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SYNTHESIS, CHARACTERIZATION AND *IN-VITRO* ANTICANCER SCREENING OF N-[2-(1-*H*-BENZIMIDAZOL-1-YL)-2-OXOETHYL]-2-BENZYLIDENE HYDRAZINECARBOXAMIDE DERIVATIVES

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ABSTRACT

Six novel compounds have been synthesized combining pharmacological potential of benzimidazole and schiff's bases ring. A new series of substituted benzimidazole derivative **3a-3f** were synthesized by cyclocondensation of 4-(2-(1*H*-benzo[d]imidazol-1-yl)-2-oxoethyl)semicarbazide with various aryl benzaldehyde derivatives. The newly synthesized compounds were characterized by IR, MASS and ^{1}H NMR spectral data. The synthesized compounds were evaluated for *in vitro* anti-cancer activity by MTT assay. Compound **(3f)** exhibited significant cytotoxicity was observed against HeLa cell line with IC_{50} value in the range of 6.13 µM respectively.

KEYWORDS

Benzimidazole, Schiff's bases and MTT assay.

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INTRODUCTION

Benzimidazole is a heterocyclic aromatic organic compound which is an important pharmacophore and a privileged structure in medicinal chemistry. This compound is bicyclic in nature which consists of the fusion of benzene and imidazole. Benzimidazole with unsubstituted NH groups, exhibit fast prototropic tautomerism which leads to equilibrium mixtures of asymmetrically substituted compounds. The benzimidazole scaffold is a useful structural modification for the development of molecules of pharmaceutical or biological interest. The optimization of benzimidazole - based structures has

resulted in various drugs that are currently on the market, such as omeprazole (Proton pump inhibitor), pimobendan (Ionodilator) and mebendazole (Anthelmintic)[1].

Nowadays it is a moiety of choice which possess many pharmacological properties. The most prominent benzimidazole compound in nature is *N*-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitaminB_{12}[2]. Benzimidazole and its derivatives which possess different biological activities such as fungicidal[3], anithelmintic[4], anti-inflammatory[5] antioxidant[6], antiallergy[7], anti-HIV[8], antihypertensive[9] and antiulcer activity. Furthermore, benzimidazoles showed anticancer activity against DNA topoisomerase I and colon cancer cell lines. The need for anticancer agents that selectively kill or inhibit the growth of neoplastic cells without affecting non-cancerous host tissues is high and persistent. Thus, the aim of the current study was the synthesis of novel benzimidazole derivatives that incorporated different heterocycles of anticancer activity. They were also active in a number of solid tumor screens, e.g., HELA uterine carcinoma, PC12, SOS bone osteosarcoma, lung MB9812, lung A549 and Mcf-7 breast growth[10].

Schiff bases derived from aromatic amines and aromatic aldehydes are also a very important class of organic compounds because of their applications in many fields including biological, inorganic and analytical chemistry. The hybrid molecules composed of the combination of part of a heterocyclic ring, like benzimidazole, and part of the Schiff base may exert potential biological activities. Several synthetic methods have been reported for the synthesis of Schiff bases[11,12].

An attempt was made to synthesize some new congeners by linking Schiff bases with benzimidazole pharmacophore to evaluate their possible synergistic activity. Benzimidazole N-substituted Schiff‘s bases skeleton as bioisosteric of naturally occurring molecules, hopping to produce anticancer agents of high potency and selectivity[13,14].

MATERIALS AND METHOD

All the chemicals were of synthetic grade and commercially procured from sigma Aldrich Chemicals. The melting points of all the synthesized compounds were determined using capillary tubes with Thermionic model C-LMP-1-Campbell melting point apparatus and are uncorrected. IR spectra were determined on JASCO FTIR 4100 using KBr pellets and wave number was reported in cm^{-1}. ^{1}H NMR spectra on AV-III-400 Fourier Transform NMR instrument using $CDCl_3$ as solvent using TMS as internal standard; the chemical shifts (δ) were reported in ppm. Mass spectra were recorded on JOEL SX 102-GC MATE instrument employing electron impact ionization technique. The purity of the compounds by performing TLC over glass plates coated with silica gel with suitable (benzene: ethyl acetate, 1:4) mobile phase system and detected by iodine vapor. The reagent grade chemicals were purified by either distillation or recrystallization before use. The compounds were identified by IR, ^{1}H-NMR and MASS spectra. The physical data of compounds were presented in Table No.1.

CHEMISTRY

EXPERIMENTAL WORK

STEP 1: Synthesis of 1-(1*H*-benzo[d]imidazol-1-yl)-2-chloroethanone

Chloroacetyl chloride (0.02 mol) was added drop by drop at 0-5^{0}C to benzimidazole (0.016 mol) in dry benzene (50 ml) and stirred for 3 h. Solution was kept at room temperature where the solid separated was filtered and washed with petroleum ether and recrystallized from chloroform[15].

STEP 2: Synthesis of 4-(2-(1*H*-benzo[d]imidazol-1-yl)-2-oxoethyl) semicarbazide

A mixture of 1-(1*H*-benzo[d]imidazol-1-yl)-2-chloroethanone (0.01 mol) and semicarbazide (0.04 mol, 3 g) in absolute ethanol was refluxed for 5 h. Contents were poured into ice cold water. The resulting solid was filtered, dried and recrystallized from chloroform[16].

STEP 3: Synthesis of Schiff's Base Derivatives

Mixture of 4-(2-(1*H*-benzo[d]imidazol-1-yl)-2-oxoethyl)semicarbazide (0.01 mol) and substituted benzaldehyde (0.01 mol, 1.06 ml) in ethanol containing acetic acid (0.5 ml) was refluxed for 6 h. Excess of solvent was distilled off, concentrated and cooled. The solid thus separated was filtered, washed and recrystallized from ethanol[17].

***N*-[2-(1*H*-benzimidazol-1-yl)-2oxoethyl]-2-benzylidene hydrazinecarboxamide (3a)**

IR (KBr disc) cm^{-1}: 3340 (NH str), 3154,1595,736 (aromatic str),1610 (C=O str), 1470 (C-N str). **^{1}H-NMR (DMSO-d$_6$)**: 6.68-7.14 (m,8H,Ar-H), 3.3 (s,3H,OCH_3), 3.7 (s,2H,CH_2), 2.9 (s,2H,NH). **MS *m/z*** 322 $[M]^+$.

***N*-[2-(1*H*-benzimidazol-1-yl)-2oxoethyl]-2-(4methoxybenzylidene)hydrazine carboxamide (3b)**

IR (KBr disc) cm^{-1}: 3340 (NH stre), 3157,1585,736 (aromatic str), 1600 (C=O str),1472 (C-N str), 1083 (C-O-C). **^{1}H-NMR (DMSO-d$_6$)**: 6.64-7.22 (m,9H,Ar-H), 3.2 (s,3H,OCH_3), 3.7 (s,2H,CH_2), 2.8 (s,2H,NH), **MS *m/z*** 353 $[M]^+$.

***N*-[2-(1*H*-benzimidazol-1-yl)-2-oxoethyl]-2-(4chlorobenzylidene)hydrazinecarboxamide (3c)**

IR (KBr disc) cm^{-1}: 3330 (NH stre), 3150,1598,735 (aromatic str), 1700 (C=O str), 1478 (C-N str), 821(C-Cl). **^{1}H-NMR (DMSO-d$_6$)**: 6.52-7.26 (m,9H,Ar-H), 3.32 (s,2H,CH_2), 2.9 (s,2H,NH). **MS *m/z*** 357 $[M]^+$.

***N*-[2-(1*H*-benzimidazol-1-yl)-2-oxoethyl]-2-(4-nitrobenzylidene)hydrazinecarboxamide (3d)**

IR (KBr disc) cm^{-1}: 3340 (NH str), 3160,1598,735 (aromatic str), 1670 (C=O str), 1475 (C-N str) 1515 and 1342 (N=O). **^{1}H-NMR (DMSO-d$_6$)**: 6.33-7.20 (m,9H,Ar-H), 3.2 (s,3H,OCH_3), 3.7 (s,2H,CH_2), 2.7 (s,2H,NH). **MS *m/z*** 368 $[M]^+$.

***N*-[2-(1*H*-benzimidazol-1-yl)-2-oxoethyl]-2-(3-fluorobenzylidene)hydrazinecarboxamide (3e)**

IR (KBr disc) cm^{-1}: 3340 (NH str), 3154,1595,736 (aromatic str), 1650 (C=O str), 1470 (C-N str), 1472 (C-F). **^{1}H-NMR (DMSO-d$_6$)**: 6.57-7.10 (m,9H,Ar-H), 3.5 (s,3H,OCH_3), 3.8 (s,2H,CH_2) 2.7 (s,2H,NH). **MS *m/z*** 340 $[M]^+$.

***N*-[2-(1*H*-benzimidazol-1-yl)-2-oxoethyl]-2-[4-(dimethylamino)benzylidene]hydrazine carboxamide (3f)**

IR (KBr disc) cm^{-1}: 3340 (NH str), 3155,1580,736 (aromatic str), 1710 (C=O str), 1460 (C-N str) 1311 (CN of $N(CH_3)_2$. **^{1}H-NMR (DMSO-d$_6$)**: 6.59-7.0 (m,9H, Ar-H), 2.9 (s,6H, $N(CH_3)_2$), 3.4 (s,2H,CH_2), 2.8 (s2H,NH). **MS *m/z*** 366 $[M]^+$.

BIOLOGICAL ACTIVITY

Anticancer activity of samples determined by MTT assay method. The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune. The cells were grown in Eagles Minimum Essential Medium containing 10 % fetal bovine serum (FBS). For screening experiment, the cells were seeded into 96-well plates in 100 µl of medium containing 5% FBS, at plating density of 10,000 cells/well and incubated at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of samples. The samples were solubilized in Dimethylsulfoxide and diluted in serum free medium. After 24 h, 100 µl of the medium containing the samples at various concentration (0.1, 1, 10, 100 µM) were added and incubated at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity for 48 h. Triplicate was maintained and the medium containing without extracts were served as control. After 48 h, 15 µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^{0}C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Non linear regression graph was plotted between percentage cell inhibition and Log_{10} concentration and IC_{50} was determined using Graph Pad Prism software[18-20]. The results are tabulated in Table No.2 and Figure No.1-4.

RESULTS AND DISCUSSION

Chemistry

On the basis of literature review Novel benzimidazole containing six derivatives were synthesized. The derivatives were synthesized by a three step reaction as described in Scheme-I. In the first step, 1-(1*H*-benzo[d]imidazol-1-yl)-2-chloroethanone has been prepared from benzimidazole and chloroacetyl chloride using dry benzene as solvent followed by the treatment with semicarbazide to form 4-(2-(1*H*-benzo[d]imidazol-1-yl)-2-oxoethyl)semicarbazide. Schiff bases of benzimidazole were synthesized by the condensation of 4-(2-(1*H*-benzo[d]imidazol-1-yl)-2-oxoethyl) semicarbazide with various substituted aromatic aldehydes (step 3). All the titled compounds yielded the products in the range of 78-85%. The melting points of the compounds **3a-f** were observed in the range of 110-173^{0}C. All compounds showed only one spot of migration from the origin on TLC plates, thereby confirming their purity.

The compounds **3a-3f** showed IR characteristic peaks in the regions of 1657 cm^{-1} for C=O stretching, 3340 cm^{-1} for NH stretching, 3129 cm^{-1}, 1592 cm^{-1} and 737 cm^{-1} for aromatic stretching and 1467 cm^{-1} for C-N stretching. Compounds containing NO_2 group showed absorption bands at 1518, 1343 cm^{-1} for the N=O stretching and presence of fluoro group was confirmed by the appearance of broad peak at 1472 cm^{-1}.

The synthesized compounds were characterized using ^{1}HNMR. The compound **3b** showed singlet at δ 3.3 for three protons of OCH_3 group. The spectrum of **3f** revealed a singlet at δ 2.9 corresponding to six protons of $N(CH_3)_2$ group. The compounds **3a-3f** showed multiplet signals in the range of δ 6.52 - 7.5 for the protons of aromatic ring, singlet at δ 3.35 for two protons of CH_2 group and a singlet at δ 2.9 for one NH proton. Compounds **3a-3f** was also verified their molecular ion peaks (M+).

Biological evaluation

Anticancer screening

The *in vitro* anticancer studies were performed on 3 randomly selected compounds using MTT assay against HeLa cell line (NCCS). The results indicated that among the three compounds tested, the compounds **3d** and **3f** were found to have significant cytotoxic activity against Hela cell line and then IC_{50} values were found to be 11.09 µM and 6.13 µM.

The compound **(3d)** is expected to be highly cytotoxic activity due to the presence highly electronegative nitro group substituted aromatic ring in addition to the presence of benzimidazole linked with urea moiety[21]. The bulkier group of dimethylamino aryl substituents on the benzimidazole **(3f)** possesses excellent cytotoxic activity against HeLa cell line[13]. The compound **3c** showed lack of anticancer activity against HeLa cell line.

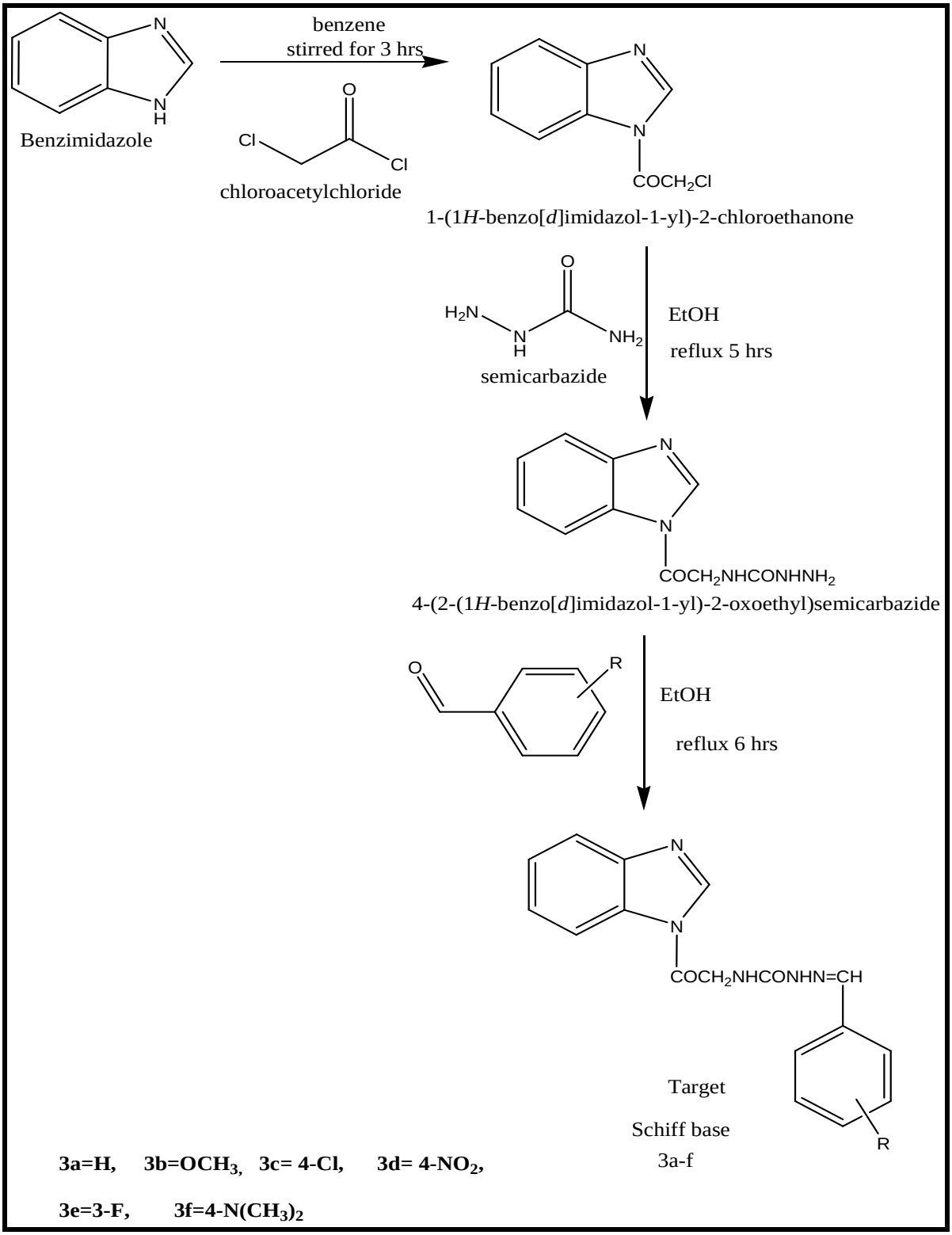
Table No.1: Physical and analytical data of synthesized compounds

S.No	Code no	Melting range (^{o}C)	*Rf	Molecular Formula	Mol.wt	Colour	% Yield	Solubility
1	**3a**	120-122	0.66	$C_{17}H_{15}N_5O_2$	321.33	Colourless	79 %	Ethanol, Ether, Acetone
2	**3b**	159-162	0.69	$C_{18}H_{17}N_5O_3$	351.36	Colourless	82%	Ethanol, Benzene
3	**3c**	141-144	0.49	$C_{17}H_{14}ClN_5O_2$	355.78	White crystals	85%	Ethanol, Benzene
4	**3d**	170-173	0.58	$C_{17}H_{14}N_6O_4$	366.33	Light yellow	78%	Ethanol, Acetone
5	**3e**	110-115	0.60	$C_{17}H_{14}FN_5O_2$	339.2	white	84%	Ethanol, Benzene
6	**3f**	140-143	0.63	$C_{19}H_{20}N_6O_2$	364.4	Whitish yellow	78%	Ethanol, Benzene, Acetone

***Mobile Phase: 3a-f** = benzene/ethyl acetate (4:1), **Detecting Agent: 3a-f**- iodine vapours

Table No.2: Percentage cell inhibition produced by titled compounds at varying concentrations

S.No	Code	Conc (µM)	% Cell Inhibition	IC_{50} (µM)
1	**3c**	0.1	12.71967	11.09
		1	22.42678	
		10	50.20921	
		100	72.80335	
2	**3d**	0.1	0.123001	111.2
		1	5.904059	
		10	15.91016	
		100	33.57934	
3	**3f**	0.1	0.585774	6.3
		1	1.004184	
		10	72.21754	
		100	86.5257	



Scheme No.1: Synthesis of N-[2-(1-*H*-Benzimidazol-1-Yl)-2-Oxoethyl]-2-Benzylidene

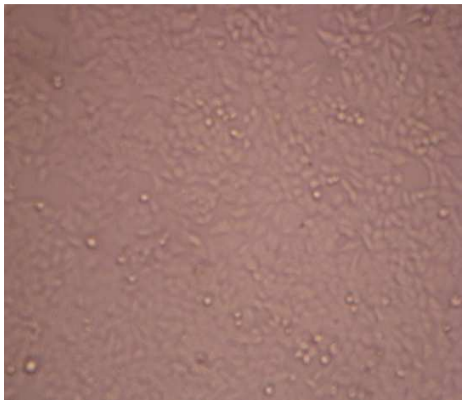


Figure No.1: Inhibition of HeLa by Compound **3f** (0.1 µM)

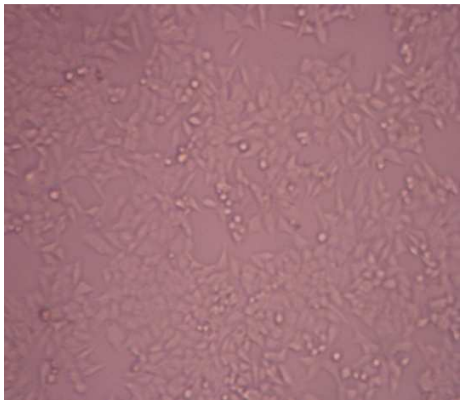


Figure No.2: Inhibition of HeLa by compound **3f** (1 µM)

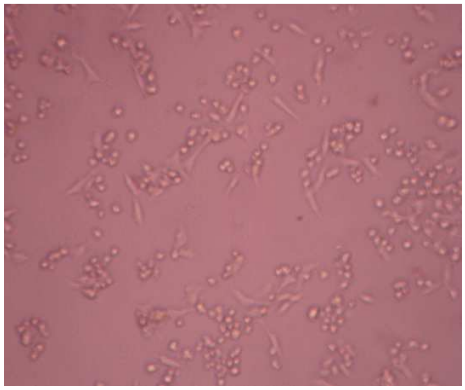


Figure No.3: Inhibition of HeLa by Compound**3f** (10 µM)

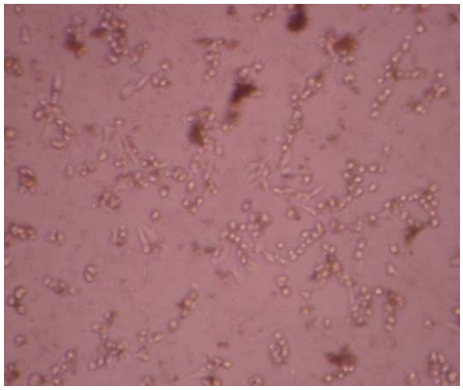


Figure No.4: Inhibition of HeLa by compound **3f** (100 µM)

CONCLUSION

Six novel derivatives of benzimidazole were synthesized, characterized by FTIR and ^{1}HNMR, MASS and evaluated for in *vitro* anticancer activities by MTT assay. Compound **(3f)** exhibited significant cytotoxicity was observed against HeLa cell line with IC_{50} value in the range of 6.13 µM respectively. The result obtained, encourages me to make further research for synthesis different derivatives and *in vivo* trials in experimental animals to broaden their Pharmacological assessment, may provide a new analogue that can overcome drug resistance, prolonged treatment, complex drug regimen and side effects involved.

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BIBLIOGRAPHY

1. Barker H A, Smyth R D, Weissbach H, Toohey J I, Ladd J N and Volcani B E. Isolation and properties of crystalline cobamide coenzymes containing Benzimidazole or 5,6-Dimethylbenzimidazole, *J Biolgical. Chem*, 235(2), 1960, 235(2), 480-488.

2. Hranjec M, Starcevic K, Piantanida I, Kralj M, Marjanovic M, Hasani M, Westman G and Zamola G K. Synthesis, antitumor evaluation and DNA binding studies of novel amidino benzimidazolyl substituted derivatives of furyl-phenyl and thienyl phenylacrylates, naphthofurans and naphthothiophenes, *Eur. J. Med. Chem*, 43, 2008, 2877-2890.
3. Ansari K F and Lal C. Synthesis and evaluation of some new Benzimidazole Derivatives as Potential Antimicrobial Agents, *Eur. J. Med. Chem*, 44(5), 2009, 2294-2299.
4. Townsend L B and Wise D S. The Synthesis and Chemistry of Certain Anthelmintic Benzimidazoles, *Parasitology,* 6(4), 1990, 6(4), 107-112.
5. Mariappan G, Bhuyan NR, Pradeep K, Deepak K and Murali K. Synthesis and biological evaluation of Mannich bases of benzimidazole derivatives. *Ind. J. Chem*. 2011; 50, 1216-1219.
6. Kus C, Kilcigil G A, Ozbey S and Kaynak F B. Synthesis and antioxidant properties of novel *N*-methyl-1,3,4-thiadiazol-2-amine and 4-methyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives of benzimidazole class, *Bioorg. Med. Chem. Lett*, 16, 2008, 4294-4303.
7. Richards M L, Lio S C, Sinha A, Banie H et al. Substituted 2-phenyl benzimidazole derivatives: novel compounds that suppress key markers of allergy, *Eur. J. Med. Chem*, 41, 2006, 950-969.
8. Kerimov G, Kilcigil L, Caneke B, Altanlar N and Scan M M. Synthesis, antifungal and antioxidant screening of some novel benzimidazole derivatives, *J. Enz. Inhib. Med. Chem,* 22, 2007, 696-701.
9. Kumar J R, Jawahar J and Pathak D P. Synthesis of benzimidazole derivatives as antihypertensive agents, *E-J. Chem,* 3, 2006, 278-285.
10. Ramla M M, Omar M A, E L Khamry AM and EL Diwani HI. Synthesis and antitumor activity of 1-substituted-2- methyl-5-nitrobenz imidazoles, *Bioorg Med Chem,* 14, 2006, 7324-7332.
11. Canpolat E and Kaya M. Studies on Mononuclear Chelates Derived from Substituted Schi Base Ligands (Part 4): Synthesis and Characterization of a New 5-Hydroxysalicyliden-P-Aminoacetophenoneoxime and Its Complexes with Co(II), Ni(II), Cu(II) and Zn(II), *Turk. J. Chem*, 29, 2005, 409-415.
12. Croot P L and Johansson M. Determination of Iron Speciation by Cathodic Stripping Voltammetry in Seawater Using the Competing Ligand 2-(2-Thiazolylazo)-*p*-cresol (TAC), *Electroanalysis*, 12, 2000, 565-576.
13. Walia R, Dhamija K, Vandana, Akhtar J and Lamba H S. Synthesis of novel substituted benzimidazole Derivatives as potential antimicrobial agents, *IJPCBS*, 2(3), 2012, 293-298.
14. Walia R, Hedaitullah, Naaz S, Iqbal K and Lamba H S. Benzimidazole derivatives - an Overview, *International journal of research in pharmacy and chemistry,* 1(3), 2011, 565-574.
15. Rajasekaran S, Gopalkrishna Rao and Chatterjee A. Synthesis, Anti-Inflammatory and Anti-oxidant activity of some substituted Benzimidazole Derivatives, *Inter J of Drug Development and Res*, 4(3), 2012, 112-116.
16. Salahuddin, Mohammad S, Avijit M and Mohammad M A. Synthesis, characterization and antimicrobial activity of 1,3,4-oxadiazole bearing 1H-benzimidazole derivatives, *Arabian J Chem*, 1, 2012, 1-6.
17. Salimon, Salih N, Yousif E, Hameed and Hiba I. Synthesis, Characterization and Biological Activity of Schiff Bases of 2, 5-Dimercapto-1,3,4-thiadiazole, *Australian Jour of Basic and App Sci*, 4(7), 2006, 2016-2021.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J Immun Meth*, 65, 1983, 55-63.
19. Monks A et al. Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines, *J Nat Canc Inst*, 83, 1991, 757-766.
20. Singh G S, Mbukwa E and Pheko T. Synthesis and antimicrobial activity of new 2-azetidinones

from *N*-(salicylidene)amines and 2-diazo-1,2-diarylethanones, *Arkivoc*, 9, 2007, 80-90.

21. Asma N, Samreen S, Tabinda F, Humaira M S and Bushra M. Synthesis, characterization, biological evaluation and QSAR of some Schiff base esters: Promising new antitumor, antioxidant and anti-inflammatory agents, *Pak. J. Pharm. Sci*, 26(1), 2013, 113-124.