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SYNTHESIS, CHARACTERISATION AND *IN VITRO* ANTICANCER ACTIVITY OF SOME BENZIMIDOTHIAZOLIDINONE DERIVATIVES

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ABSTRACT

Benzimidazole derivatives plays an important role in medical field with so many pharmacological activities. A series of novel benzimidazole substituted Schiff bases were synthesized by reaction of 2-amino benzimidazoles with corresponding aromatic aldehydes. The prepared Schiff bases were reacted with thioglycolic acid to form correspondingthiazolidinone derivatives. The synthesized title compounds were characterized by means of their FT-IR, ¹HNMR and ESI MS spectroscopy. The above synthesized title compounds were tested for *in vitro* anticancer activity against HCT-116 cell line and PaCa MIA cell line. Among the tested compounds, the compounds 3NB(3-[4-(1*H*-benzimidazol-2-yl)phenyl]-5-(3-nitrophenyl)-1,3-thiazolidin-4-one) and PFB(3-[4(1H-benzimidazol-2-yl)phenyl]-5-(4-fluorophenyl)-1,3-thiazolidin-4-one) exerted significant activity in HCT-116 celllines at micro molar concentration. Compound **PFB** (3-[4(1H-benzimidazol-2-yl) phenyl]-5-(4-fluoro phenyl)-1, 3-thiazolidin-4-one), and compound 2CB3-[4-(1*H*-benzimidazol-2-yl)phenyl]-5-(2- chloro phenyl)-1,3- thiazolidin-4-one have significant activity against PACA MIA cell lines. This may due to presence of electronegative atoms in the benzimidothiazolidinone ringprobably interacts with a specific target molecule to inhibit cell growth.

KEYWORDS: Benzimidazole, Schiff reaction, benzimidothiazolidinone, anticancer, MTT assay.

INTRODUCTION

Benzimidazole contains a phenyl ring fused to an imidazole ring, Benzimidazole Schiff base derivatives become an important benzoheterocyclics. These derivatives are of particular interest because they have a wide range of activities like antiepileptic, antidiabetic, antiviral anticancer etc.[1,2]

Benzimidazole Schiff base derivatives are an important class of benzoheterocycles which has received considerable attention in recent years. Benzimidazole is one of the most important key building blocks for a variety of biologically important molecules. The fact that benzimidazole nuclei is very important pharmaco - -phore in modern drug discovery as well as in medicinal chemistry and that benzimidazole residue is a constituent of vitamin B 12 support their potential use as therapeutics.[3]

Schiff bases derived from aromatic amines and aromatic aldehyde have a wide variety of applications in many fields.[4] Schiff bases, are formed when any primary amine reacts with an aldehyde or a ketone under specific conditions. They are having the general formula RRC=NR. They are crystalline or oily substances that are insoluble in water and soluble in organic solvents.[5,6]

Thiazole is an important heterocyclic ring in anticancer drug research area Interest in the antineoplastic activity of thiazoles increased after the discovery of the thiazole carboxamide moiety containing natural chemotherapeutic agent's tiazofurin, distamycin, netropsin, and thianetropsin.[7,8]

Need for anticancer agents that selectively kill or inhibit the growth ofneoplastic cells without affecting non cancerous host tissues is high and persistent. The aim of the current study was the synthesis of novel benzimidazole derivatives that incorporated different heterocycles of anticancer activity.[9]

In this work combination chemotherapy existing antineoplastic agents with different mechanism of action in one strategy employed to combat this disease. Thus a single molecule containing two functional groups is beneficial in cancer treatment. In this paper the antitumour effects of benzimido-thiazolidinone derivativesis evaluated.

METHODS

All the reagents and chemicals were obtained from Aldrich Chemicals Company (USA) and Merck were used as received. 1H NMR was recorded on a Bruker DPX 300 MHz NMR instrument at ambient temperature in DMSO-*d*6. Chemical shift values are reported in parts per million in δ scale using tetramethy- lsilane (TMS) as the internal standard. Mass spectral data correspond to ESIMS and are given in m/z unit. Melting points were recorded on a SPAC-A Service (India). Open capillary melting point apparatus (Laboratory device) and are uncorrected. Infrared spectra were recorded on a Bruker FTIR Zn. Se (α ATR). Spectra were calibrated against the polystyrene absorption at 1601 cm-1. Samples were scanned in KBr discs. Analytical thin layer chromatography was performed on Merck 20 cm × 20 cm silica gel 60-F254 plates using appropriate solvents. Log *P* values were determined using Alchemy-2000 and Scilog P soft wares(Tripos Co).

STEP-1

General Procedure for 2-Aminophenyl Benzimidazole

Place 2.11g (0.01 mole) Para amino benzoic acid, 1.08g (0.01 mole) of ortho phenylenediamine and 20ml 0.4N HCL in a 100 ml round bottom flask (RBF) attached to a reflux condenser. The content of the reaction mixture reflux along with stirring on a magnetic stirrer for about 5 hr. The reaction mixture should ultimately be almost clear and homogenious, transfer the filterate to a 100 ml beaker and allow it to cool to about 5ºC. Add ammonia solution drop wise in order to adjust the PH. Product will form while stirring. Filter the crystals at the pump, wash with water and dried. Yield (77%) as pale pink coloured solid. M.P. 197°C.[10,11]

STEP-2

Preparation of Schiff Bases

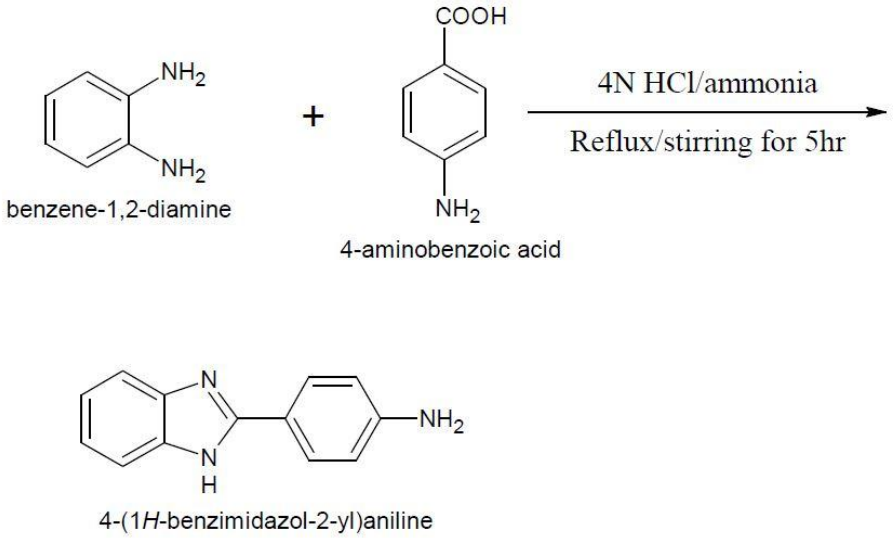
Place 2.09g (0.01 mole) Amino phenyl benzimidazole, 1.06g (0.01 mole) of corresponding benzaldehyde and 50ml ethanol in a 100 ml round bottom flask (RBF) attached to a reflux condenser introduce 1 ml glacial acetic acid drop by drop while the reaction mixture was warming, the content of the reaction mixture reflux on a water bath for 3h, the reaction mixture should ultimately be almost clear and homogenious, transfer the filterate to a 100ml wide-mouthed conical flask containing crushed ice and stirring, Product will formwhile stirring the flask, Filter the crystals at the pump, wash with water and dried.[12]

STEP-3

Preparation of Thiazolidin-4-Ones

Place 0.01 mole of Schiff bases, 0.01 mole of thioglycolic acid and 20ml 1, 4-dioxane in a 100 ml round bottom flask (RBF) attached to a reflux condenser introduce 0.05g zinc chloride as catalyst. Reflux the content of the reaction mixture on a water bath for 8h, the reaction mixture should ultimately be almost clear and homogenious, transfer thefilterate to a 100 ml wide-mouthed conical flask containing crushed ice and stirring, Product will form while stirring the flask, Filter the crystals at the pump, wash with water and dried.

Schemes



Scheme 1: Synthesis of 2- Amino phenyl benzimidazole.

Biological Evaluation

Methodology

The human pancreatic cancer cell line (MIA PaCa-2), human colorectal adeno carcinoma (HCT116) were obtained from National Centre for Cell Science (NCCS), Pune. All the cell lines were grown in Dulbecco's Modified Eagles Medium containing 10% fetal bovine serum (FBS) and maintained at 370C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.[13]

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x105 cells/ml. one hundred

microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 370C, 5% CO2, 95% air and 100% relative humidity. After 24 h the cellswere treated with serial concentrations of the test samples. They were initially dissolvedin neat dimethylsulfoxide (DMSO) to prepare the stock (200 mM) and stored frozen priorto use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 10 fold serial dilutions were made to provide a total of four drugconcentrations. Aliquots of 100 µl of these different drug dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final drug concentrations. Following drug addition the plates were incubated for an additional 48 h at 370C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate dehydro- genase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple form azan. Therefore, the amount of form azan produced is directly proportional to the number of viable cells. After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 370C for 4h. The medium with MTT was then flicked off and the formed form azan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cellinhibition was determined using the following formula. % cell Inhibition = 100- Abs (sample)/Abs (control) x100. Nonlinear regression graph was plotted between % Cell inhibition and Log10 concentration and IC50 was determined using Graph Pad Prism software.

RESULTS AND DISCUSSION

Chemistry

The synthetic strategy to synthesis the target compounds is depicted in Schemes. 2-(4-aminophenyl)-benzimida -zole was treated with different substituted aromatic aldehydes to produce Schiff base. The resulting Schiff bases were subjected to addition reactions with thioglycolic acid in the presence of zinc chloride to form title compounds of Benzimidothiazolidinone derivatives. The target compounds were prepared and the purity of the compounds were ascertained by routine TLC, Chloroform: Ethanol (4: 1) and the consistency in melting points were checked by open capillary tube method and were

uncorrected. The molecular weight, percentage yield of all the synthesized compounds was calculated. compounds are confirmed by means of their IR, ^{1}H NMR, and ESIMS. All the compounds shows the characteristic absorption of IR in the range of 3300, 1700 and 1250 cm^{-1} for N-H, C=O and C-N str. Moreover ^{1}H NMR signal of all the compounds about singlet in the range of [8.2 - 11.5 (δ ppm)] for NH proton of imidazole ring and signals about singlet for CH2 protons of thiazolidinone at [3.6 – 4.3 (δ ppm)]. The mass spectra exhibited intense molecular ion peak of benzimidothiazolidinone. The spectral results of all the synthesized compounds are:

IR spectral analysis

R4NB: (2919, s) C-H str (Asym), (2851, s) C-H str (Sym), (3450, m) N-H str (1710, s) C=O str, (1269, s) C-N str (678, s) C-S str.

MCB: (2922, s) C-H str (Asym), (2849, s) C-H str (sym), (3619, m) N-H str (1690, s) C=O str, (1269, s) C-N str, (678, s) C-S str.

RPMB: (2918, s) C-H str (Asym), (2849, s) C-H str (Sym), (3619, m) N-H str (1674, s) C=O str, (1269, s) C-N str, (678, s) C-S str.

RPFB: (2918, s) C-H str (Asym), (2849, s) C-H str (Sym), (3619, m) N-H str(1673, s) C=O str, (1220, s) C-F str (689, s) C-S str.

RPBB: (2919, s) C-H str (Asym), (2850, s) C-H str (Sym), (3619, m) N-H str (1672, s) C=O str, (1269, s) C-N str, (609, s) C-Br str.

3NB: (2919, s) C-H str (Asym), (2851, s) C-H str (Sym) (3450, m) N-H str(1710, s) C=O str, (1269, s) C-N str, (678, s) C-S str.

^{1}HNMR spectral analysis

BNZ

δ 3.64 (s, 2H, CH2 of thiazolidinone) δ 4.09 (s, 1H, CH of thiazolidinone) δ 6.03 – 6.69 (m, 4H, ArH of benzimidazole) δ 7.47 – 7.78 (m, 4H, ArH) δ 9.64 (s, 1H, NH)
OMB:
δ 2.24 (s, 3H, OCH3 of anisole) δ 4.39 (s, 2H, CH2 of thiazolidinone) δ 5.70 (s, 1H, CH of thiazolidinone) δ 6.90 – 7.10 (m, 4H, ArH of anisole) δ 7.12 – 7.25 (m, 4H, ArH) δ 7.53 – 7.65 (m, 4H, ArH of benzimidazole) δ 8.23 (s, 1H, NH)

PMB:
δ 1.31 (s, 3H, CH3 of toluene)
δ 3.48 (s, 2H, CH2 of thiazolidinone)
δ 4.60 (s, 1H, CH of thiazolidinone)
δ 6.17 – 6.93 (m, 4H, ArH of toluene)
δ 7.04 – 7.23 (m, 4H, ArH of benzimidazole)
δ 7.49 – 7.83 (m, 4H, ArH)
δ 11.04 (s, 1H, NH)
DPB:
δ 2.10 (s, 6H, N(CH3)2 of Dimethylbenzamine)
δ 3.38 (s, 2H, CH2 of thiazolidinone)
δ 4.38 (s, 1H, CH of thiazolidinone)
δ 7.11 – 7.16 (m, 4H, ArH of Dimethylbenzamine)
δ 7.26 – 7.56 (m, 4H, ArH)
δ 7.63 – 7.68 (m, 4H, ArH of benzimidazole)
δ 8.20 (s, 1H, NH)

In Vitro Cytotoxic Activity

To evaluate the *in vitro* cytotoxic potency of newly synthesized compounds an established MTT assay was performed according to the Mosmann's method based on the reduction of the soluble 3-(4,5-methyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) in to a blue-purple formazan product, mainly by mitochondrial reductase activity in side living cells.

In this study, HCT-116 and PaCa MIA cell lines was used. The cells were treated with different concentrations (0.1-100μM) of the newly synthesized compounds. The absorbance was measured by spectrophotometer at 570 nm. The test result is expressed as the concentration of test compounds which inhibits the cell growth by 50% (IC50), each Experiment was performed at least 3 times [there was a good reproducibility between replicate wells with the standard errors below 10%].

HCT-116 Cell line

Ic 50 of 3NB:15.26μM

PBB: 63.67 μM

PaCa MIA cell line

2CB:121.8 μM

PFB:231.5 μM

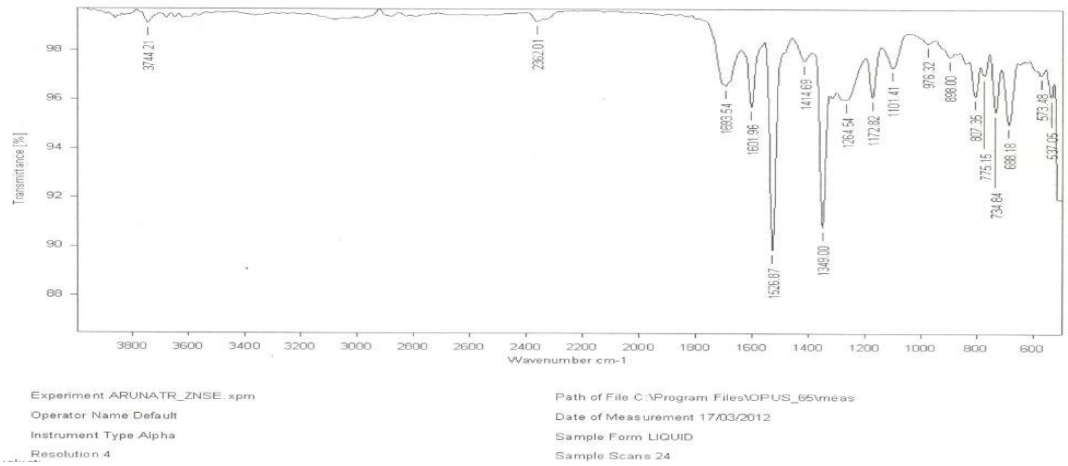


Fig. 1: Infra red spectrum.

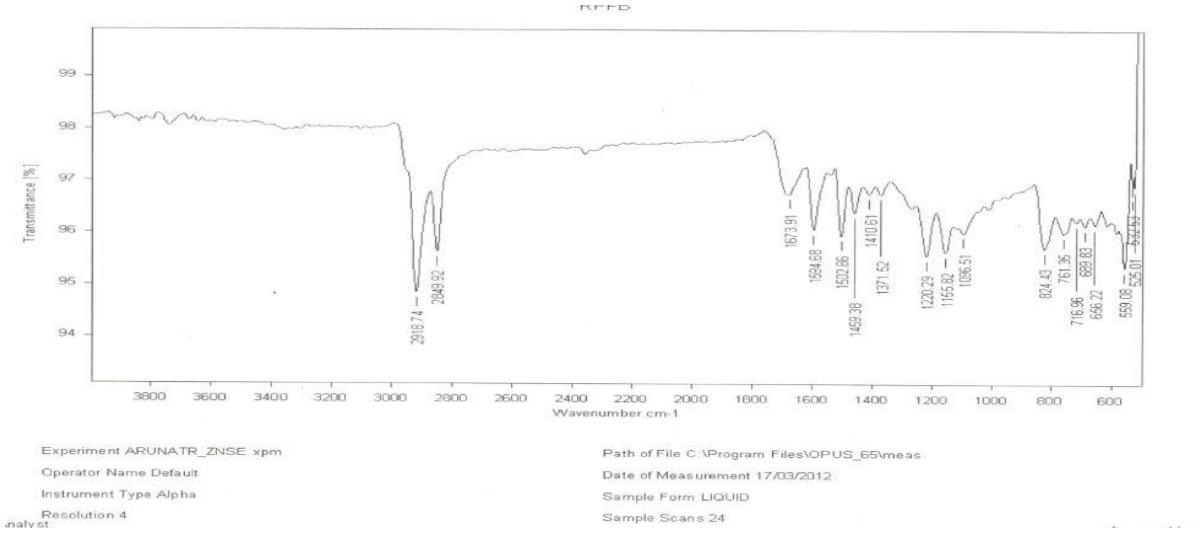
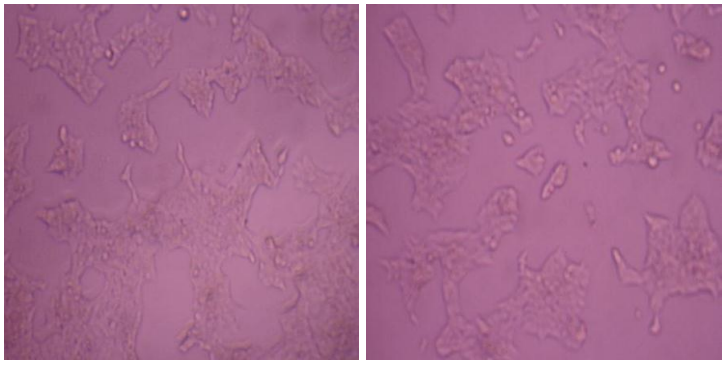
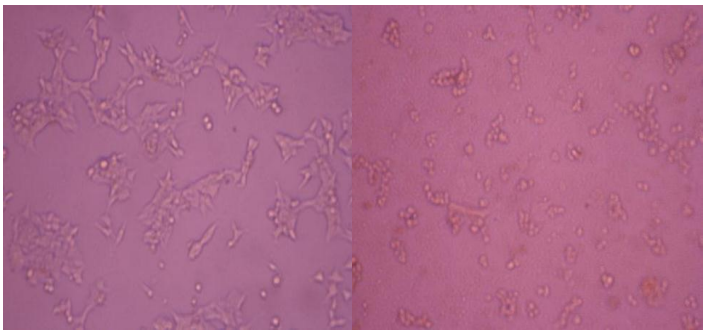


Fig. 2: Infra red spectrum.

Fig. 3: Mass spectrum.



0.1µm **1µm**



10µm **100µm**

Fig. 4: Images of anticancer activity of 3NB on HCT-116.

CONCLUSION

In summary, simple, environment friendly, cost-effective, and convenient method for the synthesis of Benzimidothiazolidinone derivatives was achieved with specific reagents and conditions. 11 new compounds of Benzimidothiazolidinone derivatives have been synthesized, characterized by means of their IR, 1H NMR, and ESIMS and reported. All newly synthesized Benzimidothiazolidinone derivatives were evaluated *in vitro* cytotoxic activities against, HCT-116 and PaCa MIA cell lines. Cytotoxicity values (IC50) are summarized in table. Hence, it is concluded that this remarkable SAR and *in vitro* results were indicating in this series, active compound **3NB** (3-[4-(1*H*-benzimidazol-2-yl) phenyl]-5-(3-nitrophenyl)-1, 3-thiazolidin-4-one) has more active against HCT-116 with IC50 values of 15.26µM. Moreover compound **PFB** (3-[4(1H-benzimidazol-2-yl) phenyl]-5-(4-fluorophenyl)-1,3-thiazolidin-4-one) has the IC50 values of 231µM and against PaCa MIA cell lines respectively. This may due to presence of electronegative atoms in the benzimidothiazolidinone ring probably interacts with a specific target molecule to inhibit cell growth and emerged as a promising anti proliferative agent. Thus there is an ample scope for

further study of this series of compounds. In future we will aim to understand interaction of this compound with molecular targets such as DNA as well as the mechanism of cell death.

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REFERENCES

1. Anshul Chawla, Ramandeep Kaur, Anju Goyal. Importance of microwave reactions in the synthesis of novel benzimidazole derivatives. *A Review, J. Chem. pharm res.*, 2011;
2. L.Srikanth, V.Varun Raj, N. Raghunandan, L.Venkateshwerlu. Recent advances and potential pharmacolo -gical activities of benzimidazole derivatives. *Der pharma chemica*., 2011; 3(2): 172-193. (6): 925-944.
3. Marijana Hranjec, Kristina Starcevic, Sandra Kraljevic Pavelic, Grace Karminski Zamola. Synthesis, spectro scopic characterization and antiproliferative evaluation *in vitro* of novel Schiff bases related to benzimidazoles. *European Journal of Medicinal chemistry*, 2011; 46: 2274-2279.
4. Umesh K.Singh, Surendra N Pandeya, Sandeep K.Sethia, M.Pandey et al. Synthesis and biological evaluation of some Sulfonamide Schiff's bases. *International journal of pharmaceutical sciences and drug research*, 2010; 2(3): 216-218.
5. Vendelin Macho, Milan Kralik, Jozef Hudec, Jarmila Cingelova. One stage preparation of Schiff's bases from nitroarenes, aldehydes and carbon monoxide atpresence of water. *Journal of molecular Catalysis*. *Chemical*, 2004; 209: 69-73.
6. Sweta Singh, Himanshu Ojha, Anjani.K, Tiwari, Nitin Kumar, Bachcha Singh, Anil Kumar Mishra. Design, Synthesis, and *In vitro* Anti proliferative activity of benzimidazole analogues for Radio pharmaceutical efficacy. *Cancer biotherapy and Radiopharmaceuticals*, 2010; 25.
7. Nadeem Siddiqui, Bhanupriya Bhrigu, Devender Pathak, M.Shamsher Alam, Ruhi Ali. Cytotoxicity and enzymes estimation of some newer benzimidazoles. *Annals of Biological Research*, 2011; 2(3): 194-199.
8. Ozkayyusuf, Incesu Zerrin, Isikadag Iihan. Synthesis and anticancer activity of some Dithiocarbamate based bisthiazoles.

9. Zienab M Nofal, Elsyed A.Soliman, Somaia S, Abd el-karim, Magdy I. El Zahar, Aladdin M Srour, Shalinisethumadhavan, Timothy J. Maher. Novel benzimidazole derivatives as expected anticancer agents. *Actapoloniapharmaceutica-Drug Research*, 2011; 68: 519.
10. Panneer Selvam.T, P.P. Radhika, S.Janagaraj, A.Siva Kumar. Synthesis of novel 2 substitute dbenzimidazole derivatives as potential antimicrobial agents. *Research in Biotechnology*, 2011; 2(3): 50-57.
11. Santosh S.Chhajed, Chandrasekhar D, Upasani. Synthesis and Antioxidant Activity of some novel 2-substituted analogues of benzimidazoles. *Journal of pharmaceutical research*, Jan 2011; 4(2): 340-343.
12. Y.S Chhonker, B.Veenu, S.R. Hashim, NiranjanKaushik, Devendra Kumar, Pradeep Kumar. Synthesis and pharmacological evaluation of some new 2- phenyl benzimidazoles. derivatives and their Schiff's bases. *E-Journal of Chemistry*, 2009; 6(S1): 342-346.
13. Hanan M.Refaat. Synthesis and anticancer activity of some novel 2-Substituted benzimidazole derivatives. *European Journal of Medicinal chemistry*, Jan-2010; 45: 2949-2956.