Original Article

Synthesis Of Novel Benzimidazole-Pyrazoline Hybrid Molecules As Antibacterial And Anticancer Agent

Gopal Krishna Padhy[1*],Deepankar Rath[2], Pallishree Bhukta[3], Ladi Alik Kumar[4], Kamini Sethy[5], Jagadeesh Panda[6], Saroj K. Raul[7],Ajaya K. Behera[8]

[1*]School of Pharmacy, Centurion University of Technology and Management, Odisha, India;
[2]School of Pharmacy, Centurion University of Technology and Management, Odisha, India;
[3]School of Pharmacy, Centurion University of Technology and Management, Odisha, India;
[4]School of Pharmacy, Centurion University of Technology and Management, Odisha, India;
[5]School of Pharmacy, Centurion University of Technology and Management, Odisha, India;
[6]Raghu College of Pharmacy, Dakamarri, Visakhapatnam 531162, India;
[7]Maharajah's College of Pharmacy, Phool Baugh, Vizianagaram 535002, India;
[8]Organic Synthesis Laboratory, School of Chemistry, Sambalpur University, JyotiVihar, Burla 768019, India.

***Corresponding Author:** Gopal Krishna Padhy

*School of Pharmacy, Centurion University of Technology and Management, Odisha, India,
Email: gopalmedchem@gmail.com;gopalkrishna.padhy@cutm.ac.in
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Abstract

Appearance of resistance to available antibacterial and anticancer drugs became a major challenge in the recent time. Hence, finding of new antibacterial and anticancer compounds become highly essential. Pyrazolines and benzimidazoles were reported to have potent antibacterial and anticancer activity. Therefore,a hybrid tactic has been used, combining the core structure of tentatively active benzimidazole and pyrazoline moiety in to a single molecule. Targeted compounds were obtained by the cyclizing N-substituted benzimidazole chalcones with hydrazine hydrate. The structure of compounds were characterized by taking the help of spectroscopic techniques like IR, ^{1}H, ^{13}C-NMR and mass. Compounds were evaluated for their growth inhibitory potential against human breast cancer cell line MDA-MB-231. Furthermore, they were also evaluated against gram positive and negative bacteria. Compounds **4a** (GI_{50} = 26.13μM) and 4c (GI_{50}=12.27μM) found to possess significant anticancer activity. Similarly, Compound **5a** was significantly active against Staphylococcus aureus at a concentration of 62.5 μg mL^{-1}.

Keywords: Chalcone, Benzimidazole, Pyrazoline, Anticancer, SRB assay, Antibacterial.

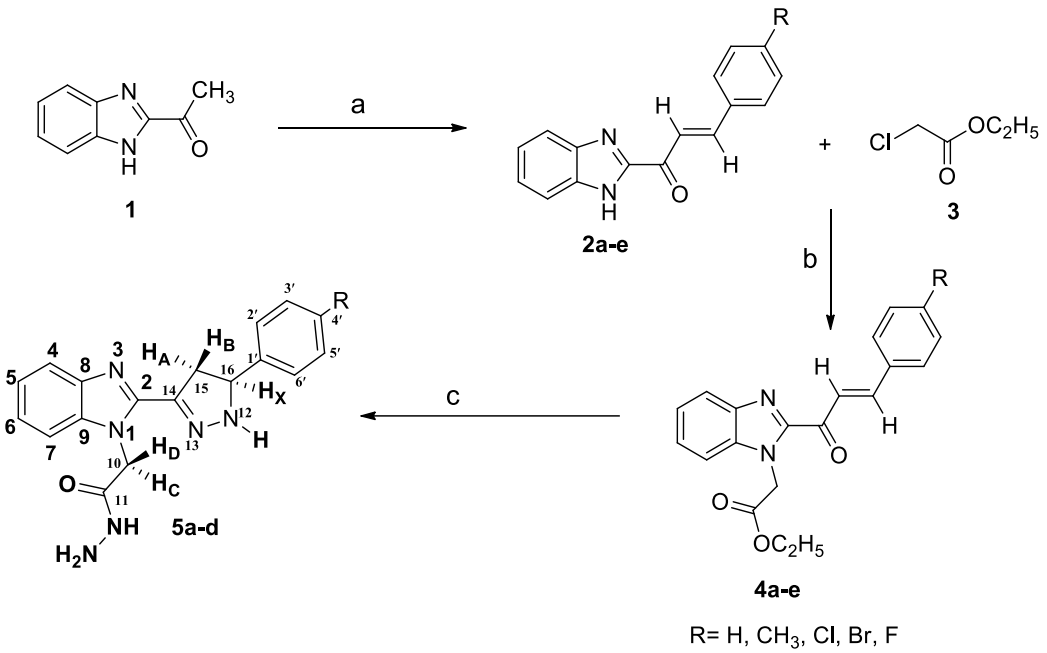
INTRODUCTION

Benzimidazoles are very valuable for the progress of molecules of therapeutic interest. It has been found that derivatives of benzimidazole have numerous therapeutic applications, including antibacterial, [1-2]anticancer, [3-5]antiviral [6] and anti-inflammatory.[7] As a result of their promising biological activities and important properties, [8-12] acid hydrazides have garnered an increasing amount of research interest. Furthermore, pyrazoline derivatives have been well documented in terms of their medicinal properties. [13-15] Thus, in the current study novel 1H-benzimidazoleacetohydrazide incorporated pyrazoline derivatives were synthesized and screened for their antibacterial and anticancer activity.

RESULTS AND DISCUSSION

Chemistry:

The desired compounds are obtained as mentioned in **scheme-1**. o-phenylenediamine and lactic acid were used to prepare 2-Acetylbenzimidazole (**1**),as per the reported method.[16]The required chalcone synthons **(2a-e)** were obtained by claisen-schmidt reaction of 2-acetylbezimidazole with substituted benzaldehydes in presence base.[17] The syntheses of novel N-l substituted benzimidazole chalocone esters (**4a-e**) were carried out by nucleophilic substitution reactions of 1H-Benzimidazole chalcones (**2a-e**) with ethyl chloroacetate (3) in presence of anhydrous K_2CO_3 and acetone. Acid hydrazides of benzimidazole linked pyrazolines (**5a-e**) are prepared by reacting benzimidazole chalcone ester (**4a-e**) with hydrazine hydrate in absolute ethanol. In this reaction the ester underwent hydrazinolysis to form hydrazide and the α, β-unsaturated keto system cyclised to form pyrazoline.



Scheme-1: (a) Ar-CHO, 10% NaOH, ethanol, stir (b) Dry acetone, K_2CO_3, reflux (c) Hydrazine hydrate 95%, alcohol, reflux

Spectral studies

The purity of the compounds were examined by TLC. ^{1}H NMR, C^{13} NMR, Mass and IR spectra of all the compounds were logged and found in full promise with the planned structures. IR data were highly informative and entirely reinforced the anticipated compounds. In chalcones **4a-e**, IR spectra demonstrated stretching of unsaturated keto system at 1656-1657 cm^{-1}, which was within the anticipated region. At 1741-1744 cm^{-1}, the carbonyl stretching was observed for the ester group. In addition, there are (C=N) stretching band at 1625-1659cm^{-1}in compounds 5**a-d**, which gave conclusive evidence of transformation of chalcone to the pyrazoline. The compounds **5a-d** also showed typical absorption bands at 3429-3298 & 3213-3219 cm^{-1} due to (-NH-NH$_2$).

In the ^{1}H NMR spectrum of compounds **4a-e**, we observed two doublets by coupling constant amid 16–20 Hz indicating the olefinic protons in E form. As evidenced by the occurrence of peak at δ 182.23-182.34 matching to Carbonyl carbon in the ^{13}C NMR spectrum of **4a-e**, the chalcones have an unsaturated carbonyl system.

Spectral studies have unambiguously assigned structures to the compounds **5a-d**, although they are racemates. Compounds **5a-d** exhibited an ABX pattern for the hydrogens of the pyrazoline ring in their ^{1}H NMR spectra. The stereochemical properties of the protons H_A, H_B & H_X have been established from the scrutiny of J value. H_A and H_X were found to be vicinal coupled at 10-10.5 Hz (J_{AX}), which indicates that these protons are in cis configuration, whereas H_B and H_X are trans coupled at 11-12.5 Hz (J_{BX}). J_{AB} value of 16.5-17.5 Hz between H_A and H_B is indicative of their germinal placement at C-4. Geminal coupling with each other and vicinal coupling with the H_X proton affected the CH_2 protons of pyrazoline, which looked as a pair of doublets of doublets at δ 3.02-3.04 ppm (H_A) and δ 3.62-3.67 ppm (H_B), respectively. As a result of vicinal coupling with the two methylene and amino protons of the pyrazoline ring, the H_X proton appeared as a triplet of doublets at δ 4.85-4.91 ppm. The acetohydrazide methylene protons on geminal coupling exhibited two distinct doublets at δ 5.3-6.08 (H_C) and δ 5.28-5.31(H_D). The amino proton of pyrazoline appeared as doublet (J = 2 Hz) at δ 8.18-8.25 due to coupling with H_X proton. For the NH-NH$_2$ protons, compounds 5a-d had two singlets that were δ 9.31-9.32 ppm and δ 4.27-4.28 ppm. These findings were further established by the presence of three sets of signals in the ^{13}C NMR spectra of pyrazolines at δ 42.18-42.21 ppm (C4 of pyrazoline), δ 42.36-46.30 ppm (CH_2 of acetohydrazide) and δ 62.44-63.21 ppm (C5 of pyrazoline).Further peaks detected were in promise with the benzimidazole ring and aromatic substituents. Additionally, the proposed structures were supported by elemental analyses and mass spectra.

Antibacterial activity

The synthesized compounds **4a-e** and **5a-d** were assessed for their in vitro antibacterial activity. Antibacterial activities of newly synthesised compounds are presented in **Table 1**. The synthesized compounds showed feebler antibacterial activities in comparison to ciprofloxacin. The minimum inhibitory concentrations of the compounds extended in between 125–500µg mL^{-1} for the chalcones **4a-e** and 62.5–500µg mL^{-1} for the pyrazolines **5a-d**. Amidst the compounds tested, compound **5a** showed significant activity (62.5 µg mL^{-1}) against S. aureus.

Table 1: In vitro antibacterial activity of compounds 4a-e and 5a-d(µg mL^{-1})

	Gram-positive bacteria		Gram-negative bacteria	
Compound	S. aureus	B. subtilis	E. coli	P. aeruginosa
4a	>500	125	500	> 500
4b	250	500	> 500	>500
4c	250	> 500	> 500	> 500
4d	500	250	> 500	500
4e	250	500	500	>500
5a	62.5	500	125	250
5b	125	500	500	500
5c	125	500	> 500	> 500
5d	125	250	250	250
Ciprofloxacin	6.25	6.25	6.25	12.5

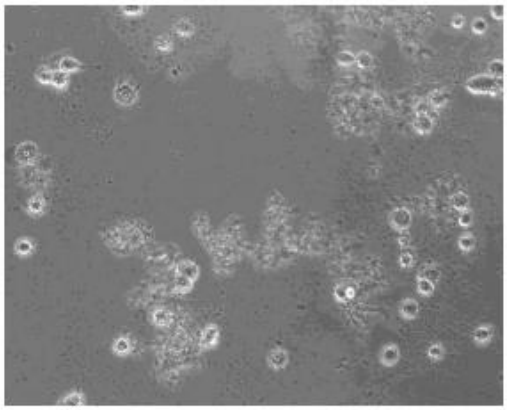
Anticancer activity

The in vitro anticancer activities (cell viability assay) of selected compounds were screened by SRB assay against Human breast cancer cell line MDA-MB-231. Compounds **4a** (GI_{50} = 26.13 µM) and **4c** (GI_{50} = 12.27µM) clearly inhibited the proliferation of Human breast cancer cell line MDA-MB-23 as shown in (**Figure 1**). All the other compounds found inactive (GI_{50}>100 µM) as compared to standard drug adriamycin. The results obtained from cytotoxicity testing are represented in (**Table 2**).

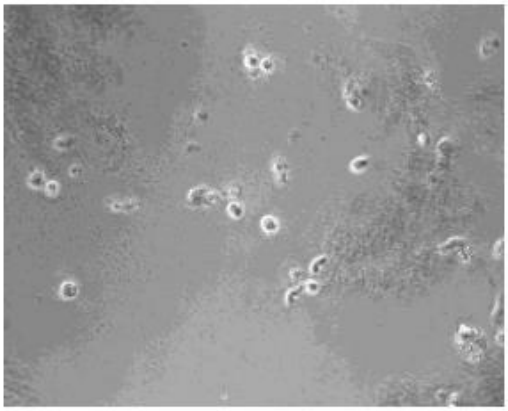
Table 2: In vitro anticancer activity of compounds 4a, 4c, 5a, 5b and 5d (GI_{50} µM)

Compound	GI_{50} (µM)
4a	26.13
4c	12.27
5a	> 100
5b	> 100
5d	> 100

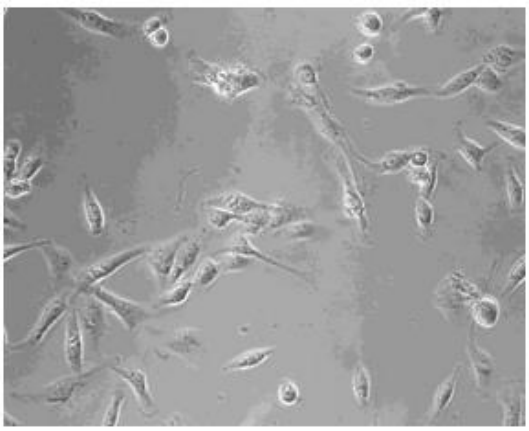
Positive control Adriamycin (GI_{50}= 0.04 µM)



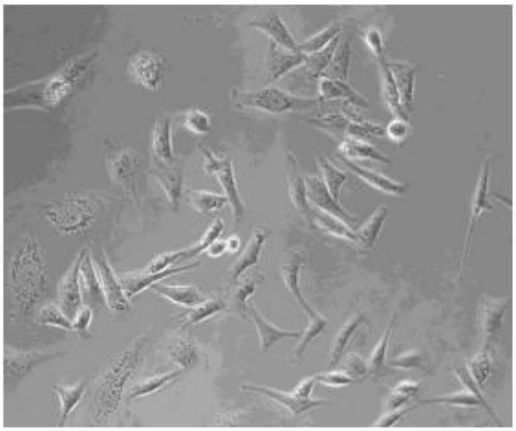
Compound 4a



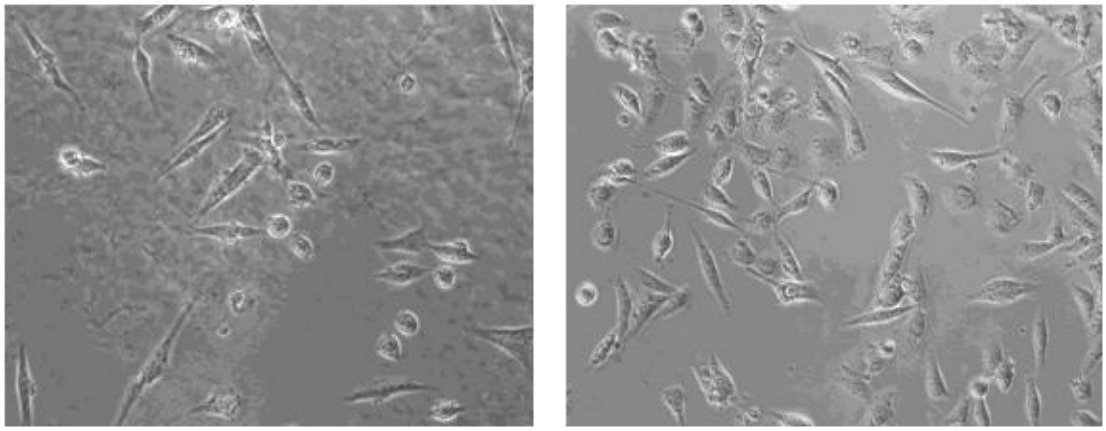
Compound 4c



Compound 5a



Compound 5b



Compound 5d **Negative control (DMSO)**

Figure 1: Images of Phase Contrast Microscopy. Cells were treated with 100 µM for 48 h. Negative Control Included cells treated with DMSO.

EXPERIMENTAL

Materials and Instrumentation

Merck (India) and Finar (India) provided the chemicals used. On AVANCE III 500 MHz (AV 500) spectrometer (Bruker), ^{1}H and ^{13}C NMR spectra were documented. ALPHA-T Fourier-transform IR spectrometer (Bruker) was used to measure the IR spectra. Mass spectra were determined using an 6410 LC-MS Spectrometer (Agilent) or 410 Prostar Binary LC-MS (Varian Inc.). Melting points were recorded by the open tube capillary method and are uncorrected. TLC under iodine vapors/UV light monitored the completion of the reaction.

Synthesis of Ethyl 2-(2-(4-(substituted phenyl)acryloyl)-1H-benzo[d]imidazol-1-yl)acetate (4a-e)

In 40 mL of dry acetone, 10 mmol of benzimidazole chalcone **3a-e** was dissolved, followed by the addition of 15 mmol of anhydrous K_2CO_3.The mixture was then added with ethyl chloroacetate (20 mmol) and heated under reflux. Using TLC (ethyl acetate-benzene, 1:8), the progress of the reaction was observed. Reaction mixture was cooled and added into crushed ice after it had finished reacting (12-14 hours).Afterwards, the solid obtained was filtered and recrystallized in rectified spirit.

Ethyl 2-(2-cinnamoyl-1H-benzo[d]imidazol-1-yl)acetate(4a)

Cream colour solid;m.p.112-114 ^{o}C; Yield: 76%; FTIR (KBr) cm^{-1}1741 (C=O), 1656 (C=O), 1595 (C=N);^{1}H-NMR (DMSO d_6):8.19 (1H, d, J= 20 Hz, H_α), 7.93 (1H, d, J= 8.0 Hz, Ar–H), 7.90-7.84 (4H, m, H_β, Ar-H), 7.51-7.48 (4H, m, Ar–H), 7.44-7.40 (1H, m, Ar-H), 5.52 (2H, s, –CH2-) 4.19 (2H, q, J= 7.25 Hz , -O$\underline{CH_2}$-CH_3), 1,24 (3H, t, J= 7.25 Hz , -OCH_2-$\underline{CH_3}$); ^{13}C NMR (DMSO d6):182.34, 168.67, 147.10, 144.78, 141.52, 137.19, 134.68, 131.63, 129.64, 129.43, 126.61, 124.37, 122.76, 121.85, 111.97, 61.61, 47.29, 14.54; mass m/z: 335.0 [M+1]

Ethyl 2-(2-(3-(p-tolyl)acryloyl)-1H-benzo[d]imidazol-1-yl)acetate(4b)

Buff colour solid;m.p.140-142 ^{0}C, Yield: 70%; FTIR (KBr) cm^{-1}1744 (C=O), 1656 (C=O), 1593 (C=N);^{1}H-NMR (DMSOd_6):8.14 (1H, d, J= 16.5 Hz, H_α), 7.92 (1H, d, J= 8.0 Hz, Ar–H), 7.87-7.84 (2H, m, H_β, Ar-H), 7.76 (2H, d, J= 8.0 Hz, Ar–H), 7.51-7.48 (1H, m, Ar-H), 7.43-7.40 (1H, m, Ar-H), 7.31(2H, d, J= 8.0 Hz, Ar–H), 5.51 (2H, s, –CH_2-) 4.18 (2H, q, J= 7.25 Hz , -O$\underline{CH_2}$-CH_3), 2.37 (3H, s, –CH_3) 1.23 (3H, t, J= 7.25 Hz , -OCH_2-$\underline{CH_3}$);^{13}C NMR (DMSO d6): 182.33, 168.68, 144.85, 141.88, 141.52, 137.18, 132.00, 130.27, 129.48, 126.53, 124.32, 121.81, 121.74, 111.95, 61.59, 47.28, 21.61, 14.54; mass m/z: 349.0 [M+1]

Ethyl 2-(2-(3-(4-chlorophenyl)acryloyl)-1H-benzo[d]imidazol-1-yl)acetate(4c)

Buff colour solid; m.p.180-182^{0}C, Yield: 80%; FTIR (KBr) cm^{-1}1743 (C=O), 1657 (C=O), 1595 (C=N);^{1}H-NMR (DMSO d_6):8.19 (1H, d, J= 16.0 Hz, H_α), 7.93-7.84 (5H, m, Ar–H), 7.55 (1H, d, J= 8.5 Hz, Ar-H), 7.52-7.48 (1H, m, Ar-H), 7.43-7.40 (1H, m, Ar-H), 5.52 (2H, s, –CH_2) 4.18 (2H, q, J= 7.0 Hz , -O$\underline{CH_2}$-CH_3), 2.37 (3H, s, –CH_3) 1.23 (3H, t, J= 7.0 Hz , -OCH_2-$\underline{CH_3}$); ^{13}C NMR (DMSO d6): 182.23, 168.66, 147.03, 143.28, 141.52, 137.20, 136.09, 133.65, 131.13, 129.66, 126.67, 124.39, 123.47, 121.86, 112.00, 61.61, 47.28, 14.55; mass m/z: 369.0 [M+1]

Ethyl 2-(2-(3-(4-bromophenyl)acryloyl)-1H-benzo[d]imidazol-1-yl)acetate(4d)

Buff colour solid; m.p.170-172 ^{0}C, Yield: 80%; FTIR (KBr) cm^{-1}1743 (C=O), 1656 (C=O), 1595 (C=N);^{1}H-NMR (DMSO d_6):8.21 (1H, d, J= 16.0 Hz, H_α), 7.93 (1H, d, J= 8.0 Hz, Ar-H) 7.87-7.81 (4H, m, H_β, Ar–H), 7.69 (1H, d, J= 8.5 Hz, Ar-H), 7.50 (1H, t, J= 7.25 Hz, Ar-H), 7.24 (1H, t, J= 7.25,Ar-H), 5.52 (2H, s, –CH_2-), 4.18 (2H, q, J= 7.0 Hz , -O$\underline{CH_2}$-CH_3), 1.23 (3H, t, J= 7.25 Hz , -OCH_2-$\underline{CH_3}$); mass m/z: 383.1 [M+1]

Ethyl 2-(2-(3-(4-fluorophenyl)acryloyl)-1H-benzo[d]imidazol-1-yl)acetate(4e)
Cream colour solid;m.p.152-154 C, Yield: 75%; FTIR (KBr) cm^{-1}1744 (C=O), 1657 (C=O), 1589 (C=N) ; ^{1}H-NMR (DMSO d_6):8.14 (1H, d, J= 16.0 Hz, H_α), 7.97-7.94 (2H, m, Ar–H), 7.92 (1H, d, J= 8.5 Hz, Ar-H), 7.88 (1H, d, J= 16.6 Hz, H_β), 7.85 (1H, d, J= 8.5 Hz, Ar-H), 7.50 (1H, t, Ar-H), 7.42 (1H, t, Ar-H), 7.348-7.313 (2H,m, Ar-H), 5.51 (2H, s, –CH_2-), 4.18 (2H, q, J= 7.0 Hz , -O$\underline{CH_2}$-CH_3), 1.23 (3H, t, J= 7.0 Hz , -OCH_2-$\underline{CH_3}$); ^{13}C NMR (DMSO d6): 182.29, 168.66, 165.17, 163.18, 147.08, 143.57, 141.52, 137.19 131.93, 131.86, 131.42, 131.39, 126.60, 124.36, 122.68, 121.84, 116.76, 116.58, 111.98, 61.60, 47.28, 14.54; mass m/z: 369.0 [M+1].

Synthesis of 2-(2-(5-(substituted phenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-1H-benzo[d] imidazole-1-yl) acetohydrazide (5a-d)
Benzimidazole chalcone ester **4a-e** (2 mmol) was dissolved in ethanol (15 ml) and hydrazine hydrate (4 mmol) was added dropwise. TLC was used to monitor the progress of the reaction (ethyl acetate-benzene, 1:3). After the reaction was completed (4-5 hours), the resultant mixture was cooled. Precipitate obtained was filtered and washed with rectified spirit.

2-(2-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-benzo[d]imidazol-1-yl)acetohydrazide (5a)
Colourless solid; Yield: 80 %;m.p.: 226-228 ºC; FTIR (KBr) cm^{-1} 3309 & 3213 (NH-NH_2), 1658 (C=O), 1625 (C=N); ^{1}H-NMR (DMSO-d_6): 9.32 (1H, s, –N$\underline{H}$-NH_2), 8.23 (1H, d, J = 5 Hz, -NH of pyrazoline) 7.63 (1H, d,J = 8 Hz, H-4), 7.42-7.34 (5H, m, H-7, H-2′, H-6′, H-3′ and H-5′), 7.30-7.19 (3H, m, H-4′, H-5 and H-6), 5.36 (1H, d, J_{DC} = 17.5 Hz , H_D), 5.29 (1H, d, J_{CD} = 17.5 Hz , H_C), 4.89 (1H, td, J = 5 Hz, J= 10 Hz, H_X), 4.27 (2H, s, –NH-N$\underline{H}_2$), 3.65 (1H, dd, J_{BX} = 12.5, J_{AB} = 17.5Hz, H_B) 3.04 (1H, dd, J_{AX} = 10.0, J_{AB} = 20.0 Hz, H_A) ; ^{13}C NMR (DMSO d_6): 166.97 (C11), 146.93 (C2), 142.98 (C1′), 142.87 (C14), 142.48 (C8), 137.24 (C9), 128.97 (C3′ and C5′), 127.79 (C4′), 127.06 (C2′ and C6′), 123.41 (C6), 122.45 (C5), 119.54 (C4), 110.60 (C7), 63.21 (C16 pyrazoline), 46.39 (C10), 42.20 (C15 pyrazoline); mass m/z: 335.0 [M+H].

2-(2-(5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)-1H-benzo[d]imidazol-1-yl)acetohydrazide(5b)
Colourless solid; Yield: 86%;m.p. 222-224 º C; FTIR (KBr) cm^{-1} 3219 (br, NH-NH_2), 1658 (C=O), 1625 (C=N); ^{1}H-NMR (DMSO-d_6):9.32 (1H, s, –N$\underline{H}$-NH_2), 8.18 (1H, d, J = 3.0 Hz, -NH of pyrazoline) 7.64 (1H, d, J = 7.5 Hz, H-4), 7.42 (1H, d, J = 8 Hz, H-7) 7.28-7.22 (6H, m, H-2′, H-6′, H-5, H-6, H-3′ and H-5′), 5.37 (1H, d, J_{DC} = 17 Hz , H_D), 5.29 (1H, d, J_{CD} = 16.5 Hz, H_C), 4.85 (1H, td, J = 11 Hz , J = 2.5 Hz, H_X), 4.27 (2H, s, –NH-N$\underline{H}_2$), 3.62 (1H, dd, J_{BX} = 11, J_{AB} = 16.5Hz, H_B) 3.02 (1H, dd, J_{AX} = 10.5, J_{AB} = 16.5 Hz, H_A), 2.36 (3H, s, –CH_3); 13C NMR (DMSO d_6): 166.97 (C11), 146.95 (C2), 142.87 (C14 pyrazoline), 142.46 (C8), 139.97 (C1′), 137.23 (C9), 136.89 (C4′), 129.49 (C3′ and C5′), 126.97 (C2′ and C6′), 123.38 (C6), 122.43 (C5), 119.52 (C4), 110.59 (C7), 62.98 (C16 pyrazoline), 46.36 (C10), 42.19 (C15 pyrazoline); mass m/z: 349.0 [M+H].

2-(2-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-1H-benzo[d]imidazol-1-yl)aceto- hydrazide (5c)
Colourless solid;Yield:62.78 %;m.p. 227-229 ºC; FTIR (KBr) cm^{-1} 3298 (br, NH-NH_2), 1659 (C=O), 1620 (C=N); ^{1}H-NMR (DMSO-d_6): 9.32 (1H, s, –N$\underline{H}$-NH_2), 8.25 (1H, d, J = 2.0 Hz, -NH of pyrazoline) 7.64 (1H, d, J = 8 Hz, H-4), 7.43-7.39 (5H, m, H-7, H-2′, H-6′, H-3′ and H-5′), 7.28-7.19 (2H, m, H-5 and H-6), 5.36 (1H, d, J_{DC} = 16.5 Hz , H_D), 5.31 (1H, d, J_{CD} = 17 Hz , H_C), 4.91 (1H, td, J = 10.7 Hz , J = 2.3, H_X), 4.27 (2H, s, –NH-N$\underline{H}_2$), 3.67 (1H, dd, J_{BX} = 11.2, J_{AB} = 16.7Hz, H_B) 3.02 (1H, dd, J_{AX} = 10.2, J_{AB} = 16.7 Hz, H_A); ^{13}C NMR (DMSO d_6): 166.93 (C11), 146.80 (C2), 142.84 (C14 pyrazoline), 142.61 (C8), 142.03 (C1′), 137.22 (C9), 132.29 (C4′), 128.98 (C2′ and C6′), 128.91 (C3′ and C5′), 123.46 (C6), 122.47 (C5), 119.55 (C4), 110.62 (C7), 62.44 (C16 pyrazoline), 46.38 (C10), 42.21 (C15 pyrazoline); mass m/z: 368.5 [M+H].

2-(2-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-1H-benzo[d]imidazol-1-yl)aceto- hydrazide (5d)
Colourless solid; Yield: 84 %; m.p. 216-218 ºC; FTIR (KBr) cm^{-1}3429 & 3297 (NH-NH_2), 1657 (C=O), 1620 (C=N); ^{1}H-NMR (DMSO-d_6):9.31 (1H, s, –$\underline{NH}$-NH_2), 8.25 (1H, d, J= 2.5 Hz, -NH of pyrazoline) 7.64 (1H, d, 7.5 Hz, H-4), 7.56 (2H, d, J = 8.5 Hz,H-2′ and H-6′), 7.42 (1H, d, J = 8 Hz, H-7), 7.35 (2H, d, J=8.5 Hz, H-3′ and H-5′), 7.25-7.19 (2H, m, H-5 and H-6), 5.36 (1H, d, J_{DC} = 16.5 Hz, H_D), 5.28 (1H, d, J_{CD} = 16.5 Hz , H_C), 4.89 (1H, td, J = 10.5 Hz , J = 2.5, H_X), 4.28 (2H, s, –NH-N$\underline{H}_2$), 3.67 (1H, dd, J_{BX} = 11.5, J_{AB} = 16.5 Hz, H_B) 3.02 (1H, dd, J_{AX} = 10.0, J_{AB} = 16.5 Hz, H_A); ^{13}C NMR (DMSO d_6): 166.93 (C11), 146.79 (C2), 142.84 (C14 pyrazoline), 142.61 (C8), 142.46 (C1′), 137.22 (C9), 131.83 (C3′ and C5′), 129.35 (C2′ and C6′), 123.47 (C6), 122.48 (C5), 120.79 (C4′), 119.56 (C4), 110.62 (C7), 62.49 (C16 pyrazoline), 46.39 (C10), 42.18 (C15 pyrazoline); mass m/z: 412.9 [M+H].

Antibacterial activity
In vitro antimicrobial activity of the compounds was evaluated against four bacterial strains, including Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. IMTECH, Chandigarh, India-160036, provided the cultures. A two-fold serial dilution method was used to determine MICs using nutrient broth.[18]After incubation for 24 hours at 35°C, the growth was watched. Positive control was Ciprofloxacin, while negative control was DMSO. The antibacterial activity was performed in duplicate.

Anticancer activity

The SRB assay[19] was used to determine the in vitro cytotoxicity (cell viability assay) against the human breast cancer cell line MDA-MB-231.In RPMI 1640 medium with 2 mM L-glutamine and 10% fetal bovine serum, the cell line was grown. Cells were seeded into 96-well microtiter plates in 90 mL medium at 5000 cells per well for 24 hours at 37°C with 5% CO_2 and 95% air. The plate was then incubated for an additional 48 hours after the addition of drugs (0.1–100 µM).Then, 25 mL of 10% tri chloroacetic acid was added to the culture plate and the cells were incubated at 4°C for 60 minutes. The plate was washed five times with tap water after discarding the supernatant. As a last step, 50 mL of sulforhodamine solution was added to individual well for staining. Stained cells were then dissolved in 10 mM trizma base and the absorbance was determined at 515 nm. % viability was determined for each compound at individual concentration using the formula: (Absorbance of Test/Absorbance of control) × 100. The GI_{50} (Concentration required to cause 50% inhibition in growth) for the synthesized compounds were calculated using GraphPad Prism v.4.03 software.

CONCLUSION

Nine novel benzimidazole derivatives having chalcone or pyrazoline motif were synthesized starting from 2-Acetylbenzimidazole. The compounds were evaluated for their antibacterial and anticancer activity. The chalcone derivatives were found to be moderately active in inhibiting the growth of bacteria. More over chalcone **4c**, significantly affected the proliferation of cancer cell line at a concentration of 12.27µM.The pyrazoline derivative **5a** was found to be significantly active against Gram positive bacteria. However, pyrazolines were found to be inactive during anticancer study.

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