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## SYNTHESIS AND INVITRO ANTICANCER SCREENING OF SOME NOVEL BENZOTRIAZOLE DERIVATIVES

\*Sreedevi R, Kamalabhai Amma V K, Babu G, Biju C R

Devaki Amma Memorial College of Pharmacy, Pulliparamba, Chelembra,  
Malappuram, India – 673 634.

### 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 <sup>1</sup>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<sup>1</sup>, 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<sup>8</sup>.

### 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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### Author for Correspondence:

Sreedevi R,  
Devaki Amma Memorial College of Pharmacy, Pulliparamba,  
Chelembra, Malappuram, India – 673 634.  
E-mail: [sreedevi.r19@gmail.com](mailto:sreedevi.r19@gmail.com)

uncorrected. The IR spectra were measured on ATR Zn-Se Bruker FTIR in the range of 4000-400 $\text{cm}^{-1}$ . The  $^1\text{H-NMR}$  of the synthesised compounds was recorded in  $\text{CDCl}_3$  on Bruker Ultra Shield DPX 400 spectrometer. Chemical shift were reported in  $\delta(\text{ppm})$  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 $^\circ\text{C}$ , 5%  $\text{CO}_2$ , 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, 15 $\mu\text{l}$  of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 $^\circ\text{C}$  for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 $\mu\text{l}$  of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula,

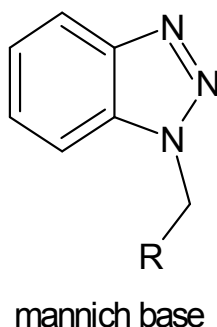
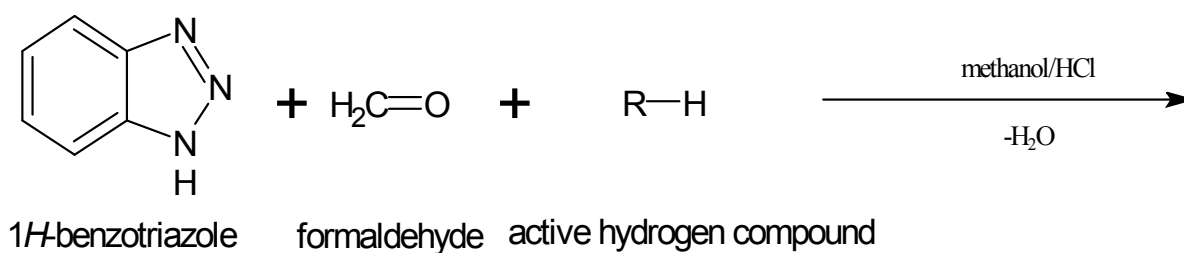
$$\% \text{ cell Inhibition} = 100 - \text{Abs (sample)}/\text{Abs (control)} \times 100.$$

Nonlinear regression graph was plotted between % Cell inhibition and  $\text{Log}_{10}$  concentration and  $\text{IC}_{50}$  was determined using Graph Pad Prism software<sup>17</sup>

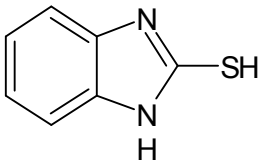
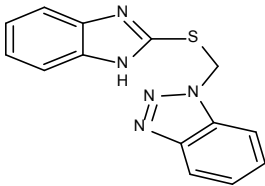
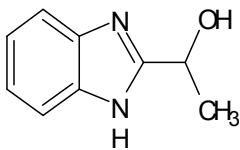
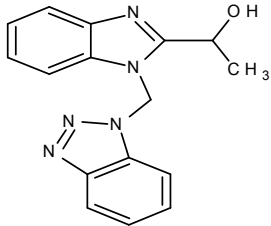
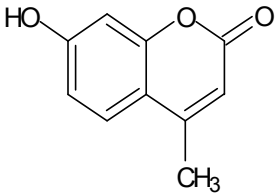
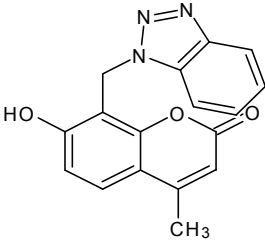
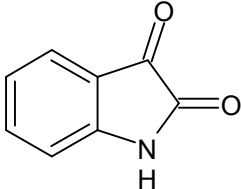
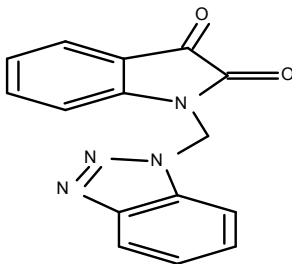
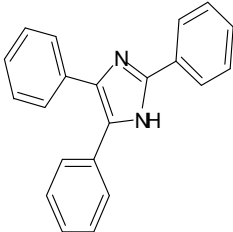
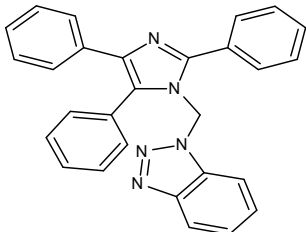
#### Synthesis of mannich bases of benzotriazole

##### 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<sup>4</sup>.



**Table No. 01: List of synthesized compounds**

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

**Results and discussion****Table No. 02: Physicochemical data of synthesized compounds**

Compounds	Molecular formula	Molecular weight	Melting point	Percentage yield	R <sub>f</sub> value
BTZ-MBZ	C <sub>14</sub> H <sub>11</sub> N <sub>5</sub> S	281.344	114 C	55	0.6
BTZ-HBZ	C <sub>19</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub>	293.33	127 C	49	0.9
BTZ-MHC	C <sub>17</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub>	307.309	175 C	62	0.56
BTZ-IN	C <sub>15</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	278.271	85-90 C	54	0.62
BTZ-TPI	C <sub>28</sub> H <sub>21</sub> N <sub>5</sub>	427.51	81 C	58	0.44

**Spectral data of synthesized compounds**

The synthesized compounds are confirmed with IR and <sup>1</sup>H NMR spectra [Table 3]. A new singlet peak of CH<sub>2</sub> 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	<sup>1</sup> 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 <sub>2</sub> ),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 <sub>3</sub> ), 3.38 (s,2H,CH <sub>2</sub> ),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 <sub>3</sub> ), 2.49 (s, 2H, C-CH <sub>2</sub> -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(AI-CHbend)	2.93(s,2H,CH <sub>2</sub> ),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 <sub>2</sub> -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 (IC<sub>50</sub> of 16.76μM) against HCT 116 [Table 5] and IC<sub>50</sub> of 15.26μM against MDA-MB-468 [Table 4], when compared with the standard drug.

**Table No. 04: IC<sub>50</sub> values of tested compounds in MDA-MB-468**

Compound Code	% Cell Inhibition				IC <sub>50</sub> 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<sub>50</sub> values of tested compounds in HCT116**

Compound Code	% Cell Inhibition				IC <sub>50</sub> 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

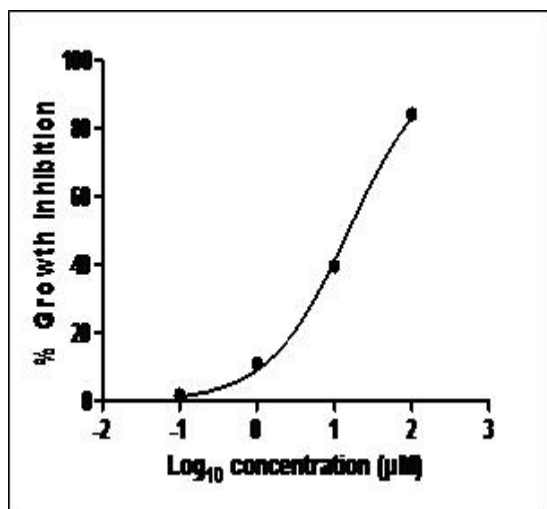


Fig. No. 01: %cell inhibition of BTZ-MHC on MDA-MB468

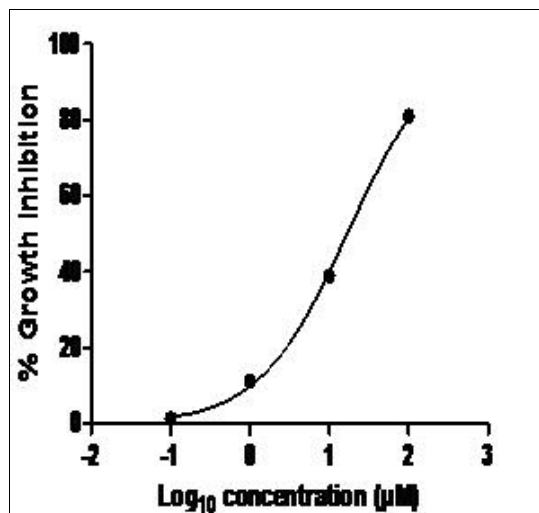


Fig. No. 02: %cell inhibition of BTZ-MHC on HCT116

### 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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### Reference

1. Jun wan, Peng-Cheng LV, Na-Na Tian, Hai-Liang Zhu: Facile synthesis of novel benzotriazole derivatives and their antibacterial activities. *J.Chem. Sci* 2010; 122: 597-606.
2. Marija D. Lazarevic, Janos Csanadi, Ljiljana Klisarova: Synthesis of benzotriazole derivatives. *Bulletin of Chemists and Technologists of Macedonia* 1995; 14:19-22.
3. V.Ravichandran, S.Mohan and K.Suresh kumar: Synthesis and antimicrobial activity of mannich bases of isatin and its derivatives with 2-[(2,6-dichlorophenyl)amino]phenyl acetic acid. *ARKIVOC* 2007; (xiv): 51-57.
4. T B Shah, A Gupte, M R Patel, V S Chaudhari, H Patel, V C Patel: Synthesis and in vitro study of biological activity of heterocyclic N-Mannich bases. *Indian Journal of Chemistry* 2009; 48B: 88-96.
5. Jun Wan, Xia Yan Cuiping Ma , Sai Bi ,Hai-Liang Zhu: Synthesis, structure characterization, and biological evaluation of some new 1,2,3-benzotriazole derivatives. *Med Chem Research* 2010; 19:970–983.
6. Jun Wan, Peng-Cheng L V, NA-NA Tian and Hai-Liang Zhu: Facial synthesis of novel benzotriazole derivatives and their antibacterial activities. *J.Chem. Sci* 2010; 122: 597-606.
7. Jie Fu, Ying Yang, Xue-Wei Zhang, Wen-Jun Mao, Zhi-Ming Zhang, Hai-Liang Zhu: Discovery of 1H-benzo[d][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate as a potential antiproliferative agent by inhibiting histone deacetylase. *Bioorganic & Medicinal Chemistry* 2010;18: 8457–8462.
8. B Shivarama Holla, B Veerendra, M.K Shivananda, Boja Poojary: Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1, 2,4 triazoles. *European Journal of Medicinal Chemistry* 2003; 38: 759–767.

9. Krzysztof Sztanke, Tomasz Tuzimski, Jolanta Rzymowska, Kazimierz Pasternak, Martyna Kandefer-Szerszen: Synthesis, determination of the lipophilicity, anticancer and antimicrobial properties of some fused 1,2,4-triazole derivatives. *European Journal of Medicinal Chemistry* 2008; 43: 404-419.
10. Mohamed Al Messmary, Mohamed Gebriel Elarfi and Rahim Mohamed: Synthesis and Spectral Studies of Mannich bases derived from 2-substituted benzimidazoles. *International Archieve of applied sciences and technology* 2010; 1: 84-86.
11. GiganiYaseen and JadhavSudhakar: Design, synthesis and antimicrobial activity of 2-mercaptopbenzimidazole derivatives. *International Journal of Pharma and Bio Sciences* 2010; 1:312.
12. Jun Wan, Peng-Cheng L V, NA-NA Tian and Hai-Liang Zhu: Facial synthesis of novel benzotriazole derivatives and their antibacterial activities. *J.Chem. Sci*; july 2010; 122:597-606.
13. Li Yuan Mou, Zi Yun Lin, Li Y Zhu, XiaoTianLian: A new investigation of mannich reaction, *Chinese Chemical Letters* 2001;12:471-474.
14. Lingappa.B, Girish K S, Balakrishnan Kalluraya, SatheshRai, NailoSuchephaKumari, Regioselective reaction: Synthesis of novel mannich bases derived from 3-(4,6-disubstituted-2-thiomethylpyrimidyl)-4-amino-5-mercapto-1,2,4-triazoles and their antimicrobial properties. *Indian Journal of Chemistry* 2008; 47B:1858-1864.
15. Mahesh R, Venkatesha Perumal, Microwave induced mannich reaction: Synthesis of some mannich derivatives of p-amino phenol. *Indian Journal of Chemistry*; 2004; 43B:1012-1014.
16. Sheela Joshi, NavitaKhosla, Deepak Khare and RakeshSharda: Synthesis and in vitro study of novel mannich bases as anti-bacterial agents. *Bioorganic and Medicinal chemistry Letters* 2005; 15:221-226.
17. Monks, A., et al: Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. *Journal of the National Cancer Institute*; 83: 757-766.