–	–	–	–	–	–
4b	–	–	–	–	–	–	–	–
4c	–	–	–	–	–	–	–	–
4d	8 ± 1	10 ± 3	12 ± 1	14 ± 2	11 ± 1	13 ± 3	15 ± 1	17 ± 2
4e	–	–	–	–	–	–	–	–
4f	–	–	–	–	–	–	10 ± 1	12 ± 3
4g	–	–	–	–	–	–	–	–
5a	18 ± 3	20 ± 1	22 ± 3	24 ± 1	21 ± 3	23 ± 1	25 ± 2	27 ± 1
5b	16 ± 1	17 ± 2	19 ± 1	21 ± 3	20 ± 1	22 ± 3	24 ± 1	26 ± 2
5c	12 ± 1	14 ± 3	15 ± 1	17 ± 2	16 ± 3	18 ± 1	20 ± 3	22 ± 1
5d	32 ± 1	34 ± 3	35 ± 1	37 ± 2	36 ± 3	38 ± 2	39 ± 1	41 ± 3
5e	29 ± 3	30 ± 1	32 ± 3	34 ± 3	31 ± 2	33 ± 1	34 ± 3	36 ± 1
5f	25 ± 3	27 ± 1	29 ± 3	31 ± 2	28 ± 1	30 ± 3	32 ± 1	34 ± 2
5g	22 ± 2	24 ± 3	26 ± 2	27 ± 1	29 ± 3	31 ± 1	34 ± 2	35 ± 3
7a	20 ± 3	22 ± 2	23 ± 1	25 ± 3	27 ± 1	29 ± 2	31 ± 3	33 ± 1
7b	17 ± 2	19 ± 1	21 ± 3	23 ± 2	20 ± 2	22 ± 3	24 ± 1	26 ± 2
7c	14 ± 1	16 ± 3	17 ± 3	19 ± 1	21 ± 3	18 ± 2	20 ± 3	22 ± 2
7d	34 ± 3	36 ± 1	38 ± 2	40 ± 3	37 ± 2	40 ± 3	41 ± 1	43 ± 2
7e	30 ± 1	32 ± 3	33 ± 1	35 ± 2	34 ± 3	36 ± 1	38 ± 2	40 ± 1
7f	27 ± 1	29 ± 2	30 ± 3	33 ± 1	31 ± 2	33 ± 3	35 ± 1	37 ± 2
7g	24 ± 3	26 ± 2	28 ± 1	31 ± 3	27 ± 1	29 ± 2	31 ± 2	33 ± 3
Ketoconazole	33 ± 3	35 ± 2	38 ± 1	41 ± 1	36 ± 3	39 ± 1	40 ± 2	41 ± 2
Control (DMSO)	–	–	–	–	–	–	–	–

Values were the means of three replicates ±SD

(–) No activity

LUMO energy with biological statistics, because in this study the most biologically active compounds belong to the electron-withdrawing category, and LUMO has electron-withdrawing nature as well.

Table 3 shows that, among the two most active nitro-withdrawing substituted compounds (**5d** and **7d**), compound **7d** had the highest LUMO value of −2.89 or, in a sense, lower negative LUMO value and higher bioassay

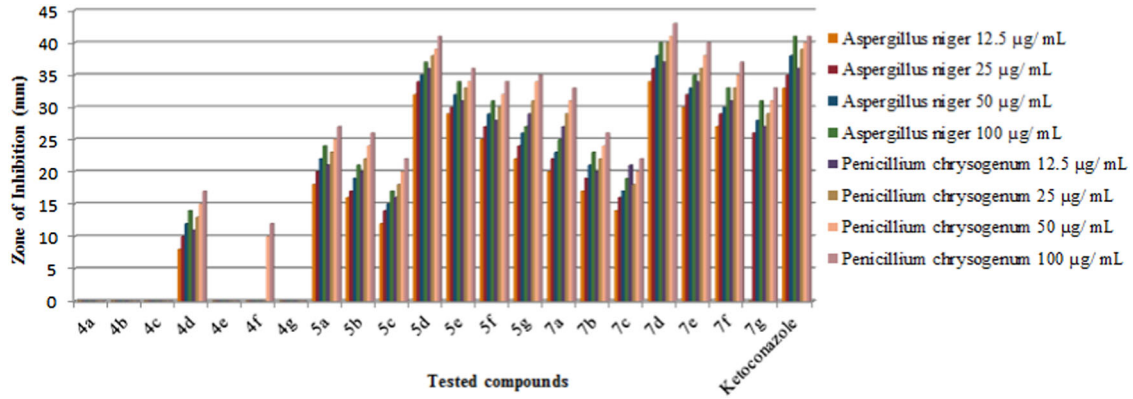


Fig. 3 The in vitro antifungal activity of compounds **4**(**a**–**g**), **5**(**a**–**g**), and **7**(**a**–**g**)

Table 3 MIC, MBC, and MFC of compounds **5d**, **7d**, and **7e**

Compound	Minimum inhibitory concentration					
	MIC (MBC/MFC) µg/mL					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K.pneumoniae*	*A. niger*	*P. chrysogenum*
5d	50 (200)	25 (100)	12.5 (50)	25 (>100)	12.5 (50)	25 (100)
7d	25 (>100)	25 (100)	6.25 (12.5)	12.5 (50)	12.5 (25)	25 (50)
7e	50 (>200)	50 (>200)	25 (100)	25 (>100)	50 (>200)	25 (100)
Chloramphenicol	6.25	6.25	6.25	12.5	–	–
Ketoconazole	–	–	–	–	12.5	6.25

Table 4 Electronic results of compounds **4**(**a**–**g**), **5**(**a**–**g**), and **7**(**a**–**g**)

Compound	HOMO (eV)	LUMO (eV)	Dipole	Ionization potential (eV)	Electron affinity (eV)
4a	−6.071	−1.369	2.800301	6.071	1.369
4b	−6.016	−1.295	3.309853	6.016	1.295
4c	−5.834	−1.181	3.349854	5.834	1.181
4d	−6.299	−2.961	4.651046	6.299	2.961
4e	−6.114	−1.396	2.005869	6.114	1.396
4f	−6.133	−1.524	1.963084	6.133	1.524
4g	−6.144	−1.573	1.959860	6.144	1.573
5a	−5.853	−1.935	3.733806	5.853	1.935
5b	−5.810	−1.883	4.013415	5.810	1.883
5c	−5.777	−1.837	3.589576	5.777	1.837
5d	−6.117	−2.947	5.881343	6.117	2.947
5e	−5.902	−1.984	3.394990	5.902	1.984
5f	−5.924	−2.022	3.419687	5.924	2.022
5g	−5.940	−2.052	3.434012	5.940	2.052
7a	−5.908	−2.150	2.661062	5.908	2.150
7b	−5.905	−2.003	2.642107	5.905	2.003
7c	−5.782	−1.954	1.818452	5.782	1.954
7d	−6.218	−2.898	7.188567	6.218	2.898
7e	−5.997	−2.101	3.002701	5.997	2.101
7f	−5.970	−2.242	3.326221	5.970	2.242
7g	−5.987	−2.261	3.474386	5.987	2.261

property than **5d** (which had a value of −2.96). In addition, the promising electron-withdrawing fluorine-attached biologically active compound **7e** showed the peak LUMO value of −2.10 and greater biological activity than compound **5e**, which has similar properties. The above results clearly indicated that, among the electron-withdrawing substituted compounds, the ones with high LUMO value exhibited prominent antimicrobial activity (Fig. 4). On the other hand, compound **7d** displayed a dipole moment of 7.18 Debye. This value was higher than that of the other active compounds. Nevertheless, compound **5d** containing the second higher dipole moment was shown to be an antimicrobial active compound. Meanwhile, the other active compounds with different LUMO and diplomat values showed different activities.

The location of LUMO orbital on the motifs is the other best way to justify the most active compound from less active ones (Reddy et. al. 2016). In this case, for compounds **5d** and **7d** the frontier molecular orbital, particularly LUMO orbital, was located on aryl group having nitro link. Where as, the same orbital is near to aryl group having fluorine association, this was happened in the compounds **5e** and **7e** (Fig. 5). Finally, the LUMO orbital location on the moieties of **5d** and **7d** also supported that those compounds have prominent biological activity than other active compounds. The theoretical computations and experimental biological data had justified each other.

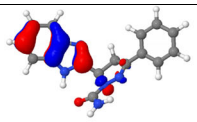
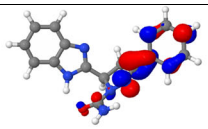
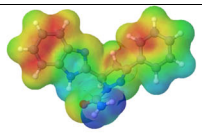
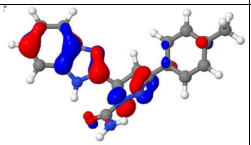
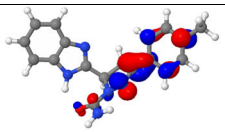
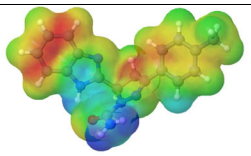
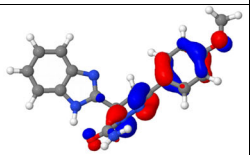
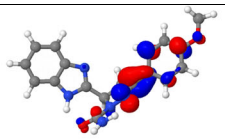
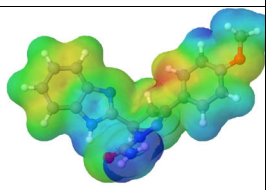
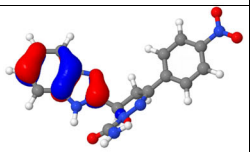
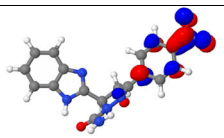
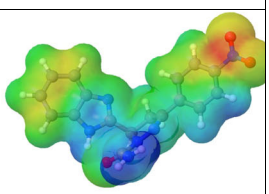
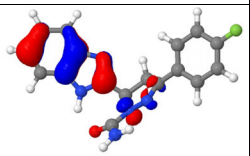
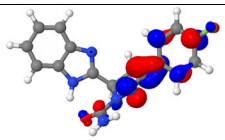
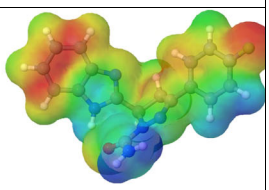
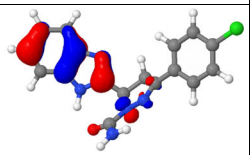
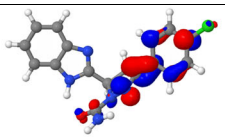
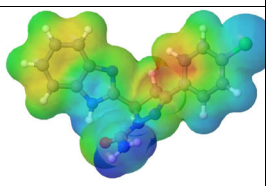
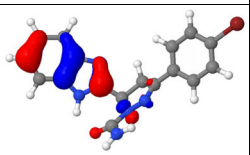
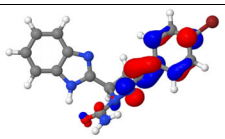
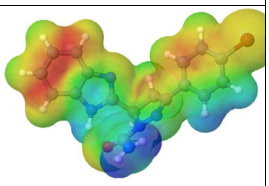
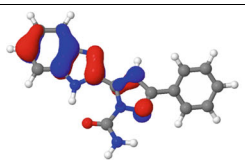
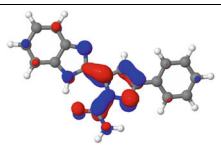
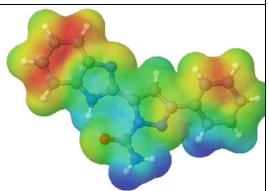
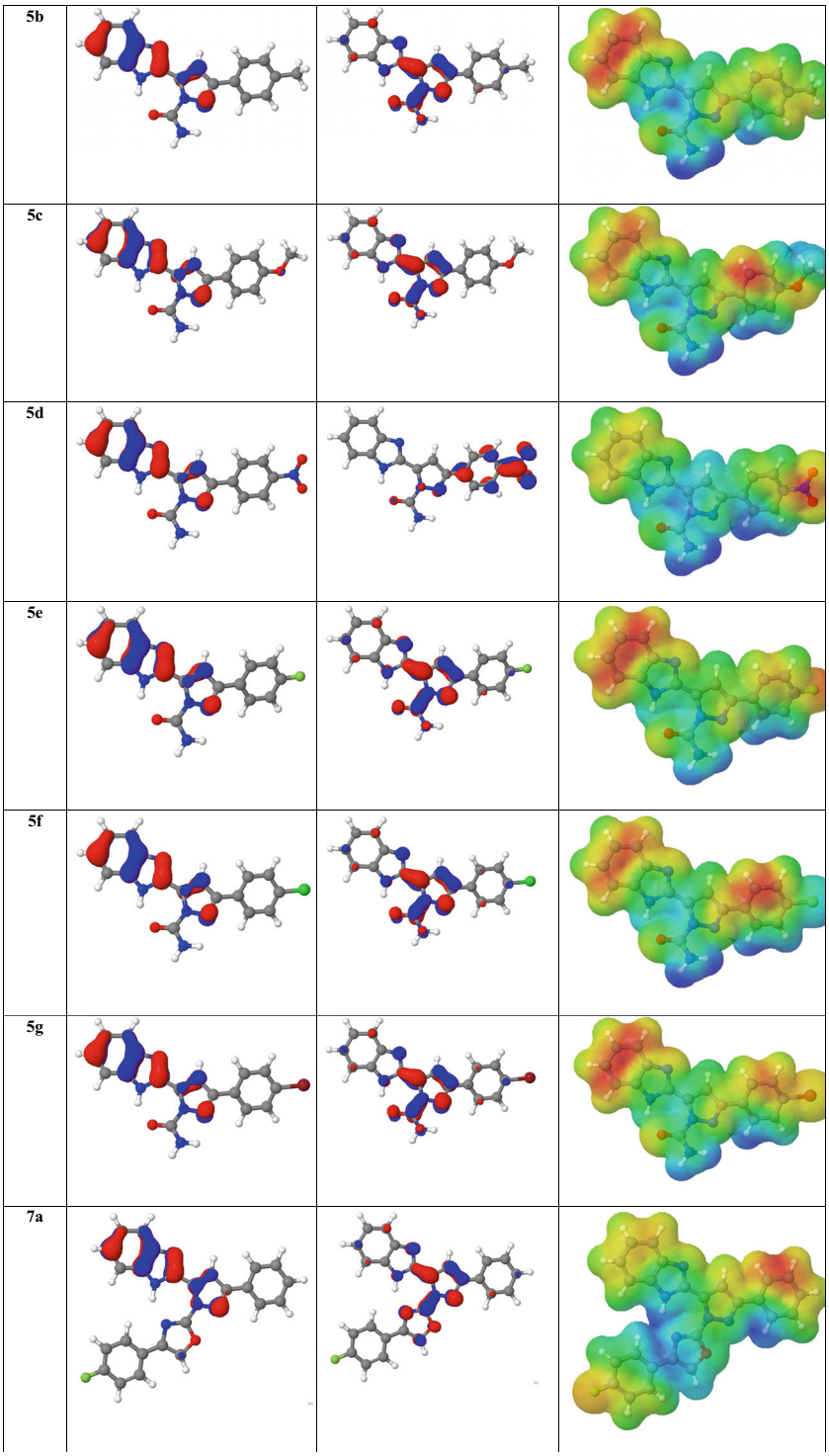
Compound	HOMO	LUMO	MEP
4a			
4b			
4c			
4d			
4e			
4f			
4g			
5a			

Fig. 4 HOMO, LUMO, and MEP diagrams of compounds **4** (**a**–**g**), **5**(**a**–**g**), and **7**(**a**–**g**)

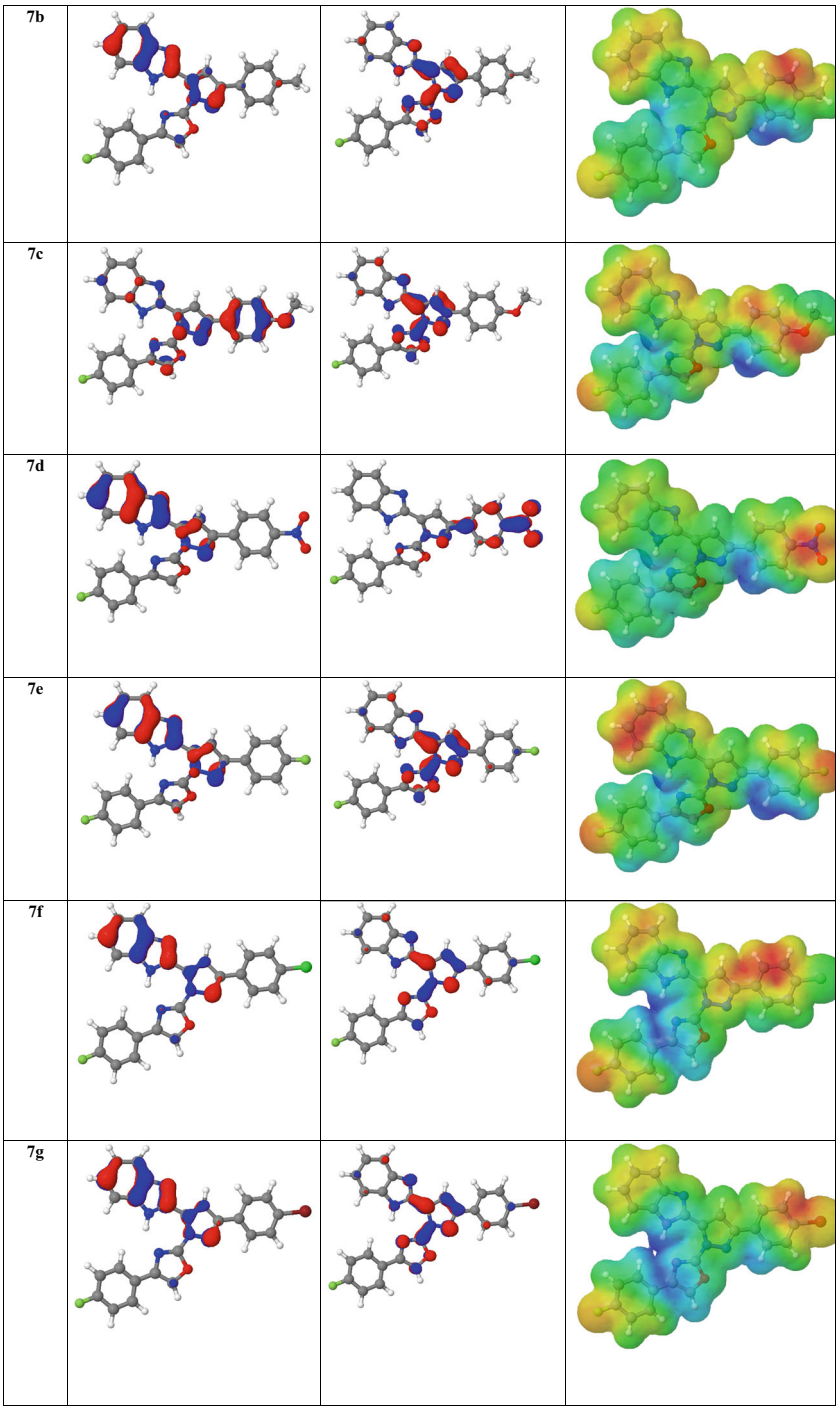
Fig. 4 (Continued)



Conclusion

In summary, a simple method for deriving benzimidazole-containing pyrazolyl oxazole derivatives was synthesized; the compounds were screened for their antimicrobial activity, and their properties were elucidated on the basis of their electronic results. To categorize the best active compounds from a group of other compounds; DFT simulation studies made clear the role of frontier molecular orbital, in particular LUMO, as a beneficial tool to study electronic and molecular structures. Consequently, the compounds having electron-withdrawing nature possessed higher antimicrobial activity than other active compounds, which was supported by the LUMO energy values. Thus,

Fig. 4 (Continued)



compounds **5d** and **7d** exhibited significant biological activity than all the active compounds. Besides, the fluoro functional group substituted cores **5e** and **7e** were also found to possess profound bioactivity. Finally, the biological data were correlated with electronic data, and these correlation results can be further used to explore the effective drugs in near future.

Experimental protocols

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The homogeneity of the compounds was checked by TLC (silica gel H, BDH, hexane/ethyl acetate, 3:1). The synthesized compounds that have been synthesized are about 95–100% purity. The ^{1}H

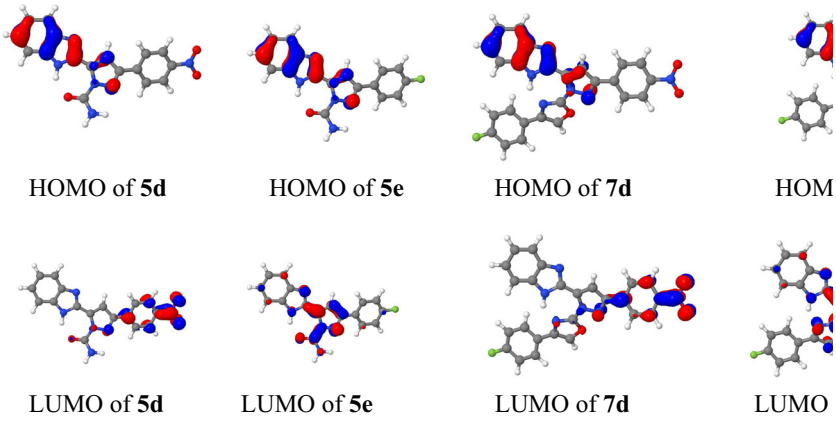


Fig. 5 Frontier orbital location of most active compounds

NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM λ-400 MHz spectrometer. The ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM spectrometer operating at λ-100 MHz. High-resolution mass spectra were recorded on micromass Q-TOF micro mass spectrometer using electro spray ionization. All chemical shifts were reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The temperature was measured by flexible probe throughout the reaction.

General procedure for the synthesis of 4,5-dihydro-5-(1*H*-benzimidazol-2-yl)-3-aryl-pyrazole-1-carbothioamide (4a–g)

To an equimolar (1 mmol) mixture of compound **3** and semicarbazide, ethanol (3 ml) and sodium hydroxide (1.5 mmol) were added. It was refluxed for 7–8 h. After completion of the reaction (monitored by TLC), the contents of the flask were poured onto crushed ice. The separated solid was collected by filtration and purified by recrystallization from isopropyl alcohol.

4,5-Dihydro-5-(1*H*-benzimidazol-2-yl)-3-phenylpyrazole-1-carboxamide (4a)

m. p. 152–154 °C; yield 82%; IR (KBr) (cm^{-1}): 3455, 3346 (NH_2), 3239 (NH), 1594 (C=N), 1375 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 3.61 (dd, 1H, H_X, $J_{AX} = 6.3$ Hz, $J_{MX} = 10.3$ Hz), 4.04 (dd, 1H, H_M, $J_{AM} = 12.3$ Hz, $J_{MX} = 10.3$ Hz), 4.25 (dd, 1H, H_A, $J_{AM} = 12.3$ Hz, $J_{AX} = 6.3$ Hz), 8.60 (bs, 2H, NH_2), 7.32–7.82 (m, 9H, Ar–H), 10.25 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 40.6 (C-4), 54.1 (C-5), 116.5, 124.7, 129.4, 129.8, 132.2, 137.7, 139.5, 142.7, 152.9, 156.7 (aromatic carbons), 155.9 (C=O) ppm; MS (EI) *m/z*: 305.1277 [M^+]; Anal. Calcd. for $C_{17}H_{15}N_5O$: C, 66.87; H, 4.95; N, 22.94%; Found: C, 66.97; H, 4.96; N, 22.14%.

4,5-Dihydro-5-(1*H*-benzimidazol-2-yl)-3-p-tolylpyrazole-1-carboxamide (4b)

m. p. 143–145 °C; yield 78%; IR (KBr) (cm^{-1}): 3448, 3342 (NH_2), 3246 (NH), 1585 (C=N), 1343 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 2.47 (s, 3H, Ar–CH_3), 3.60 (dd, 1H, H_X, $J_{AX} = 6.1$ Hz, $J_{MX} = 10.2$ Hz), 4.18 (dd, 1H, H_M, $J_{AM} = 12.1$ Hz, $J_{MX} = 10.2$ Hz), 4.98 (dd, 1H, H_A, $J_{AM} = 12.1$ Hz, $J_{AX} = 6.1$ Hz), 8.43 (bs, 2H, NH_2), 7.33–7.87 (m, 8H, Ar–H), 10.19 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.4 (Ar–CH3), 42.9 (C-4), 56.0 (C-5), 117.2, 125.7, 128.4, 130.8, 134.3, 140.9, 141.6, 143.7, 152.6, 157.9 (aromatic carbons), 157.4 (C=O) ppm; MS (EI) *m/z*: 319.3605 [M^+]; Anal. Calcd. for $C_{18}H_{17}N_5O$: C, 67.70; H, 5.37; N, 21.93%; Found: C, 67.81; H, 5.39; N, 21.14%.

4,5-Dihydro-5-(1*H*-benzimidazol-2-yl)-3-(p-methoxyphenyl) pyrazole-1-carboxamide (4c)

m. p. 146–148 °C; yield 76%; IR (KBr) (cm^{-1}): 3450, 3341 (NH_2), 3249 (NH), 1588 (C=N), 1349 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 3.56 (dd, 1H, H_X, $J_{AX} = 6.1$ Hz, $J_{MX} = 10.2$ Hz), 3.99 (dd, 1H, H_M, $J_{AM} = 12.1$ Hz, $J_{MX} = 10.2$ Hz), 3.92 (s, 3H, Ar–OCH_3), 4.12 (dd, 1H, H_A, $J_{AM} = 12.1$ Hz, $J_{AX} = 6.1$ Hz), 8.38 (bs, 2H, NH_2), 7.17–8.06 (m, 8H, Ar–H), 10.13 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.5 (C-4), 56.2 (Ar–OCH_3), 56.1 (C-5), 115.7, 116.9, 125.0, 129.1, 129.4, 140.3, 142.9, 151.7, 156.8, 163.8 (aromatic carbons), 156.5 (C=O) ppm; MS (EI) *m/z*: 335.3599 [M^+]; Anal. Calcd. for $C_{18}H_{17}N_5O_2$: C, 64.47; H, 5.11; N, 20.88%; Found: C, 64.35; H, 5.10; N, 21.02%.

4, 5-Dihydro-5-(1*H*-benzimidazol-2-yl)-3-(p-nitrophenyl) pyrazole-1-carboxamide (4d)

m. p. 178–180 °C; yield 90%; IR (KBr) (cm^{-1}): 3436, 3343 (NH_2), 3241 (NH), 1580 (C=N), 1348 (C=O); ^1H NMR

(400 MHz, DMSO-d_6): δ 3.76 (dd, 1H, H_X, $J_{AX} = 6.8$ Hz, $J_{MX} = 10.8$ Hz), 4.23 (dd, 1H, H_M, $J_{AM} = 12.8$ Hz, $J_{MX} = 10.8$ Hz), 4.63 (dd, 1H, H_A, $J_{AM} = 12.8$ Hz, $J_{AX} = 6.8$ Hz), 8.76 (bs, 2H, NH_2), 7.37–8.48 (m, 8H, Ar–H), 10.59 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.2 (C-4), 54.9 (C-5), 116.7, 124.9, 127.5, 128.4, 140.1, 142.7, 143.0, 151.4, 152.9, 156.4 (aromatic carbons), 156.3 (C=O) ppm; MS (EI) *m/z*: 350.3315 [M^+]; Anal. Calcd. for $C_{17}H_{14}N_6O_3$: C, 58.28; H, 4.03; N, 23.99%; Found: C, 58.36; H, 4.06; N, 24.23%.

4, 5-Dihydro-5-(1*H*-benzimidazol-2-yl)-3-(p-fluorophenyl) pyrazole-1-carboxamide (4e)

m. p. 172–174 °C; yield 87%; IR (KBr) (cm^{-1}): 3447, 3339 (NH_2), 3247 (NH), 1582 (C=N), 1343 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.13 (dd, 1H, H_X, $J_{AX} = 6.7$ Hz, $J_{MX} = 10.7$ Hz), 4.14 (dd, 1H, H_M, $J_{AM} = 12.6$ Hz, $J_{MX} = 10.7$ Hz), 4.57 (dd, 1H, H_A, $J_{AM} = 12.6$ Hz, $J_{AX} = 6.7$ Hz), 8.71 (bs, 2H, NH_2), 7.36–8.19 (m, 8H, Ar–H), 10.50 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.8 (C-4), 117.1, 116.6, 125.1, 130.2, 133.4, 140.5, 143.2, 151.9, 157.1, 166.4 (aromatic carbons), 156.8 (C=O) ppm; MS (EI) *m/z*: 323.3244 [M^+]; Anal. Calcd. for $C_{17}H_{14}FN_5O$: C, 63.15; H, 4.36; N, 21.66%; Found: C, 63.23; H, 4.39; N, 21.88%.

4, 5-Dihydro-5-(1*H*-benzimidazol-2-yl)-3-(p-chlorophenyl) pyrazole-1-carboxamide (4f)

m. p. 164–166 °C; yield 85%; IR (KBr) (cm^{-1}): 3437, 3329 (NH_2), 3238 (NH), 1571 (C=N), 1332 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.69 (dd, 1H, H_X, $J_{AX} = 6.5$ Hz, $J_{MX} = 10.5$ Hz), 4.09 (dd, 1H, H_M, $J_{AM} = 12.5$ Hz, $J_{MX} = 10.5$ Hz), 4.43 (dd, 1H, H_A, $J_{AM} = 12.5$ Hz, $J_{AX} = 6.5$ Hz), 8.69 (bs, 2H, NH_2), 7.35–7.98 (m, 8H, Ar–H), 10.37 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 40.9 (C-4), 54.5 (C-5), 116.5, 124.7, 129.6, 130.0, 132.3, 137.9, 139.7, 142.8, 153.0, 157.1 (aromatic carbons), 156.0 (C=O) ppm; MS (EI) *m/z*: 339.7790 [M^+]; Anal. Calcd. for $C_{17}H_{14}ClN_5O$: C, 60.09; H, 4.15; N, 20.61%; Found: C, 60.21; H, 4.18; N, 20.83%.

4,5-Dihydro-5-(1*H*-benzimidazol-2-yl)-3-(p-bromophenyl) pyrazole-1-carboxamide (4g)

m. p. 158–160 °C; yield 84%; IR (KBr) (cm^{-1}): 3457, 3349 (NH_2), 3258 (NH), 1592 (C=N), 1353 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.65 (dd, 1H, H_X, $J_{AX} = 6.3$ Hz, $J_{MX} = 10.4$ Hz), 4.07 (dd, 1H, H_M, $J_{AM} = 12.4$ Hz, $J_{MX} = 10.4$ Hz), 4.31 (dd, 1H, H_A, $J_{AM} = 12.4$ Hz, $J_{AX} = 6.3$ Hz), 8.65 (bs, 2H, NH_2), 7.34–7.90 (m, 8H, Ar–H), 10.30 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.3 (C-4), 55.8 (C-5), 116.9, 125.4, 130.5, 133.6, 126.4, 136.3, 140.7, 143.5, 152.4, 157.5 (aromatic carbons), 157.0 (C=O) ppm; MS (EI) *m/z*: 384.2300 [M^+]; Anal. Calcd. for $C_{17}H_{14}BrN_5O$: C, 53.14; H, 3.67; N, 18.23%; Found: C, 53.03.; H, 3.69; N, 18.45%.

General procedure for the synthesis of 5-(1*H*-benzimidazol-2-yl)-3-aryl-1H-pyrazole-1-carboxamide (5a–g)

A solution of compound **4** (1 mmol) and chloranil (1.2 mmol) in xylene (10 ml) was refluxed for 24 h. Then, it was treated with 5% NaOH solution. The organic layer was separated, repeatedly washed with water, and dried over an Na_2SO_4. The solvent was removed in vacuo. The solid obtained was purified by recrystallization from isopropyl alcohol.

5-(1*H*-benzimidazol-2-yl)-3-phenyl-1*H*-pyrazole-1-carboxamide (5a)

m. p. 147–149 °C; yield 86%; IR (KBr) (cm^{-1}): 3447, 3339 (NH_2), 3247 (NH), 1581 (C=N), 1342 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.42 (s, 1H, C_4-H), 7.39–8.10 (m, 9H, Ar–H), 8.69 (bs, 2H, NH_2), 10.19 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.4 (C-4), 116.9, 124.2, 128.4, 129.6, 130.4, 131.2, 134.4, 142.4, 151.6, 155.8 (aromatic carbons), 152.8 (C=O) ppm; MS (EI) *m/z*: 303.3180 [M^+]; Anal. Calcd. for $C_{17}H_{13}N_5O$: C, 67.32; H, 4.32; N, 23.09 %; Found: C, 67.43; H, 4.33; N, 23.28 %.

5-(1*H*-benzimidazol-2-yl)-3-p-tolyl-1*H*-pyrazole-1-carboxamide (5b)

m. p. 155–157 °C; yield 80%; IR (KBr) (cm^{-1}): 3448, 3340 (NH_2), 3249 (NH), 1584 (C=N), 1343 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.47 (s, 3H, Ar–CH_3), 6.81 (s, 1H, C_4–H), 7.34–8.17 (m, 8H, Ar–H), 8.62 (bs, 2H, NH_2), 10.16 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.4 (Ar–CH_3), 107.3 (C-4), 117.9, 125.3, 126.4, 130.8, 131.2, 132.3, 133.9, 144.0, 152.8, 157.4 (aromatic carbons), 153.5 (C=O) ppm; MS (EI) *m/z*: 344.3888 [M^+]; Anal. Calcd. for $C_{18}H_{15}N_5O$: C, C, 68.13; H, 4.76; N, 22.07 %; Found: C, 68.21; H, 4.74; N, 22.29 %.

5-(1*H*-benzimidazol-2-yl)-3-(p-methoxyphenyl)-1*H*-pyrazole-1-carboxamide (5c)

m. p. 132–134 °C; yield 79%; IR (KBr) (cm^{-1}): 3453, 3345 (NH_2), 3254 (NH), 1587 (C=N), 1348 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.92 (s, 3H, Ar–OCH_3), 6.74 (s, 1H, C_4–H), 7.32–8.12 (m, 8H, Ar–H), 8.60 (bs, 2H, NH_2), 10.13 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6):

δ 106.2 (C-4), 56.2 (Ar–OCH_3), 115.4, 117.6, 124.8, 126.3, 129.2, 131.5, 143.1, 152.1, 156.6, 161.4 (aromatic carbons), 153.3 (C=O) ppm; MS (EI) *m/z*: 333.3440 [M^+]; Anal. Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.86; H, 4.54; N, 21.01 %; Found: C, 64.97; H, 4.51; N, 21.21 %.

5-(1*H*-benzimidazol-2-yl)-3-(p-nitrophenyl)-1*H*-pyrazole-1-carboxamide (5d)

m. p. 173–175 °C; yield 93%; IR (KBr) (cm^{-1}): 3436, 3325 (NH_2), 3232 (NH), 1570 (C=N), 1331 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.97 (s, 1H, C_4-H), 7.37–8.21 (m, 8H, Ar–H), 8.79 (bs, 2H, NH_2), 10.24 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.9 (C-4), 117.4, 124.6, 125.7, 127.3, 130.9, 140.2, 142.9, 148.7, 151.8, 156.3 (aromatic carbons), 153.1 (C=O) ppm; MS (EI) *m/z*: 348.3156 [M^+]; Anal. Calcd. for $C_{17}H_{12}N_6O_3$: C, 58.62; H, 3.47; N, 24.13; %; Found: C, 58.74; H, 3.49; N, 24.35; %.

5-(1*H*-benzimidazol-2-yl)-3-(p-fluorophenyl)-1*H*-pyrazole-1-carboxamide (5e)

m. p. 168–170 °C; yield 91%; IR (KBr) (cm^{-1}): 3432, 3324 (NH_2), 3233 (NH), 1576 (C=N), 1331 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.95 (s, 1H, C_4–H), 7.35–8.19 (m, 8H, Ar–H), 8.76 (bs, 2H, NH_2), 10.23 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 106.7 (C-4), 117.1, 117.7, 124.9, 129.5, 131.5, 132.4, 143.5, 152.5, 156.9, 163.3 (aromatic carbons), 153.4 (C=O) ppm; MS (EI) *m/z*: 321.3085 [M^+]; Anal. Calcd. for $C_{17}H_{12}FN_5O$: C, 63.55; H, 3.76; N, 21.80 %; Found: C, 63.66; H, 3.79; N, 22.00 %.

5-(1*H*-benzimidazol-2-yl)-3-(p-chlorophenyl)-1*H*-pyrazole-1-carboxamide (5f)

m. p. 164–166 °C; yield 89%; IR (KBr) (cm^{-1}): 3427, 3319 (NH_2), 3217 (NH), 1561 (C=N), 1322 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.93 (s, 1H, C_4–H), 7.34–8.17 (m, 8H, Ar–H), 8.74 (bs, 2H, NH_2), 10.19 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.7 (C-4), 117.2, 124.5, 129.3, 129.1, 130.5, 132.3, 130.8, 131.7, 142.5, 151.8, 156.0 (aromatic carbons), 152.9 (C=O) ppm; MS (EI) *m/z*: 337.7631 [M^+]; Anal. Calcd. for $C_{17}H_{12}ClN_5O$: C, 60.45; H, 3.58; N, 20.73%; Found: C, 60.60; H, 3.59; N, 20.90%.

5-(1*H*-benzimidazol-2-yl)-3-(p-bromophenyl)-1*H*-pyrazole-1-carboxamide (5g)

m. p. 157–159 °C; yield 85%; IR (KBr) (cm^{-1}): 3469, 3362 (NH_2), 3268 (NH), 1602 (C=N), 1363 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.83 (s, 1H, C_4–H), 7.32–8.13 (m, 8H, Ar–H), 8.67 (bs, 2H, NH_2), 10.15 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 107.0 (C-4), 117.8, 124.6, 125.1, 129.7, 130.8, 133.4, 143.7, 152.6, 157.0 (aromatic carbons), 153.2 (C=O) ppm; MS (EI) *m/z*: 344.3888 [M^+]; Anal. Calcd. for $C_{17}H_{12}BrN_5O$: C, 53.42; H, 3.16; N, 18.32%; Found: C, 53.51; H, 3.17; N, 18.52%.

General procedure for the synthesis of 2-(5-(1*H*-benzimidazol-2-yl)-3-aryl-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)oxazole (7a–g)

The compound **5** (0.5 mmol), *p*-fluorophenacyl bromide (**6**) (0.6 mmol), $PdCl_2$ (0.05 mmol), $CuBr_2$ (0.1 mmol), $K_2S_2O_8$ (0.6 mol), $NaHCO_3$ (0.75 mmol), and DCE (1 mL) were added to a Schlenk tube at 120 °C stirred for overnight. After completion of the reaction, the reaction mixture was cooled to room temperature, diluted in diethyl ether, and washed with aqueous $NaHCO_3$ solution. The aqueous phase was re-extracted with diethyl ether. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuum. The solid separated was filtered and purified by column chromatography using ethyl acetate/hexane (1:1) as eluent.

2-(5-(1*H*-benzimidazol-2-yl)-3-phenyl-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)oxazole (7a)

m. p. 176–178 °C; yield 92%; IR (KBr) (cm^{-1}): 3253 (NH), 1648 (C=C), 1589 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.88 (s, 1H, C_4–H), 7.40–8.03 (m, 14H, Ar–H, C_5'-H), 10.13 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.3 (C-4), 116.3, 117.4, 124.2, 127.7, 128.7, 129.6, 130.3, 131.0, 131.7, 134.1, 140.6, 141.3, 142.7, 144.1, 151.8, 155.8, 164.2 (aromatic carbons) ppm; MS (EI) *m/z*: 421.4258 [M^+]; Anal. Calcd. for $C_{25}H_{16}FN_5O$: C, 71.25; H, 3.83; N, 16.62%; Found: C, 71.16; H, 3.81; N, 16.43%.

2-(5-(1*H*-benzimidazol-2-yl)-3-p-tolyl-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)oxazole (7b)

m. p. 161–163 °C; yield 81%; IR (KBr) (cm^{-1}): 3256 (NH), 1651 (C=C), 1591 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.41 (s, 3H, Ar–CH_3), 6.99 (s, 1H, C_4–H), 7.27–8.14 (m, 14H, Ar–H, C_5'-H), 10.10 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.5 (CH_3), 106.0 (C-4), 117.2, 118.3, 125.2, 126.4, 127.5, 132.0, 132.3, 132.5, 133.0, 133.9, 141.4, 141.9, 142.1, 144.3, 152.8, 156.9, 165.0 (aromatic carbons) ppm; MS (EI) *m/z*: 435.1495 [M^+]; Anal. Calcd. for $C_{26}H_{18}FN_5O$: C, 71.71; H, 4.17; N, 16.08%; Found: C, 71.71; H, 4.17; N, 16.08%.

2-(5-(1*H*-benzimidazol-2-yl)-3-(p-methoxyphenyl)-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)oxazole (7c)

m. p. 172–174 °C; yield 88%; IR (KBr) (cm^{-1}): 3262 (NH), 1655 (C=C), 1595 (C=N); ^{1}H NMR (400 MHz, DMSO-

d_6): δ 6.95 (s, 1H, C_4-H), 7.23–8.10 (m, 14H, Ar–H, C_5'–H), 10.08 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.7 (OCH_3), 105.7 (C-4), 115.4, 116.8, 117.9, 124.7, 125.4, 126.9, 128.3, 130.9, 131.6, 140.9, 141.1, 143.3, 145.1, 152.3, 156.3, 161.2, 164.5 (aromatic carbons) ppm; MS (EI) *m/z*: 451.4518 [M^+]; Anal. Calcd. for $C_{26}H_{18}FN_5O_2$: C, 69.17; H, 4.02; N, 15.51 %; Found: C, 69.29; H, 4.05; N, 15.68 %.

2-(5-(1*H*-benzimidazol-2-yl)-3-(p-nitrophenyl)-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)oxazole (7d)

m. p. 221–223 °C; yield 83%; IR (KBr) (cm^{-1}): 3252 (NH), 1646 (C=C), 1587 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.94 (s, 1H, C_4–H), 7.38–8.27 (m, 14H, Ar–H, C_5'–H), 10.21 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.6 (C-4), 116.7, 117.7, 124.6, 125.3, 126.8, 128.1, 130.8, 131.4, 140.9, 141.5, 141.7, 143.1, 149.3, 152.1, 156.2, 164.4 (aromatic carbons) ppm; MS (EI) *m/z*: 344.3888 [M^+]; Anal. Calcd. for $C_{25}H_{15}FN_6O_3$: C, 64.38; H, 3.24; N, 18.02%; Found: C, 64.49; H, 3.21; N, 18.14%.

2-(5-(1*H*-benzimidazol-2-yl)-3-(p-fluorophenyl)-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl)oxazole (7e)

m. p. 214–216 °C; yield 79%; IR (KBr) (cm^{-1}): 3250 (NH), 1644 (C=C), 1586 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.96 (s, 1H, C_4–H), 7.34–8.23 (m, 14H, Ar–H, C_5'–H), 10.19 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.8 (C-4), 116.9, 117.3, 118.0, 124.8, 127.2, 129.3, 131.7, 131.8, 132.3, 134.2, 141.3, 141.9, 143.4, 143.7, 152.4, 156.5, 164.6, 164.7 (aromatic carbons) ppm; MS (EI) *m/z*: 439.4163 [M^+]; Anal. Calcd. for $C_{25}H_{15}F_2N_5O$: C, 68.33; H, 3.44; N, 15.94; Found: C, 68.41; H, 3.42; N, 16.44%.

2-(5-(1*H*-benzimidazol-2-yl)-3-(p-chlorophenyl)-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl) oxazole (7f)

m. p. 205–207 °C; yield 87%; IR (KBr) (cm^{-1}): 3243 (NH), 1642 (C=C), 1581 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.91 (s, 1H, C_4–H), 7.32–8.21 (m, 14H, Ar–H, C_5'–H), 10.17 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.4 (C-4), 116.5, 117.6, 124.4, 127.9, 129.9, 130.6, 131.2, 131.9, 132.3, 135.4, 140.8, 141.5, 142.9, 144.3, 151.9, 156.1, 164.3 (aromatic carbons) ppm; MS (EI) *m/z*: 455.8709 [M^+]; Anal. Calcd. for $C_{25}H_{15}ClFN_5O$: C, 65.87; H, 3.32; N, 15.36%; Found: C, 65.98; H, 3.34; N, 15.57%.

2-(5-(1*H*-benzimidazol-2-yl)-3-(p-bromophenyl)-1*H*-pyrazol-1-yl)-4-(4-fluorophenyl) oxazole (7g)

m. p. 196–198 °C; yield 82%; IR (KBr) (cm^{-1}): 3266 (NH), 1657 (C=C), 1598 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.98 (s, 1H, C_4–H), 7.30–8.18 (m, 14H, Ar–H, C_5'–H), 10.14 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.9 (C-4), 117.1, 118.2, 124.9, 125.1, 127.3, 129.4, 131.8, 131.9, 133.1, 133.4, 141.7, 142.0, 143.5, 144.1, 152.6, 156.7, 164.8 (aromatic carbons) ppm; MS (EI) *m/z*: 500.3219 [M^+]; Anal. Calcd. for $C_{25}H_{15}BrFN_5O$: C, 60.01; H, 3.02; N, 14.00 %; Found: C, C, 60.13; H, 3.04; N, 14.22%.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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