Research Article

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SYNTHESIS AND INVITRO ANTICANCER SCREENING OF SOME NOVEL BENZOTRIAZOLE DERIVATIVES

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Abstract

Mannich bases of benzotriazoles were synthesised and are evaluated for invitro anticancer studies. Different active hydrogen compounds were selected and are reacted with benzotriazole and formaldehyde; with the removal of water molecule the desired mannich bases are obtained. The synthesised compounds were then established on the basis of IR and ^{1}HNMR spectral data and screened for anticancer activity on Human colorectal adenocarcinoma (HCT116) and on Human breast cancer cell line (MDA-MB-468). The derivatives showed moderate activity on both cell lines.

Keywords: Mannich base, Active hydrogen compound, HCT116, MDA-MB-468.

Introduction

Heterocyclic compounds have gained immense importance in human life because of their variety of application particularly these compounds have been successfully tested against several diseases and therefore have required medicinal importance. Nitrogen heterocycles have received special attention in pharmaceutical chemistry due to their diverse Medicinal potential. The study of Benzotriazole derivatives has been a developing field within the realm of heterocyclic chemistry for the past several decades because of their ready accessibility through synthesis, wide range of chemical reactivity and manifold biological activities such as anticancer, antibacterial[1], antifungal, antiinflammatory, antihypertensive, analgesic, anthelmintic, anti filarial, anti asthmatic, diuretic etc with the advantage of low toxicity, high oral availability and broad spectrum activity.

Mannich bases also have been reported as potential biological agents. By transforming amino compounds into N-mannich base forms, increases the lipophilicity of the parent amines at physiological pH values by depressing their protonation, resulting in enhanced bio membrane passage properties. Since cancer is one of the most life threatening disease nowadays, this work focused on the anticancer screening of mannich bases coupled with benzotriazole, which could furnish better therapeutic results[8].

Materials and methods

Synthesis and Characterization

All the chemicals and reagents used in this research work were of analytical or synthetic grade. Melting points of the synthesized compounds were determined by open capillary method and are

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uncorrected. The IR spectra were measured on ATR Zn-Se Bruker FTIR in the range of 4000-400cm^{-1}. The ^{1}H-NMR of the synthesised compounds was recorded in $CDCl_3$ on Bruker Ultra Shield DPX 400 spectrometer. Chemical shift were reported in δ(ppm) relative to Tetra methyl silane (TMS) as internal standard. The reactions were monitored by thin layer chromatography over precoated preactivated glass plates with solvent system Chloroform: methanol (9:1).

Pharmacological screening (MTT ASSAY)

The human colorectal adenocarcinoma (HCT116) and human breast cancer cell lines (MDA-MB-468) were obtained from National Centre for Cell Science (NCCS), Pune. All the cell lines were grown in Dulbecco's Modified Eagles Medium containing 10% foetal bovine serum (FBS) and maintained at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity.

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells. After 48h of incubation of cell lines treated with standard as well as the synthesized compounds, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^{0}C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula,

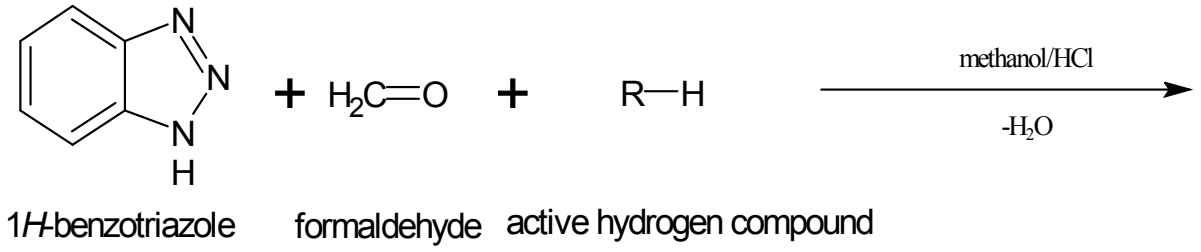
% cell Inhibition = 100 - Abs (sample)/Abs (control) x100.

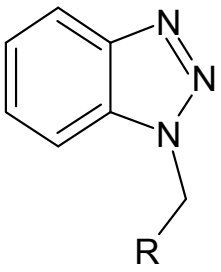
Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC_{50} was determined using Graph Pad Prism software [17]

Synthesis of mannich bases of benzotriazole

General procedure

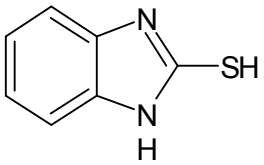
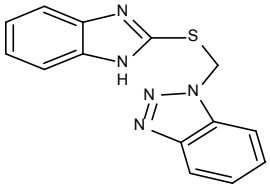
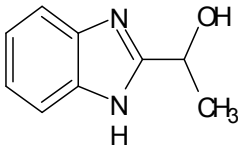
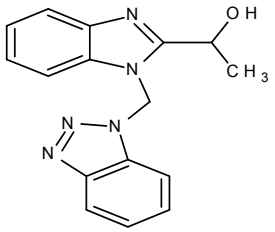
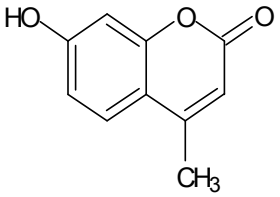
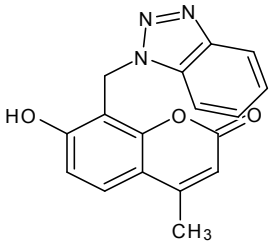
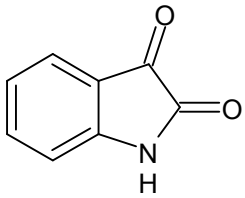
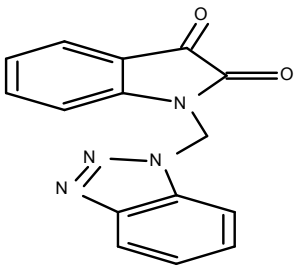
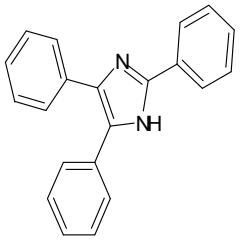
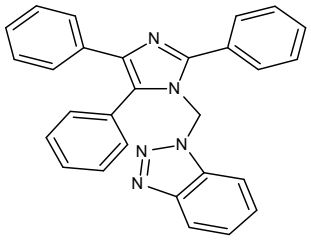
An Equimolar (0.01mol) mixture of benzotriazole, active hydrogen containing compound (2-mercapto benzimidazole, 4-methyl 7-hydroxy coumarin, 2,4,5-triphenyl imidazole, Isatin, 2-hydroxyethyl benzimidazole) [Table 1] and formaldehyde were magnetically stirred and refluxed for 3 hrs with 5ml of methanol in acidic condition. The mixture was kept overnight and the product obtained was washed with acetone and ether, recrystallised from ethanol[4].





mannich base

Table No. 01: List of synthesized compounds

Compound code	R-H	Mannich base
BTZ-MBZ 1-[(1*H*-benzimidazol-2-ylsulfanyl)methyl]-1*H*-benzotriazole		
BTZ-HBZ 1-[1-(1*H*benzotriazol-1-ylmethyl)-1*H*-benzimidazol-2yl]ethanol		
BTZ-MHC 8-(1*H*-benzotriazol-1-ylmethyl)7hydroxy-4-methyl-coumarin**)**		
BTZ-IN 1-(1*H*-benzotriazol-1-ylmethyl)-1*H*-indole-2,3-dione		
BTZ-TPI 1-(1H-benzotriazol-yl methyl)- 2,4,5-triphenyl-1*H*-imidazole.		

Results and discussion

Table No. 02: Physicochemical data of synthesized compounds

Compounds	Molecular formula	Molecular weight	Melting point	Percentage yield	R_f value
BTZ-MBZ	$C_{14}H_{11}N_5S$	281.344	114▫C	55	0.6
BTZ-HBZ	$C_{19}H_{20}N_4O_2$	293.33	127▫C	49	0.9
BTZ-MHC	$C_{17}H_{13}N_3O_3$	307.309	175▫C	62	0.56
BTZ-IN	$C_{15}H_{10}N_4O_2$	278.271	85-90▫C	54	0.62
BTZ-TPI	$C_{28}H_{21}N_5$	427.51	81▫C	58	0.44

Spectral data of synthesized compounds

The synthesized compounds are confirmed with IR and ^{1}H NMR spectra [Table 3]. A new singlet peak of CH_2 is observed in NMR spectra of all the synthesized compounds, which is not found in the parent compound and hence confirmed the formation of mannich base.

Table No. 03: spectral values of synthesized compounds

Compound	Mass value	IR spectra	^{1}H NMR spectra
BTZ-MBZ	81.344	3388(NH),740(CS),1359(C-N), 1511(C=N), 688(ArCHbend),2362(ArCHstr)	3.2(s,2H,CH_2),7.13-7.46(m,4H,ArH of benzimidazole),7.89-8.1(m,4H,ArH of benzotriazole)
BTZ-HBZ	293.33	1656(C=N),1478(N-C-N), 3382(OH), 736(Ar-CHbend), 2362(Ar-CHstr)	1.60 (d, 3H, 3 Hz, CH_3), 3.38 (s,2H,CH_2),4.55 (q, 1H, 27.9 Hz, CH),6.03(s, 1H, OH),7.23 – 7.60 (m, 4H, ArH of benzimidazole),7.90 – 8.07 (m, 4H, ArH of benzotriazole)
BTZ-MHC	307.309	1546(C=N),1462(C-N), 2362(Ar-CHstr), 696(Ar-CHbend	2.07 (d, 3H, 0.4 Hz, CH_3), 2.49 (s, 2H, C-CH_2-N),6.11(s, 1H, Phenolic OH), 6.69 (d, 1H, 1.2 Hz, ArH of Phenolic),6.77 (d, 1H, 8.8 Hz, ArH of Phenolic)7.56 – 7.59 (m, 4H, ArH of benzotriazole), 10.48 (s, 1H, H enol)
BTZ-IN	278.271	1735(C=O),1331(N-C-N), 2989(Ar-CHstr),742(Ar-CHbend) 1459(Al-CHbend)	2.93(s,2H,CH_2),7.26-7.38(m,4H, ArH of benzotriazole, 8.6(s,1H, NH)
BTZ-TPI	427.51	1553(C=N),1398(N-C-N), 2363(Ar-CHstr), 642(Ar-CHbend)	3.59 (s, 2H, N-CH_2-N), 7.19 (m, 4H, ArH of imidazole), 7.59-7.77 (m, 10H, ArH of imidazole), 7.89-7.90 (m, 4H, ArH of benzotriazole)

Pharmacological screening

All the synthesized compounds showed moderate cytotoxic activity towards both the cell lines. Among them BTZ-MHC exhibited comparatively good activity ($1C_{50}$ of 16.76µM) against HCT 116 [Table 5] and IC_{50} of 15.26µM against MDA-MB-468 [Table 4], when compared with the standard drug.

Table No. 04: IC_{50} values of tested compounds in MDA**-MB-468**

Compound Code	% Cell Inhibition				IC_{50} Value(µM)
	0.1µM	1 µM	10 µM	100 µM	
BTZ-MHC	1.904	11.142	39.714	84.285	15.26
BTZ-TPI	1.0623	2.1265	9.75613	82.60871	38.65
BTZ-HBZ	1.0481	7.1694	18.2081	69.2712	42.84
BTZ-MBZ	0.9632	8.213	16.236	60.2136	51.263
BTZ-IN	1.4312	11.3234	38.9912	80.9825	67.6

Table No. 05: IC_{50} values of tested compounds in HCT116

Compound Code	% Cell Inhibition				IC_{50} value (µM)
	0.1µM	1 µM	10 µM	100 µM	
BTZ-MHC	1.0412	8.2356	39.4255	84.97	16.76
BTZ-TPI	0.9523	1.4285	8.9523	63.7142	63.67
BTZ-HBZ	4.571	11.24	25.91	66.38	39.27
BTZ-MBZ	1.859	11.79	18.97	75.35	23.21
BTZ-IN	1.113	7.256	19.231	65.132	64.213

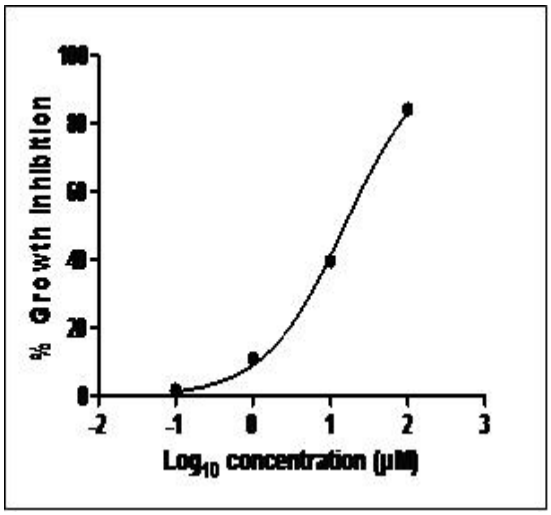


Fig. No. 01:%cell inhibition of BTZ-MHC on MDA-MB468

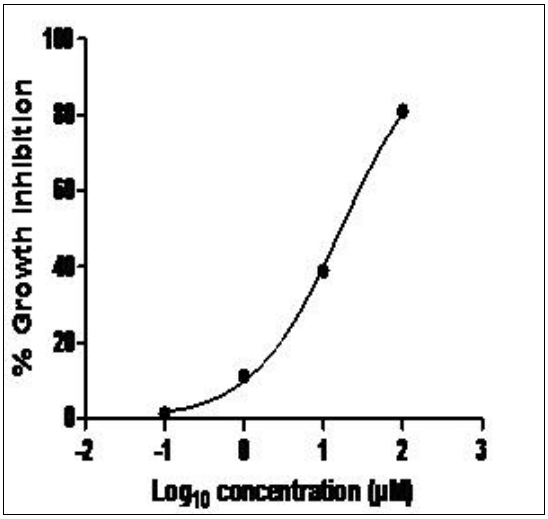


Fig. No. 02: %cell inhibition of BTZ- MHC on HCT116

Summary and conclusion

The present work describes the synthesis of novel mannich bases of benzotriazole along with their invitro anticancer studies. The purity of the compounds and the completion of reaction thus synthesized was ascertained by consistency in melting point and by TLC and the structures of the synthesised compounds were assigned on the basis of the spectral data.

The anticancer activity of mannich bases was screened by MTT assay on HCT116 and MDA-MB-468 cell lines and the report shows that all the compounds have considerable cytotoxic activity towards both the cell lines and hence we can consider these derivatives as future leads for anticancer drug discovery.

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