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Design, synthesis and biological profile of heterocyclic benzimidazole analogues as prospective antimicrobial and antiproliferative agents

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Abstract

Background: Nitrogen containing heterocycles are widely used and investigated by pharmaceutical industry, as they are important in discovery and designing of new drug molecules. Drugs with a benzimidazole nucleus possess exclusive structural features and electron-rich atmosphere, which enable them to bind to a number of biologically important targets and result in a wide range of activities. This has served as the basis of the present study whereby new scaffolds with benzimidazole moiety were designed and synthesized.

Methods: The structures of synthesized compounds were confirmed by physicochemical and spectral means. The synthesized compounds were screened for their antimicrobial and antiproliferative activities by tube dilution and Sulforhodamine B (SRB) assays, respectively.

Results and conclusion: The in vitro biological screening results revealed that compound **Z24** exhibited promising antimicrobial and anticancer activities which are comparable to standards.

Keywords: Antibacterial, Antifungal, Anticancer, 2-Mercaptobenzimidazole, SAR

Introduction

The increased incidences of drug resistance caused by extensive use of antibiotics and immunosuppressive drugs have emerged as a key issue in treatment of microbial infections. There is, therefore, a need for search of efficient, less toxic and structurally new molecules for treatment of these diseases. The discovery and development of drugs with benzimidazole moiety is now an important and attractive subject of interest given their huge therapeutic values [1]. Heterocyclic compounds offer a high degree of structural diversity and have proven to be broadly and economically useful as therapeutic agents like quinoline-branched amines and dimers [2], 8-substituted quinolines [3], 2,5- and 4,5-dihydroisoxazole [4].

The structural isosters of nucleotides, benzimidazole and heterocycles, which interact with biopolymer through the fused heterocyclic nuclei in their structure, may possess potential activity of chemotherapeutics with lower toxicity. It is well established that heterocyclic compounds with nitrogen and sulphur exhibit a wide scope of biological activities. 2-Mercaptobenzimidazole, for example, has been reported for their wide range of pharmacological and clinical applications [5]. On the other hand, azomethine group which is present in various natural and non-natural compounds, is also an important scaffold critical for biological activity of Schiff bases. Owing to their broad spectrum of biological profile, Schiff bases derived from benzimidazole compounds are extensively studied [6].

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Colorectal cancer (CRC) is one of the most common malignancies and a noteworthy reason for growth related mortality around the world. According to World Health Organization (WHO), CRC is the third most regular malignancy, with 1,361,000 cases worldwide. Unfortunately, about 25% of CRC cases are only identified at stage IV (with far off metastases) and nearly half of CRC patients suffered from metastasis in the midst of their lifetime. The treatment outcomes for these patients remain inauspicious whilst approximately half of the patients responded to traditional chemotherapy, most encountered resistance at some stage of treatment, and reoccurrence of the tumors regularly take after. This could be due to cancer stem cells (CSCs) which give rise to heterogeneity within and between tumors [7].

In recent years, remarkable attention has been directed towards the advancement of benzimidazole heterocyclic molecules as antihistaminic (H_1-receptor antagonist, e.g. bilastine, 5-HT_3 antagonist, e.g. lerisetron) [8], antimicrobial (antibiotic, e.g. ridinilazole) [9, 10], antiulcer (proton pump inhibitor (PPI), e.g. ilaprazole) [11, 12], antihypertensive (calcium channel blocker, e.g. mibefradil) [13], antiviral (non-structural protein inhibitor (NS5A), e.g. samatasvir) [14, 15], antiparasitic (specifically anthelmintic, e.g. flubendazole) [16], antipsychotic (D_2 receptor antagonist, e.g. clopimozide) [17], analgesic (opioid analgesic, e.g. clonitazene) [18], phosphodiesterase inhibitor (PDE3 inhibitor e.g. adibendan) [19, 20] and anticancer (aromatase inhibitor, e.g. liarozole, histone deacetylase inhibitor (HDAC), e.g. pracinostat) [21, 22] agents (Fig. 1).

Prompted by the aforementioned facts and literature on pharmacologically active heterocyclic benzimidazole nucleus (as reviewed in Fig. 2), the present work aimed to synthesize a new series of benzimidazole compounds and evaluate their biological activities.

Results and discussion

Chemistry

The synthesis of benzimidazole derivatives (**Z1**–**Z30**) by multistep procedure was shown in Scheme 1. The 1*H*-benzo[*d*]imidazol-2-yl 2-chloroethanethioate (intermediate-**i**) was synthesized by the reaction of chloroacetyl chloride with 2-mercaptobenzimidazole, which on further reaction with corresponding anilines in presence of ethanolic solvent yielded the title compounds (**Z1**–**Z15**). The reaction of above synthesized intermediate-**i** with hydrazine hydrate yielded intermediate-**ii**. The intermediate-**ii** on reaction with substituted aldehydes in ethanol resulted in development of title compounds (**Z16**–**Z30**) with appreciable yields. The physicochemical properties of compounds (**Z1**–**Z30**) are shown in Table 1.

Spectral characterization data

The assigned molecular scaffolds of the benzimidazole derivatives were authenticated by Infrared (IR), Nuclear Magnetic Resonance (NMR) (proton, carbon), Mass spectrometry (MS) and elemental analysis. The spectroanalytical data has been presented in Table 2. The IR spectra of compounds exhibited the characteristic secondary (–C=N–) absorption bands around 1345–1254 cm^{-1} while the tertiary (–C=N–) bands was observed at 1386–1337 cm^{-1}. Appearance of IR stretching vibrations at 1600 and 1450 cm^{-1} in the spectra of compounds showed the presence of aromatic –C=C– and the peaks at 3112–3068 cm^{-1}. The N–N peak in the spectra of synthesized derivatives was observed at 1168–1163 cm^{-1}. The presence of ketonic stretching vibrations was observed at 1732–1698 cm^{-1}. In the synthesized derivatives, the methylene group (–CH_2–) showed the vibrations at 2935–2915 cm^{-1} and 2884–2865 cm^{-1}. The compounds (**Z1**–**Z3**, **Z6**, **Z10** and **Z16**–**Z18**) showed the characteristic NO_2 group stretching vibrations in the range of 1512–1484 cm^{-1}. The compounds (**Z4**, **Z5**, **Z21** and **Z24**) exhibited the peaks of Br at 700–600 cm^{-1} while compounds (**Z6**–**Z8**, **Z10**, **Z27** and **Z28**) showed peaks in the range of 744–738 cm^{-1} and the fluorine group indicated its peak at 1012–1007 cm^{-1}. The methyl group present in the compounds (**Z9**, **Z13** and **Z20**) showed the bands at 2948–2870 cm^{-1}. The N–CH_3 stretching band in the compounds (**Z14**, **Z15** and **Z26**) was observed at 2857–2843 cm^{-1}. The phenolic group present in the compounds (**Z19**, **Z20** and **Z22**) showed its peaks in the range of 3648–3645 cm^{-1}. The aldehydic group was confirmed by the appearance of absorption bands at 1728 cm^{-1} in the compound **Z25**. The presence of OCH_3 in compounds (**Z19**, **Z20**, **Z23** and **Z30**) was observed at peaks in the range of 2839–2818 cm^{-1}. The ^{1}H-NMR spectra of the synthesized compounds have been recorded in dimethyl sulfoxide (DMSO) solvent. Multiplet signals at 6.62–8.66 δ ppm indicated the aromatic protons. The presence of singlet signal at 1.04–2.12 δ ppm indicated the presence of NH group while the singlet signal at 5.61–7.18 δ ppm confirmed the presence of NH group in imidazole ring. The appearance of singlet signals at 2.00–3.82 δ ppm indicated the presence of –CH_2 in the compounds. The appearance of singlet signal at 1.21–3.63 δ ppm indicated the presence of CH_3 in **Z9**, **Z13**–**Z15**, **Z20** and **Z26**. The singlet signal at 3.59–3.75 δ ppm confirmed the appearance of OCH_3 group in the compounds (**Z19**, **Z23** and **Z30**) while the singlet signal at 1.21 δ ppm indicated the presence of OC_2H-$_5$ in compound **Z20**. ^{13}C-NMR spectral analyses exhibit the appearance of the carbon atoms in synthesized molecular structures is shown in experimental section. The elemental analyses of compounds were around ±0.3% of the theoretical results.

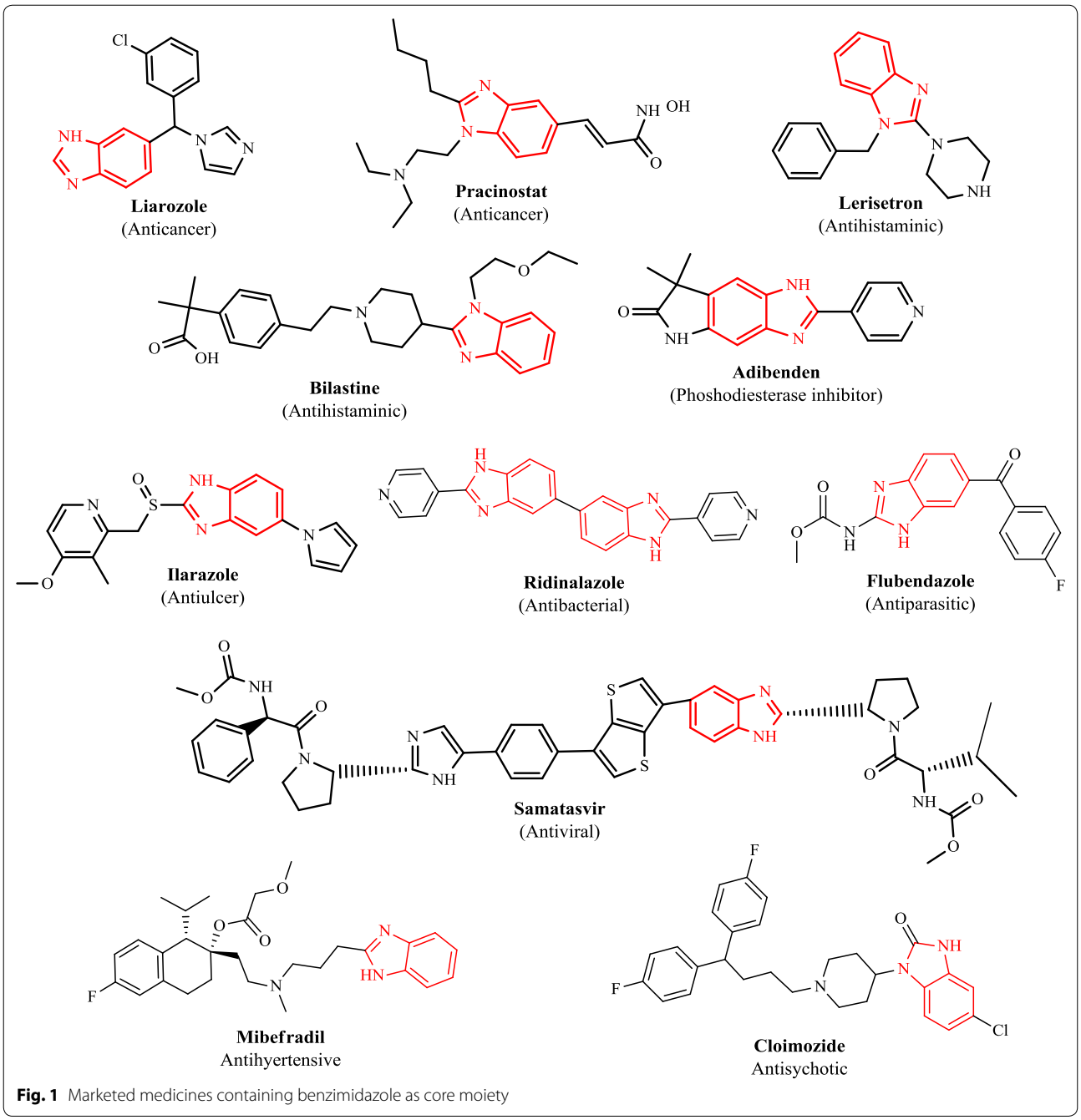


Fig. 1 Marketed medicines containing benzimidazole as core moiety

Antimicrobial and anticancer screening results

The synthesized benzimidazole compounds were screened for antimicrobial potential by tube dilution method using cefadroxil (antibacterial) and fluconazole (antifungal) as standard drugs against the represented microbial species. Furthermore, the anticancer activity of synthesized compounds against human colorectal carcinoma [HCT116 (ATCC CCL-247)] cancer cell line was assessed by using SRB assay with 5-fluorouracil (5-FU) being included as the standard anticancer drug. Biological screening results revealed that compound **Z24** displayed highest antimicrobial activity against Gram positive and Gram negative microorganisms ($MIC_{sa,\ st}$ = 30.10 µM, $MIC_{kp,ec}$ = 15.05 µM and $MIC_{ca,\ an}$ = 3.76 µM). Besides, **Z24** also elicited anticancer activity against HCT116 cell line (IC_{50} = 0.46 µM) which was more potent than the standard drug. The antimicrobial

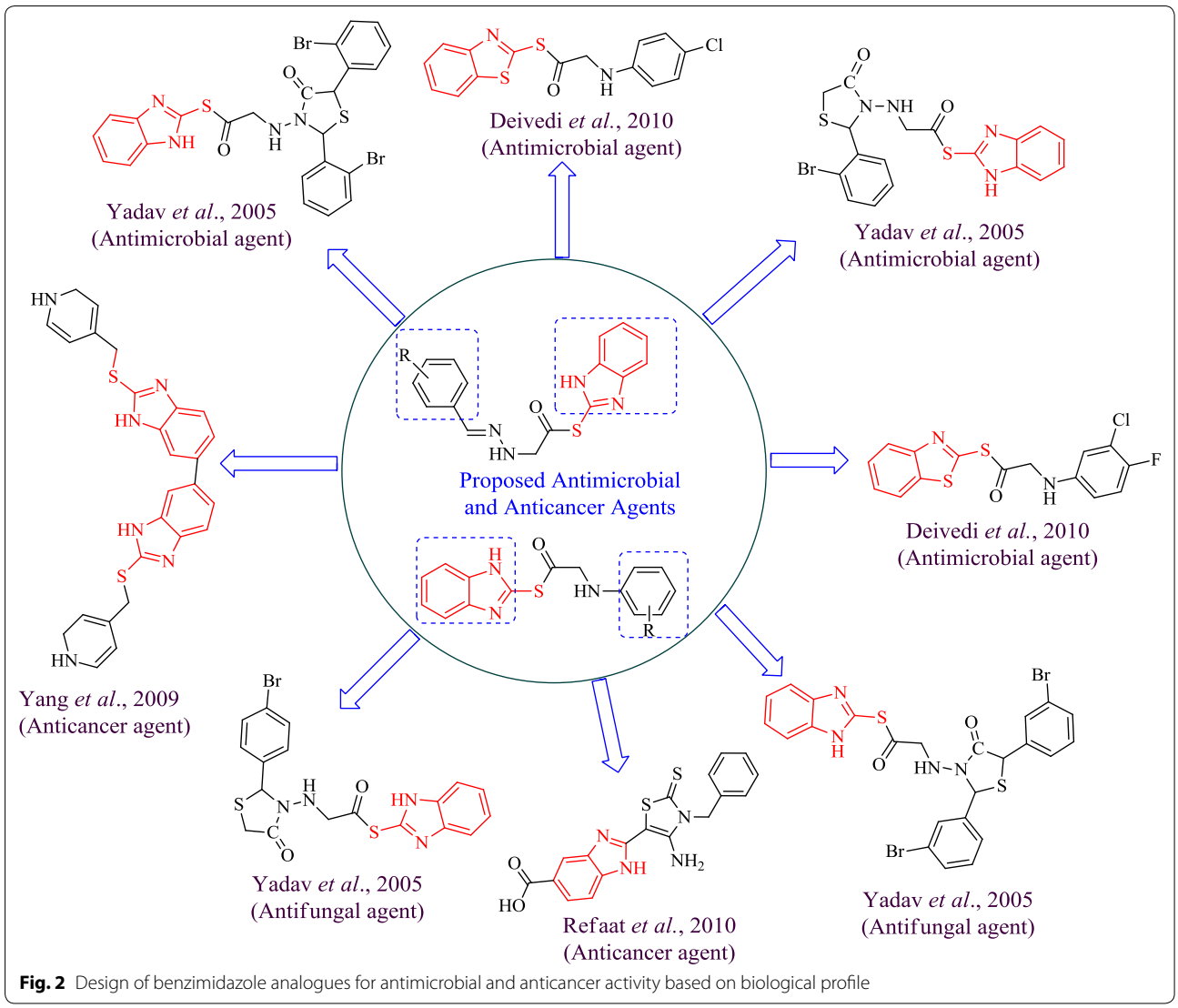


Fig. 2 Design of benzimidazole analogues for antimicrobial and anticancer activity based on biological profile

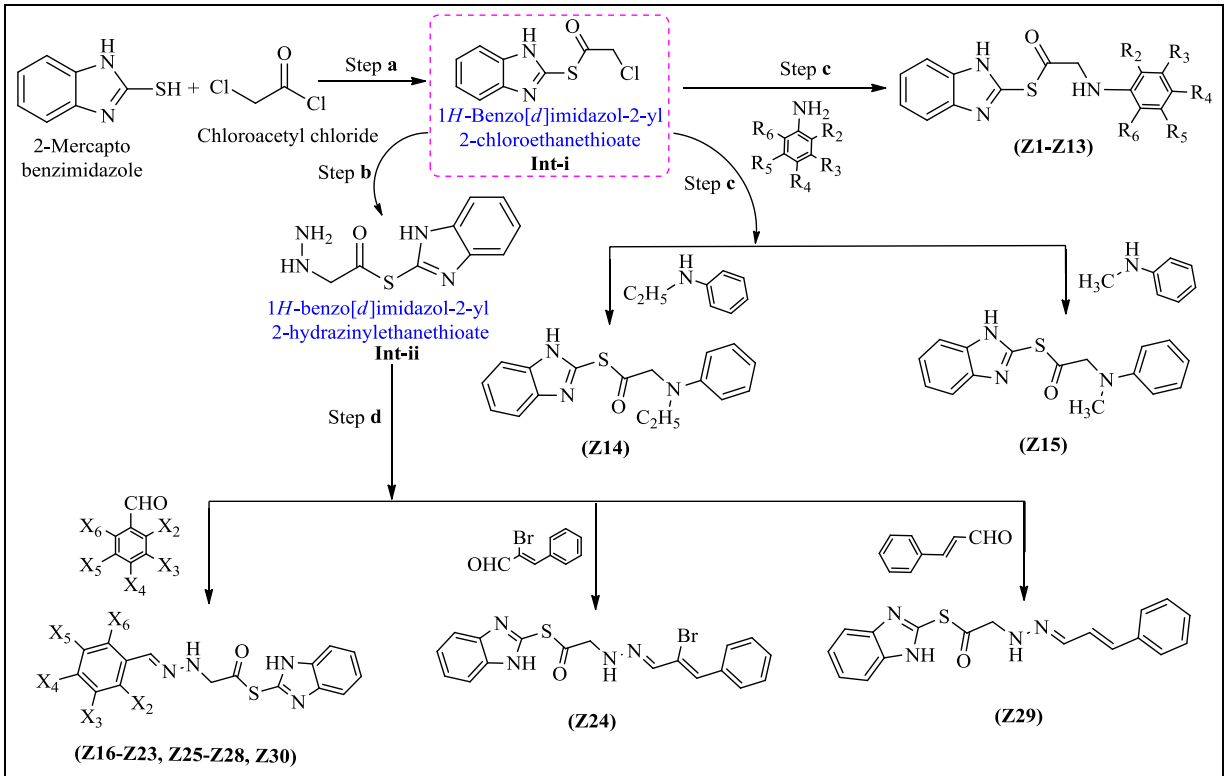
screening results are presented in Table 3 and Figs. 3, 4 whereas the anticancer results are presented in Table 4.

Structure activity relationship (SAR)

The substitution of halogenated α, β-unsaturated aldehyde i.e. 2-bromo-3-phenylacrylaldehyde (compound **Z24**) displayed an important role in improving the antiproliferative and antimicrobial activities as compared to (compound **Z29**) its non-halogenated α, β unsaturated aldehyde. Structure activity relationship study is shown in Fig. 5.

Methods/experimental

The starting material were purchased from different sources and used without further purification for the synthesis and analytical purpose. The reaction steps were confirmed by TLC (thin layer chromatography). Melting point was determined using labtech melting point equipment. An infrared spectrum was recorded [Attenuated total reflection (ATR), range of 4000–600 cm^{-1}] on Bruker 12060280 spectrometer. Proton and carbon-NMR (δ, ppm) spectral analyses were determined by Bruker Avance III at 600 NMR and 150 MHz, respectively spectrometer in deuterated solvent. Waters Micromass Q-ToF Micro instrument was used for MS spectra. Elemental analyses were carried out by C, H and N analyzer (Perkin-Elmer 2400) around $\pm 0.3\%$ of the theoretical results. The tested microorganism i.e. Gram positive, Gram negative and fungal species were procured from the Institute of Microbial Technology and Gene bank, Chandigarh for the in vitro antimicrobial activity.



Comp.	R_2	R_3	R_4	R_5	R_6	Comp.	X_2	X_3	X_4	X_5	X_6
Z1	NO_2	H	NO_2	H	H	**Z16**	NO_2	H	H	H	H
Z2	NO_2	H	H	H	H	**Z17**	H	NO_2	H	H	H
Z3	H	NO_2	H	H	H	**Z18**	H	H	NO_2	H	H
Z4	H	Br	H	H	H	**Z19**	H	OCH_3	OH	H	H
Z5	H	H	Br	H	H	**Z20**	H	OC_2H_5	OH	H	H
Z6	NO_2	H	Cl	H	H	**Z21**	H	H	Br	H	H
Z7	Cl	H	H	H	H	**Z22**	OH	H	H	H	H
Z8	H	Cl	H	H	H	**Z23**	H	OCH_3	OCH_3	H	H
Z9	CH_3	H	H	H	CH_3	**Z25**	H	H	CHO	H	H
Z10	Cl	H	NO_2	H	H	**Z26**	H	H	$N(CH_3)_2$	H	H
Z11	F	H	H	H	H	**Z27**	Cl	H	Cl	H	H
Z12	H	H	F	H	H	**Z28**	-	Cl	-	-	-
Z13	H	H	CH_3	H	H	**Z30**	OCH_3	H	H	H	H

Reaction condition:
Step **a**: Ethanol, Anhydrous K_2CO_3; Step **b**: $NH_2NH_2.H_2O$, Ethanol;
Step **c**: Ethanol, Different substituted anilines; Step **d**: Ethanol, Glacial acetic acid, Different substituted aldehydes

Scheme 1 Synthesis of heterocyclic benzimidazole derivatives

Table 1 The physicochemical properties of synthesized benzimidazole derivatives

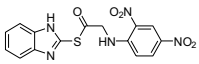
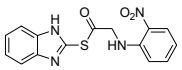
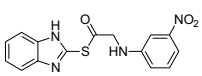
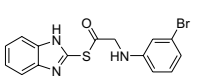
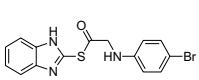
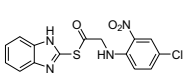
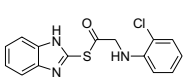
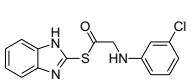
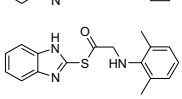
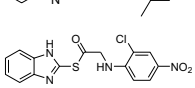
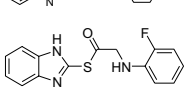
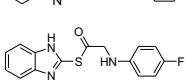
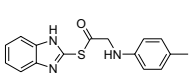
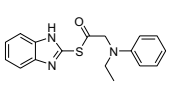
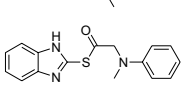
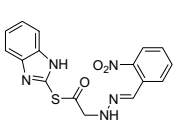
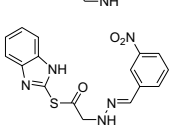
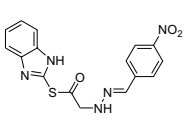
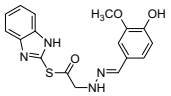
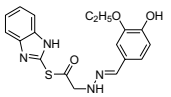
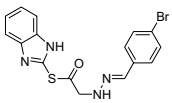
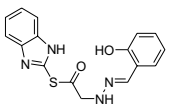
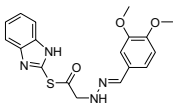
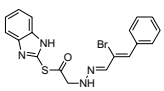
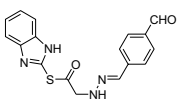
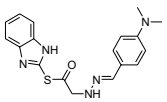
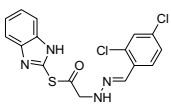
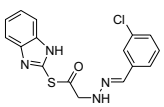
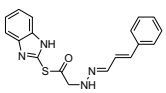
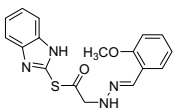
Comp.	IUPAC name	Mol. structure	Mol. formula	R*f* value[a]	m. p. (°C)	% yield
Z1	*1H-Benzo[d]imidazol-2-yl 2-(2,4-dinitrophenyl-amino) ethanethioate*		$C_{15}H_{11}N_5O_5S$	0.48	180–183	83.64
Z2	*1H-Benzo[d]imidazol-2-yl 2-(2-nitrophenylamino)ethanethioate*		$C_{15}H_{12}N_4O_3S$	0.82	212–215	73.93
Z3	*1H-Benzo[d]imidazol-2-yl 2-(3-nitrophenylamino)ethanethioate*		$C_{15}H_{12}N_4O_3S$	0.75	217–220	85.74
Z4	*1H-Benzo[d]imidazol-2-yl 2-(3-bromophenylamino)ethanethioate*		$C_{15}H_{12}N_3OSBr$	0.47	257–260	73.19
Z5	*1H-Benzo[d]imidazol-2-yl 2-(4-bromophenylamino)ethanethioate*		$C_{15}H_{12}N_3OSBr$	0.65	260–263	57.48
Z6	*1H-Benzo[d]imidazol-2-yl 2-(4-chloro-2-nitrophenylamino) ethanethioate*		$C_{15}H_{11}N_4O_3SCl$	0.66	140–143	94.19
Z7	*1H-Benzo[d]imidazol-2-yl 2-(2-chlorophenylamino)ethanethioate*		$C_{15}H_{12}N_3OSCl$	0.60	277–280	60.63
Z8	*1H-Benzo[d]imidazol-2-yl 2-(3-chlorophenylamino)ethanethioate*		$C_{15}H_{12}N_3OSCl$	0.68	265–268	65.87
Z9	*1H-Benzo[d]imidazol-2-yl 2-(2,6-dimethyyphenylamino) ethanethioate*		$C_{17}H_{17}N_3OS$	0.59	274–277	65.52
Z10	*1H-Benzo[d]imidazol-2-yl 2-(2-chloro-4-nitrophenylamino) ethanethioate*		$C_{15}H_{11}N_4O_3SCl$	0.74	180–183	91.45
Z11	*1H-Benzo[d]imidazol-2-yl 2-(2-florophenylamino)ethanethioate*		$C_{15}H_{12}N_3OSF$	0.73	272–275	72.43
Z12	*1H-Benzo[d]imidazol-2-yl 2-(4-florophenylamino)ethanethioate*		$C_{15}H_{12}N_3OSF$	0.55	270–273	68.60
Z13	*1H-Benzo[d]imidazol-2-yl 2-(4-methylphenylamino)ethanethioate*		$C_{16}H_{15}N_3OS$	0.61	266–269	64.13
Z14	*1H-Benzo[d]imidazol-2-yl 2 (methyl (phenyl)amino)ethanethioate*		$C_{17}H_{17}N_3OS$	0.70	245–248	53.40
Z15	*H-Benzo[d]imidazol-2-yl 2-(ethy l(phenyl)amino)ethanethioate*		$C_{16}H_{15}N_3OS$	0.75	261–264	56.95
Z16	*1H-Benzo[d]imidazol-2-yl 2-(2-(2-nitrobenzylidene)hydrazinyl) ethanethioate*		$C_{16}H_{13}N_5O_3S$	0.58	258–260	59.40
Z17	*1H-Benzo[d]imidazol-2-yl 2-(2-(3-nitrobenzylidene)hydrazinyl) ethanethioate*		$C_{16}H_{13}N_5O_3S$	0.54	262–265	82.89
Z18	*1H-Benzo[d]imidazol-2-yl 2-(2-(4-nitrobenzylidene)hydrazinyl) ethanethioate*		$C_{16}H_{13}N_5O_3S$	0.59	250–253	65.04

Table 1 (continued)

Comp.	IUPAC name	Mol. structure	Mol. formula	R*f* value[a]	m. p. (°C)	% yield
Z19	*1H-Benzo[d]imidazol-2-yl 2-(2-(4-hydroxy-3-methoxybenzylidene) hydrazinyl)ethanethioate*		$C_{17}H_{16}N_4O_3S$	0.53	263–266	53.93
Z20	*1H-Benzo[d]imidazol-2-yl 2-(2-(3-ethoxy-4-hydoxybenzylidene) hydrazinyl)ethanethioate*		$C_{18}H_{18}N_4O_3S$	0.52	269–272	60.65
Z21	*1H-Benzo[d]imidazol-2-yl 2-(2-(4-bromobenzylidene)hydrazinyl) ethanethioate*		$C_{16}H_{13}N_4OSBr$	0.50	277–280	65.52
Z22	*1H-Benzo[d]imidazol-2-yl 2-(2-(2-hydroxybenzylidene)hydrazinyl) ethanethioate*		$C_{16}H_{14}N_4O_2S$	0.57	259–262	63.60
Z23	*1H-Benzo[d]imidazol-2-yl 2-(2-(3,4-dimethoxybenzylidene) hydrazinyl) ethanethioate*		$C_{18}H_{18}N_4O_3S$	0.46	264–267	72.30
Z24	*1H-Benzo[d]imidazol-2-yl 2-(2-(2-bromo-3-phenylallylidene) hydrazinyl)ethanethioate*		$C_{18}H_{15}N_4OSBr$	0.51	160–163	78.68
Z25	*1H-Benzo[d]imidazol-2-yl 2-(2-(4-(dimethylamino)benzylidene) hydrazinyl)ethanethioate*		$C_{17}H_{14}N_4O_2S$	0.57	250–253	72.58
Z26	*1H-Benzo[d]imidazol-2-yl 2-(2-(4-formylbenzylidene) hydrazinyl) ethanethioate*		$C_{18}H_{19}N_5OS$	0.65	200–203	87.45
Z27	*1H-Benzo[d]imidazol-2-yl 2-(2-(2,4-dichlorobenzylidene) hydrazinyl) ethanethioate*		$C_{16}H_{12}N_4OSCl_2$	0.49	255–257	81.69
Z28	*1H-Benzo[d]imidazol-2-yl 2-(2-(2-chlorobenzylidene)hydrazinyl) ethanethioate*		$C_{16}H_{13}N_4OSCl$	0.64	259–262	74.33
Z29	*1H-Benzo[d]imidazol-2-yl 2-(2-(3-phenylallylidene)hydrazinyl) ethanethioate*		$C_{18}H_{16}N_4OS$	0.56	264–267	64.68
Z30	*1H-Benzo[d]imidazol-2-yl 2-(2-(2-methoxylbenzylidene) hydrazinyl) ethanethioate*		$C_{17}H_{16}N_4O_2S$	0.48	267–270	57.12

[a] TLC mobile phase-ethyl acetate

Procedure for synthesis of substituted benzimidazole derivative (Z1–Z30)

Step a: Synthesis of intermediate-i

A mixture of 2-mercaptobenzimidazole (0.01 mol) and chloroacetylchloride (0.01 mol) in ethanol (30 mL) in presence of anhydrous K_2CO_3 (0.01 mol) was refluxed for 6 h. The precipitated solid was filtered, evaporated to dryness under reduced pressure to get intermediate-**i** [5].

Step b: Synthesis of intermediate-ii

The solution of intermediate-**i** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol (20 mL) was refluxed for

Table 2 Spectral data of synthesized benzimidazole derivatives

Comp.	IR (ATR cm^{-1})	^{13}C-NMR (DMSO-*d6*, δ ppm)	^{1}H-NMR (DMSO-*d6*, δ ppm)	C, H, N analyses calculated (found); MS ES+ (ToF): *m/z*—[M^{+}+1]
Z1	{3074 (C–H str.), 1461 (C=C str.) of phenyl nucleus (pn)}, 1337 (C=N str.), 1258 (C–N str.), 706 (C–S str.), 2840 (C–H str., $–CH_2–$), 1512 (C–NO_2 str.), 1698 (C=O str.)	109.33, 119.56, 122.12, 123.10, 128.46, 132.16, 134.99, 149.64, 168.06	7.03–8.66 (m, 7H, Ar–H), 7.03 (s, 1H, NH of imidazole), 3.51 (s, 2H, CH_2), 2.01 (s, 1H, NH)	C, 48.26; H, 2.97; N, 18.76; (C, 48.22; H, 2.93; N, 18.72); 374
Z2	{3076 (C–H str.), 1459 (C=C str.) pn}, 1347 (C=N str.), 1254 (C–N str.), 695 (C–S str.), 2916 (C–H str., $–CH_2–$), 1508 (C–NO_2 str.), 1705 (C=O str.)	30.61, 109.41, 115.41, 119.11, 122.23, 125.31, 130.29, 132.20, 135.60, 146.12, 168.10, 206.31	6.62–7.99 (m, 8H, Ar–H), 6.62 (s, 1H, NH of imidazole), 3.49 (s, 2H, CH_2), 2.10 (s, 1H, NH)	C, 54.87; H, 3.68; N, 17.06; (C, 54.83; H, 3.64; N, 17.02); 329
Z3	{3070 (C–H str.), 1457 (C=C str.) pn}, 1351 (C=N str.), 1334 (C–N str.), 696 (C–S str.), 2835 (C–H str., $–CH_2–$), 1507 (C–NO_2 str.), 1716 (C=O str.)	30.59, 107.01, 109.41, 109.75, 119.90, 122.23, 129.82, 132.20, 148.69, 150.01 168.10	6.99–7.44 (m, 8H, Ar–H), 6.99 (s, 1H, NH of imidazole), 3.49 (s, 2H, CH_2), 2.10 (s, 1H, NH)	C, 54.87; H, 3.68; N, 17.06; (C, 54.91; H, 3.72; N, 17.10); 329
Z4	{3078 (C–H str.), 1461 (C=C str.) pn}, 1351 (C=N str.), 1333 (C–N str.), 698 (C–S str.), 2915 (C–H str., $–CH_2–$), 654 (C–Br str.), 1719 (C=O str.)	109.40, 119.56, 122.22, 128.46, 132.16, 149.64, 168.06	6.82–7.11 (m, 8H, Ar–H), 6.82 (s, 1H, NH of imidazole), 3.63 (s, 2H, CH_2), 1.05 (s, 1H, NH)	C, 49.73; H, 3.34; N, 11.60; (C, 49.77; H, 3.38; N, 11.64); 363
Z5	{3092 (C–H str.), 1462 (C=C str.) pn}, 1353 (C=N str.), 1335 (C–N str.), 701 (C–S str.), 2850 (C–H str., $–CH_2–$), 656 (C–Br str.), 1714 (C=O str.)	109.40, 119.32, 122.22, 128.34, 132.18, 134.99, 149.64, 168.06	6.65–7.25 (m, 8H, Ar–H), 6.65 (s, 1H, NH of imidazole), 3.77 (s, 2H, CH_2), 2.12 (s, 1H, NH)	C, 49.73; H, 3.34; N, 11.60; (C, 49.77; H, 3.38; N, 11.64); 363
Z6	{3083 (C–H str.), 1602 (C=C str.) pn}, 1356 (C=N str.), 1340 (C–N str.), 704 (C–S str.), 2850 (C–H str., $–CH_2–$), 763 (C–Cl str.), 1503 (C–NO_2 str.), 1716 (C=O str.)	109.37, 118.37, 121.02, 122.15, 123.94, 132.20, 135.41, 144.95, 168.10	7.06–7.22 (m, 8H, Ar–H), 7.06 (s, 1H, NH of imidazole), 3.51 (s, 2H, CH_2), 2.12 (s, 1H, NH)	C, 49.66; H, 3.06; N, 15.44; (C, 49.70; H, 3.10; N, 15.48); 363
Z7	{3071 (C–H str.), 1454 (C=C str.) pn}, 1352 (C=N str.), 1334 (C–N str.), 691 (C–S str.), 2924 (C–H str., $–CH_2–$), 739 (C–Cl str.), 1715 (C=O str.)	109.41, 119.65, 122.23, 128.56, 132.19, 134.99, 149.46, 168.09	7.16–7.22 (m, 8H, Ar–H), 7.16 (s, 1H, NH of imidazole), 3.61 (s, 2H, CH_2), 2.12 (s, 1H, NH)	C, 56.69; H, 3.81; N, 13.22; (C, 56.65; H, 3.85; N, 13.18); 318
Z8	{3082 (C–H str.), 1459 (C=C str.) pn}, 1352 (C=N str.), 1334 (C–N str.), 695 (C–S str.), 2842 (C–H str., $–CH_2–$), 738 (C–Cl str.), 1714 (C=O str)	109.40, 119.85, 122.21, 128.56, 132.18, 134.79, 149.46, 168.08	7.03–7.70 (m, 8H, Ar–H), 7.03 (s, 1H, NH of imidazole), 3.68 (s, 2H, CH_2), 1.04 (s, 1H, NH)	C, 56.69; H, 3.81; N, 13.22; (C, 56.65; H, 3.77; N, 13.126); 318
Z9	{3091 (C–H str.), 1454 (C=C str.) pn}, 1352 (C=N str., N=CH), 1334 (C–N str.), 691 (C–S str., CH_2–S), 2844 (C–H str., $–CH_2–$), 2948 (C–H str., CH_3), 1714 (C=O str., ketone)	30.59, 109.41, 119.56, 122.23, 128.41, 132.19, 134.52, 149.27, 168.10, 206.30	7.15–7.22 (m, 7H, Ar–H), 7.15 (s, 1H, NH of imidazole), 3.63 (s, 6H, $(CH_3)_2$), 2.11 (s, 1H, NH)	C, 65.57; H, 5.50; N, 13.49; (C, 65.53; H, 5.54; N, 13.45); 312
Z10	{3099 (C–H str.), 1623 (C=C str.) pn}, 1317 (C=N str.), 1305 (C–N str.), 704 (C–S str.), 2918 (C–H str., $–CH_2–$), 744 (C–Cl str.), 1484 (C–NO_2 str.), 1715 (C=O str.)	109.39, 113.46, 115.53, 122.18, 124.47, 125.51, 132.20, 135.92, 151.91, 168.10	6.91–7.99 (m, 7H, Ar–H), 6.91 (s, 1H, NH of imidazole), 3.59 (s, 2H, CH_2), 2.12 (s, 1H, NH)	C, 49.66; H, 3.06; N, 15.44; (C, 49.62; H, 3.02; N, 15.40); 363

Table 2 (continued)

Comp.	IR (ATR cm^{-1})	^{13}C-NMR (DMSO-*d6*, δ ppm)	^{1}H-NMR (DMSO-*d6*, δ ppm)	C, H, N analyses calculated (found); MS ES+ (ToF): *m/z*—[M^{+}+1]
Z11	{3070 (C–H str.), 1454 (C=C str.) pn}, 1352 (C=N str.), 1335 (C–N str.), 693 (C–S str.), 2835 (C–H str., –CH_2–), 1012 (C–F str.), 1716 (C=O str.)	30.60, 109.41, 115.21, 122.24, 132.19, 145.42, 150.32, 168.10;	7.14–7.20 (m, 8H, Ar–H), 7.14 (s, 1H, NH of imidazole), 3.53 (s, 2H, CH_2), 2.10 (s, 1H, NH);	C, 59.79; H, 4.01; N, 13.94; (C, 59.75; H, 4.05; N, 13.90); 302
Z12	{3068 (C–H str.), 1454 (C=C str.) pn}, 1351 (C=N str.), 1332 (C–N str.), 691 (C–S str.), 2933 (C–H str., –CH_2–), 1007 (C–F str.), 1714 (C=O str.)	109.40, 112.47, 122.23, 125.27, 132.19, 137.90, 146.32, 168.09	7.10–7.24 (m, 8H, Ar–H), 7.10 (s, 1H, NH of imidazole), 3.69 (s, 2H, CH_2), 2.11 (s, 1H, NH)	C, 59.79; H, 4.01; N, 13.94; (C, 59.83; H, 4.04; N, 13.98); 302
Z13	{3073 (C–H str.), 1455 (C=C str.) pn}, 1351 (C=N str.), 1333 (C–N str.), 693 (C–S str.), 2844 (C–H str., –CH_2–), 2870 (C–H str., CH_3), 1713 (C=O str.)	30.62, 109.41, 114.34, 122.24, 125.86, 127.89, 132.20, 144.56, 149.64, 168.10	7.13–7.19 (m, 8H, Ar–H), 7.13 (s, 1H, NH of imidazole), 3.47 (s, 3H, CH_3), 2.10 (s, 1H, NH)	C, 64.62; H, 5.08; N, 14.13; (C, 64.66; H, 5.04; N, 14.10); 298
Z14	{3069 (C–H str.), 1454 (C=C str.) pn}, 1352 (C=N str.), 1335 (C–N str.), 691 (C–S str.), 2914 (C–H str., –CH_2–), 2850 (C–H str., N–CH_3), 1714 (C=O str.)	30.96, 109.42, 114.37, 122.25, 128.79, 132.21, 145.64, 168.11	7.14–7.20 (m, 9H, Ar–H), 7.14 (s, 1H, NH of imidazole), 3.51 (q, 2H, –CH_2), 2.10 (t, 3H, CH_3)	C, 65.57; H, 5.50; N, 13.49; (C, 65.53; H, 5.54; N, 13.45); 312
Z15	{3079 (C–H str.), 1458 (C=C str.) pn}, 1344 (C=N str.), 1327 (C–N str.), 693 (C–S str.), 2844 (C–H str., –CH_2–), 2857 (C–H str., N–CH_3), 1706 (C=O str.)	30.59, 109.41, 114.33, 122.23, 127.89, 132.19, 139.27, 144.75, 168.10	7.08–7.22 (m, 9H, Ar–H), 7.08 (s, 1H, NH of imidazole), 3.61 (s, 31H, CH_2), 2.11 (s, 3H, CH_3)	C, 64.62; H, 5.08; N, 14.13; (C, 64.58; H, 5.12; N, 14.17); 298
Z16	{3105 (C–H str.), 1455 (C=C str.) pn}, 1701 (–CO str.), 1386 (C=N str.), 1343 (C–N str.), 1165 (N–N str., hydrazide), 694 (C–S str.), 2915 (C–H str., –CH_2–), 1510 (C–NO_2 str.)	109.41, 114.33, 119.17, 122.23, 126.32, 132.19, 137.42, 144.27, 168.09	7.04–7.35 (m, 8H, Ar–H), 7.04 (s, 1H, NH of imidazole), 2.11 (s, 1H, NH), 12.62 (s, 1H, N=CH)	C, 54.08; H, 3.69; N, 19.71; (C, 54.04; H, 3.65; N, 19.75); 356
Z17	{3112 (C–H str.), 1600 (C=C str.) pn}, 1701 (–CO str.), 1355 (C=N str.), 1337 (C–N str.), 1178 (N–N str., hydrazide), 702 (C–S str.), 2844 (C–H str., –CH_2–), 1512 (C–NO_2 str.)	109.39, 122.21, 130.92, 132.18, 139.87, 141.50, 145.71, 150.72, 168.08	7.18–8.26 (m, 8H, Ar–H), 6.84 (s, 1H, NH of imidazole), 3.55 (s, 2H, CH_2), 2.12 (s, 1H, NH), 12.66 (s, 1H, N=CH)	C, 54.08; H, 3.69; N, 19.71; (C, 54.03; H, 3.73; N, 19.67); 356
Z18	{3106 (C–H str.), 1604 (C=C str.) pn}, 1703 (–CO str.), 1337 (C=N str.), 1270 (C–N str.), 1174 (N–N str., hydrazide), 697 (C–S str.), 2843 (C–H str., –CH_2–), 1509 (C–NO_2 str.)	61.89, 111.30, 122.91, 123.16, 130.29, 132.18, 139.78, 146.17, 146.54, 150.27, 191.68	7.23–8.38 (m, 8H, Ar–H), 5.61 (s, 1H, NH of imidazole), 3.57 (s, 2H, CH_2), 2.12 (s, 1H, NH), 12.63 (s, 1H, N=CH)	C, 54.08; H, 3.69; N, 19.71; (C, 54.12; H, 3.64; N, 19.68); 356
Z19	{3081 (C–H str.), 1449 (C=C str.) pn}, 1716 (–CO str.), 1351 (C=N str.), 1331 (C–N str.), 1163 (N–N str., hydrazide), 689 (C–S str.), 2924 (C–H str., –CH_2–), 3648 (O–H str.), 2839 (C–H str., –OCH_3), 1256 (C–O–C str., phenyl ether)	30.60, 109.41, 111.19, 122.23, 127.89, 130.92, 132.19, 138.79, 145.92, 168.09	7.15–7.21 (m, 7H, Ar–H), 7.15 (s, 1H, NH of imidazole), 3.58 (s, 2H, CH_2), 2.11 (s, 1H, NH), 12.60 (s, 1H, N=CH)	C, 57.29; H, 4.52; N, 15.72; (C, 57.25; H, 4.56; N, 15.76); 357

Table 2 (continued)

Comp.	IR (ATR cm^{-1})	^{13}C-NMR (DMSO-*d6*, δ ppm)	^{1}H-NMR (DMSO-*d6*, δ ppm)	C, H, N analyses calculated (found); MS ES+ (ToF): *m/z*—[M^{+}+1]
Z20	{3085 (C–H str.), 1454 (C=C str.) pn}, 1714 (–CO str.), 1352 (C=N str.), 1334 (C–N str.), 1166 (N–N str., hydrazide), 688 (C–S str.), 2852 (C–H str., $–CH_2–$), 3645 (O–H str.), 2822 (C–H str., $–OCH_3$), 1255 (C–O–C str., phenyl ether), 2901 (C–H str., CH_3)	30.59, 109.41, 122.23, 125.21, 132.19, 130.18, 137.13, 144.65, 168.09	7.03–7.86 (m, 7H, Ar–H), 7.03 (s, 1H, NH of imidazole), 3.63 (s, 1H, OH), 2.11 (s, 1H, NH), 1.21 (t, 3H, CH_3), 12.61 (s, 1H, N=CH)	C, 58.36; H, 4.90; N, 15.12; (C, 58.40; H, 4.94; N, 15.16); 371
Z21	{3109 (C–H str.), 1466 (C=C str.) pn}, phenyl nucleus), 1732 (–CO str.), 1356 (C=N str.), 1338 (C–N str.), 1178 (N–N str., hydrazide), 703 (C–S str.), 2842 (C–H str., $–CH_2–$), 658 (C–Br str.)	30.59, 109.41, 122.23, 128.61, 131.05, 132.13, 132.19, 134.95, 192.01	7.17–7.22 (m, 8H, Ar–H), 7.17 (s, 1H, NH of imidazole), 3.66 (s, 2H, CH_2), 2.11 (s, 1H, NH), 12.62 (s, 1H, N=CH)	C, 49.37; H, 3.37; N, 14.39; (C, 49.33; H, 3.33; N, 14.35); 390
Z22	{3091 (C–H str.), 1603 (C=C str.) pn}, 1703 (–CO str.), 1346 (C=N str.), 1294 (C–N str.), 1168 (N–N str., hydrazide), 690 (C–S str.), 2841 (C–H str., $–CH_2–$), 3648 (O–H str.)	109.40, 122.22, 129.51, 131.03, 134.59, 146.32, 149.66, 168.08	7.18–7.24 (m, 8H, Ar–H), 7.18 (s, 1H, NH of imidazole), 3.74 (s, 1H, OH), 2.11 (s, 1H, NH), 12.64 (s, 1H, N=CH)	C, 58.88; H, 4.32; N, 17.17; (C, 58.84; H, 4.36; N, 17.13); 327
Z23	{3075 (C–H str.), 1452 (C=C str.) pn}, 1730 (–CO str.), 1380 (C=N str.), 1345 (C–N str.), 1170 (N–N str., hydrazide), 695 (C–S str.), 2876 (C–H str., $–CH_2–$), 2835 (C–H str., $–OCH_3$), 1253 (C–O–C str., phenyl ether)	109.40, 119.38, 122.22, 123.45, 128.67, 131.55, 132.18, 147.82, 168.08	7.18–7.24 (m, 7H, Ar–H), 7.18 (s, 1H, NH of imidazole), 375 (s, 6H, $(OCH_3)_2$), 2.12 (s, 1H, NH), 12.64 (s, 1H, N=CH)	C, 58.36; H, 4.90; N, 15.12; (C, 58.40; H, 4.94; N, 15.16); 371
Z24	{3087 (C–H str.), 1601 (C=C str.) pn}, 1713 (–CO str.), 1354 (C=N str.), 1337 (C–N str.), 1176 (N–N str., hydrazide), 694 (C–S str.), 2843 (C–H str., $–CH_2–$), 684 (C–Br str.), 1623 (conjugated C=C and phenyl subst. C=C)	109.42, 122.21, 123.89, 128.66, 128.90, 130.55, 131.36, 132.23, 132.82, 150.14, 187.80	7.13–7.94 (m, 9H, Ar–H), 7.06 (s, 1H, NH of imidazole), 2.00 (s, 2H, CH_2), 7.06 (s, 1H, NH), 12.55 (s, 1H, N=CH), 7.19 (s, 1H, Br–C=CH)	C, 52.06; H, 3.64; N, 13.49; (C, 52.02; H, 3.68; N, 13.45); 416
Z25	{3070 (C–H str.), 1453 (C=C str.) pn}, 1712 (–CO str.), 1352 (C=N str.), 1334 (C–N str.), 1167 (N–N str., hydrazide), 688 (C–S str.), 2846 (C–H str., $–CH_2–$), 1728 (C–H str., CHO)	78.04, 111.42, 122.92, 129.61, 129.94, 135.88, 139.55, 145.74, 192.70	7.25–7.98 (m, 8H, Ar–H), 7.03 (s, 1H, NH of imidazole), 3.61 (s, 2H, CH_2), 2.11 (s, 1H, NH), 12.60 (s, 1H, N=CH), 10.04 (s, 1H, CHO)	C, 60.34; H, 4.17; N, 16.56; (C, 60.38; H, 4.13; N, 16.52); 384
Z26	{3105 (C–H str.), 1453 (C=C str.) pn}, 1713 (–CO str.), 1352 (C=N str.), 1332 (C–N str.), 1163 (N–N str., hydrazide), 686 (C–S str.), 2927 (C–H str., $–CH_2–$), 2843 (C–H str., N–CH_3)	30.55, 39.49, 109.40, 110.89, 122.24, 124.45, 132.22, 154.02, 189.66	7.17–7.86 (m, 8H, Ar–H), 7.11 (s, 1H, NH of imidazole), 3.78 (s, 2H, CH_2), 2.12 (s, 1H, NH), 12.65 (s, 1H, N=CH), 2.64 (s, 6H, $(CH_3)_2$)	C, 61.17; H, 5.42; N, 19.81; (C, 61.13; H, 5.46; N, 19.85); 354
Z27	{3092 (C–H str.), 1596 (C=C str.) pn}, 1710 (–CO str.), 1354 (C=N str.), 1336 (C–N str.), 1175 (N–N str., hydrazide), 700 (C–S str.), 2930 (C–H str., $–CH_2–$), 739 (C–Cl str.)	61.91, 109.39, 122.20, 127.94, 130.66, 132.18, 133.65, 135.28, 137.04, 139.60, 188.40	6.74–7.72 (m, 7H, Ar–H), 6.74 (s, 1H, NH of imidazole), 3.02 (s, 2H, CH_2), 2.11 (s, 1H, NH), 12.62 (s, 1H, N=CH)	C, 50.67; H, 3.19; N, 14.77; (C, 50.63; H, 3.15; N, 14.73); 380

Table 2 (continued)

Comp.	IR (ATR cm^{-1})	^{13}C-NMR (DMSO-*d6*, δ ppm)	^{1}H-NMR (DMSO-*d6*, δ ppm)	C, H, N analyses calculated (found); MS ES+ (ToF): *m/z*—[M^{+}+1]
Z28	{3111 (C–H str.), 1598 (C=C str.) pn}, 1703 (–CO str.), 1355 (C=N str.), 1337 (C–N str.), 1177 (N–N str., hydrazide), 701 (C–S str.), 2845 (C–H str., $–CH_2–$), 740 (C–Cl str.)	30.58, 109.41, 119.56, 122.23, 124.54, 131.23, 132.18, 147.12, 168.08	7.16–7.28 (m, 8H, Ar–H), 7.16 (s, 1H, NH of imidazole), 3.65 (s, 2H, CH_2), 2.11 (s, 1H, NH), 12.62 (s, 1H, N=CH)	C, 55.73; H, 3.80; N, 16.25; (C, 55.77; H, 3.84; N, 16.29); 345
Z29	{3110 (C–H str.), 1598 (C=C str.) pn}, 1712 (–CO str.), 1355 (C=N str.), 1337 (C–N str.), 1176 (N–N str., hydrazide), 700 (C–S str.), 2860 (C–H str., $–CH_2–$), 1616 (phenyl conjugation)	109.40, 122.21, 128.72, 132.17, 134.54, 139.42, 141.72, 147.21, 168.07	7.18–7.27 (m, 9H, Ar–H), 7.18 (s, 1H, NH of imidazole), 3.82 (s, 2H, CH_2), 2.12 (s, 1H, NH), 12.66 (s, 1H, N=CH)	C, 64.26; H, 4.79; N, 16.65; (C, 64.22; H, 4.75; N, 16.69); 337
Z30	{3103 (C–H str.), 1599 (C=C str.) pn}, 1713 (–CO str.), 1352 (C=N str.), 1335 (C–N str.), 1168 (N–N str., hydrazide), 691 (C–S str., CH_2–S), 2841 (C–H str., $–CH_2–$), 2818 (C–H str., $–OCH_3$), 1255 (C–O–C str., phenyl ether)	109.41, 115.26, 122.23, 124.30, 132.19, 141.50, 145.71, 168.09, 205.61	7.15–7.25 (m, 8H, Ar–H), 7.15 (s, 1H, NH of imidazole), 3.70 (s, 2H, CH_2), 2.10 (s, 1H, NH), 12.60 (s, 1H, N=CH), 3.59 (s, 1H, OH)	C, 59.98; H, 4.74; N, 16.46; (C, 59.94; H, 4.98; N, 16.42); 341

Table 3 Antimicrobial screening results of synthesized derivatives

Comp.	Minimum inhibitory concentration (MIC = μM)					
	Bacterial strains				Fungal strains	
	SA MTCC3160	*ST* MTCC3231	*KP* MTCC9024	*EC* MTCC443	*AN* MTCC281	*CA* MTCC227
Z1	33.49	66.97	16.74	33.49	4.18	16.74
Z2	76.13	38.06	38.06	76.13	38.06	19.03
Z3	38.06	76.13	19.03	38.06	38.06	19.03
Z4	69.02	69.02	34.51	69.02	17.26	34.51
Z5	69.02	34.51	34.51	69.02	17.26	34.51
Z6	68.91	68.91	34.45	68.91	34.45	4.31
Z7	78.67	78.67	19.67	39.33	19.67	39.33
Z8	78.67	39.33	39.33	78.67	19.67	4.92
Z9	80.28	80.28	40.14	80.28	20.07	40.14
Z10	34.45	68.91	17.23	34.45	34.45	68.91
Z11	82.97	41.49	41.49	20.74	41.49	5.18
Z12	41.49	82.97	20.74	20.74	5.18	20.74
Z13	42.03	84.06	42.03	42.03	5.25	21.02
Z14	80.28	40.14	40.14	20.07	40.14	20.07
Z15	84.06	42.03	42.03	21.02	42.03	5.25
Z16	35.17	70.34	35.17	70.34	35.17	17.59
Z17	70.34	35.17	35.17	70.34	17.59	35.17
Z18	70.34	70.34	17.59	70.34	17.59	35.17
Z19	35.07	70.15	35.07	70.15	17.54	35.07
Z20	33.75	67.49	16.87	33.75	33.75	67.49
Z21	32.11	64.22	32.11	32.11	4.01	16.05
Z22	76.59	38.30	76.59	38.30	38.30	19.15
Z23	33.75	67.49	16.87	67.49	16.87	67.49
Z24	30.10	30.10	15.05	15.05	3.76	3.76
Z25	65.21	65.21	32.60	32.60	16.30	32.60
Z26	35.37	70.74	17.69	35.37	17.69	4.42
Z27	65.91	65.91	16.48	65.91	16.48	32.96
Z28	36.25	72.51	18.13	72.51	7.25	18.13
Z29	37.16	74.32	18.58	74.32	18.58	37.16
Z30	36.72	73.44	73.44	18.36	36.72	18.36
DMSO	NA	NA	NA	NA	NA	NA
Broth control	NG	NG	NG	NG	NG	NG
Std.	34.40[a]	34.40[a]	17.20[a]	17.20[a]	10.20[b]	10.20[b]

SA, *Staphylococcus aureus*; *ST*, *Salmonella typhi*; *KP*, *Klebsiella pneumoniae*; *EC*, *Escherichia coli*; *CA*, *Candida albicans*; *AN*, *Aspergillus niger*; DMSO, Dimethyl sulfoxide; NA, No activity; NG, No growth

Std drugs: [a]Cefadroxil; [b]Fluconazole

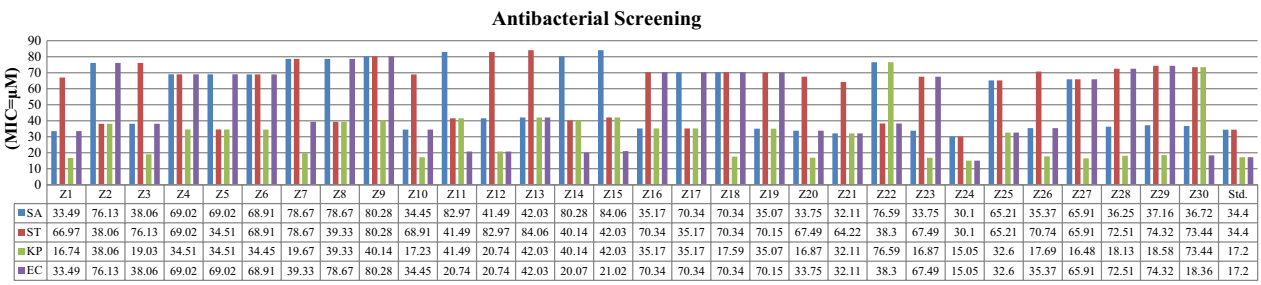
5 h. The solution was then poured in ice cold water and resulting solid was filtered, dried and recrystallized from ethanol [23].

Step c: Synthesis of title compounds Z1–Z15

An equimolar mixture of intermediate-**i** and substituted aniline in ethanol was refluxed for 4–5 h. After completion of reaction, it was poured into ice cold water and the precipitated title compound was filtered, dried and recrystallized from ethanol [24].

Step d: Synthesis of title compounds Z16–Z30

An equimolar mixture of intermediate-**ii** and substituted aromatic aldehydes with 2–3 drops of glacial acetic acid in ethanol (20 mL) was refluxed for 6 h. The resultant



	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9	Z10	Z11	Z12	Z13	Z14	Z15	Z16	Z17	Z18	Z19	Z20	Z21	Z22	Z23	Z24	Z25	Z26	Z27	Z28	Z29	Z30	Std.
SA	33.49	76.13	38.06	69.02	69.02	68.91	78.67	78.67	80.28	34.45	82.97	41.49	42.03	80.28	84.06	35.17	70.34	70.34	35.07	33.75	32.11	76.59	33.75	30.1	65.21	35.37	65.91	36.25	37.16	36.72	34.4
ST	66.97	38.06	76.13	69.02	34.51	68.91	78.67	39.33	80.28	68.91	41.49	82.97	84.06	40.14	42.03	70.34	35.17	70.34	70.15	67.49	64.22	38.3	67.49	30.1	65.21	70.74	65.91	72.51	74.32	73.44	34.4
KP	16.74	38.06	19.03	34.51	34.51	34.45	19.67	39.33	40.14	17.23	41.49	20.74	42.03	40.14	42.03	35.17	35.17	17.59	35.07	16.87	32.11	76.59	16.87	15.05	32.6	17.69	16.48	18.13	18.58	73.44	17.2
EC	33.49	76.13	38.06	69.02	69.02	68.91	39.33	78.67	80.28	34.45	20.74	20.74	42.03	20.07	21.02	70.34	70.34	70.34	70.15	33.75	32.11	38.3	67.49	15.05	32.6	35.37	65.91	72.51	74.32	18.36	17.2

Fig. 3 Antibacterial screening results against bacterial species

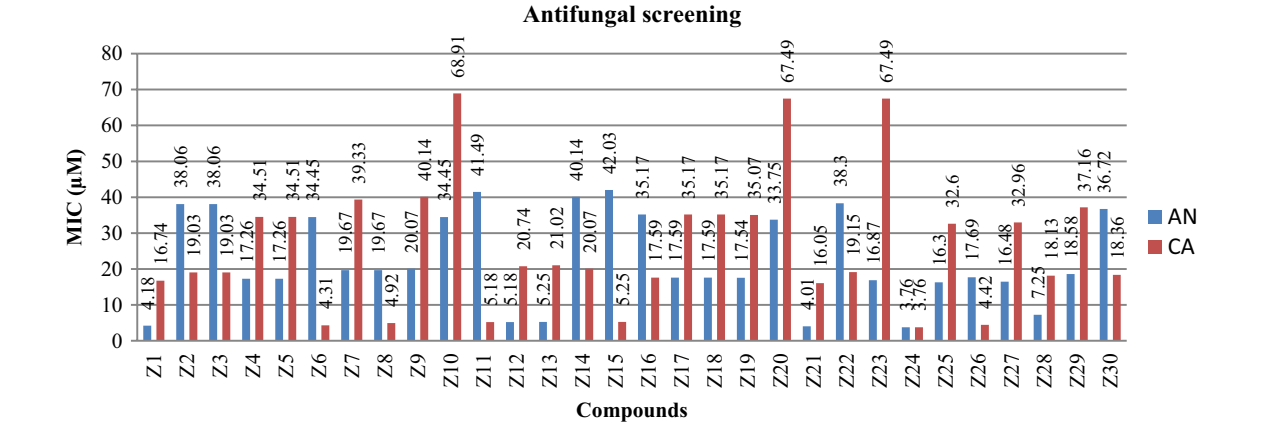


Fig. 4 Antifungal screening results against fungal species

Table 4 Anticancer screening results of synthesized derivatives

Anticancer screening			
Comp.	**IC_{50} (µM)**	**Comp.**	**IC_{50} (µM)**
Z1	214.30	**Z16**	> 281.37
Z2	> 304.51	**Z17**	140.69
Z3	> 304.51	**Z18**	121.83
Z4	220.87	**Z19**	> 280.58
Z5	220.87	**Z20**	> 269.98
Z6	179.16	**Z21**	> 256.87
Z7	> 314.66	**Z22**	> 306.37
Z8	314.66	**Z23**	> 269.98
Z9	> 321.13	**Z24**	0.46
Z10	152.70	**Z25**	78.25
Z11	> 331.90	**Z26**	198.08
Z12	> 331.90	**Z27**	67.49
Z13	336.25	**Z28**	> 290.02
Z14	> 321.13	**Z29**	252.68
Z15	> 336.25	**Z30**	> 293.77
5-Fluorouracil	8.84	**5-Fluorouracil**	8.84

precipitate was filtered and recrystallized from ethanol to yield the required compound [23].

Biological evaluation

Antimicrobial evaluation (MIC)

The antimicrobial evaluation of the synthesized derivatives was carried out by tube dilution method [25] towards selected Gram positive, Gram negative and fungal microorganisms shown in Table 3. The screening results were compared with standard drugs i.e. cefadroxil and fluconazole.

The stock solutions (100 µg/mL)

The tested compounds and standard drugs were prepared in dimethylsulfoxide and further diluted up to six concentrations [26].

Broth media

Sabouraud dextrose broth used for antifungal activity and double strength nutrient broth used for antibacterial activity.

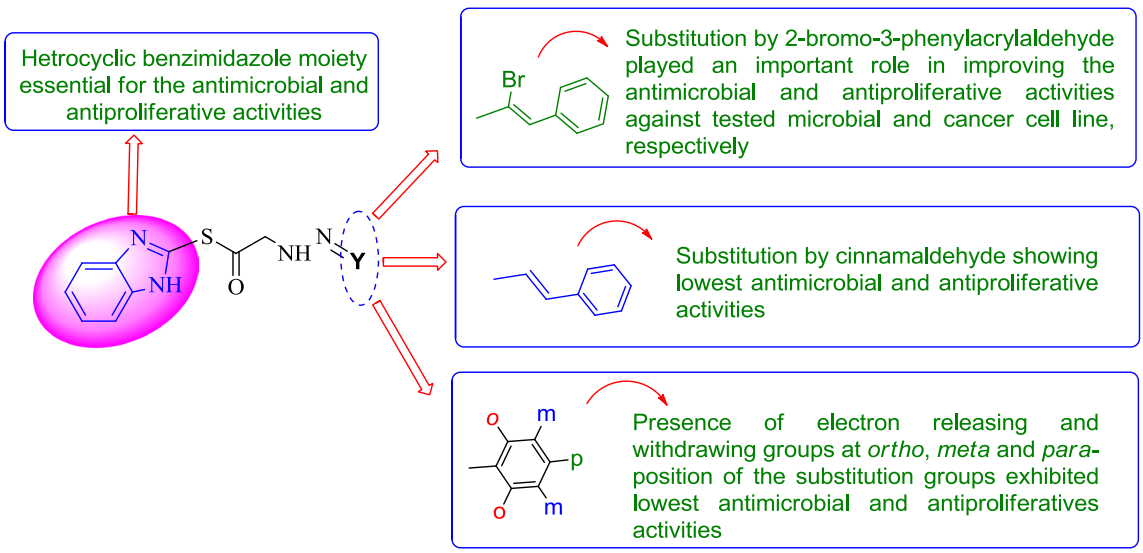


Fig. 5 Structural requirements for the antimicrobial and anticancer activities of synthesized benzimidazole derivatives

Incubation periods

The compounds were incubated at 25 ± 1 °C for 7 days (fungi—*A. niger*), at 37 ± 1 °C for 24 h (bacteria) and at 37 ± 1 °C for 48 h (fungi—*C. albicans*), respectively.

Anticancer evaluation (IC_{50})

The antiproliferative activity was determined by SRB assay. Briefly, HCT116 was seeded onto the 96 well plate at 2500 cells/well. The cells were allowed to attach overnight before being exposed to the respective compounds (0.01–100 µg/mL) for 72 h. The highest concentration of each compound tested (100 µg/mL) contained only 0.1% DMSO (non-cytotoxic). SRB assay [27] was then performed whereby the cells were fixed using trichloroacetic acid for 30 min at 4 °C and stained with 0.4% (*w/v*) SRB mixed with 1% acetic acid for 15 min. After five washes with 1% acetic acid solution, the protein-bound dye was extracted with 10 mM tris base solution. Optical density was read at 570 nm and IC_{50} (i.e. concentration required to inhibit 50% of the cells) of each compound was determined. Anticancer results were presented as mean IC_{50} of at least triplicates.

Conclusion

In this study, the synthesized benzimidazole scaffolds were authenticated by their consistent spectral data. The antimicrobial potential of synthesized compounds was assessed against different fungal and bacterial species, along with their anticancer activity against HCT116 cell line. The activity results indicated that the presence of *α*-bromo group in benzylidene portion (compound **24**) played an important role in improving the antimicrobial as well as antiproliferative activities and may be used as a lead for the discovery of new antimicrobial and anticancer agents.

Abbreviations

CRC: Colorectal Cancer; HDAC: Histone Deacetylase Inhibitor; WHO: World Health Organization; IR: Infrared; NMR: Nuclear Magnetic Resonance; MS: Mass Spectrometry; TLC: Thin Layer Chromatography; ATR: Attenuated Total Reflection; DMSO: Dimethyl Sulfoxide; SRB: Sulforhodamine B; HCT116: Human colorectal carcinoma 116; MIC: Minimum Inhibitory Concentration; 5-FU: 5-Fluorouracil; µM: micro mole; SAR: Structure Activity Relationship; MTCC : Microbial Type Culture Collection; *SA*: *Staphylococcus aureus*; *ST*: *Salmonella typhi*; *KP*: *Klebsiella pneumoniae*; *EC*: *Escherichia coli*; *CA*: *Candida albicans*; *AN*: *Aspergillus niger*.

Authors' contributions

Authors BN, ST and SK—have designed, synthesized and carried out the antimicrobial activity and KR, SML, SAAS and VM—have carried out the spectral analysis and anticancer evaluation of synthesized compounds. All authors read and approved the final manuscript.

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