DESIGN AND IN-SILICO STUDIES OF NEWER N-(SUBSTITUTED BENZOTHIAZOL-2-YL) BENZAMIDE ANALOGUES AND MOLECULAR DOCKING IN COMPARISON WITH NATIVE LIGAND ONALESPIB AS POTENT ANTICANCER AGENTS.

Mohit Agarwal[1*], Vandana Sharma[2]

Abstract:
We report here the preparation of three new benzthiazole analogues using the scaffold hoping technique. All the benzthiazole analogues were predicated for their ADME profiles and toxicity studies. All the compounds were found to follow the Lipinski's rule of 5 with safe toxicity profile (Class IV compound) against immunotoxicity, mutagenicity and toxicity. All of the compounds were designed, followed by their molecular docking against Heat Shock Protein HSP-90 (ALPHA) (PDB code: 2XJX). One of the appealing cancer targets demonstrated an efficient binding within the binding site of HSP-90. Analogue **3a** with docking score = −6.771 kcal/mol have shown stronger H-bond with amino acid (**Thr184** and **Gly97)** showing an efficient binding within the binding site of HSP-90 in comparison with the native ligand Onalespib (XJX) a potent inhibitor of Hsp90.

Keywords: Anticancer; Benzthiazole; Molecular docking; Cell lines studies; Heat Shock Protein inhibitor; Onalespib.

[1*]Research Scholar, Department of Pharmaceutical Chemistry, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan 302001, India, Email: agarwalmohit1709@gmail.com.
[2]Department of Pharmaceutical Chemistry, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan 302001, India, Email: vanu4amit@gmail.com

***Corresponding Author:** Mohit Agarwal,
*Department of Pharmaceutical Chemistry, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan 302001, India, Email: agarwalmohit1709@gmail.com.

DOI: 10.48047/ecb/2023.12.si5a.0444

1. Introduction:

Cancer refers to diseases in which abnormal cells divide uncontrollably and can invade surrounding tissues. Cancer is caused by gene mutation which governs cell development. (1). The estimated cancer cases in India for the year 2022 was found to be 14,61,427, which is expected to increase by 12.8 % in year 2025(2).Compounds having different Nitrogen-based heterocyclic molecules play a crucial role in anticancer drug development because of the vast range of therapeutic effects they exhibit. The aromatic heterocyclic analogue benzothiazole has a 1,3-thiazole a five member ring fused with benzene ring (3, 4). There are various targets of benzthiazole scaffold that show remarkable and prevalent biological and pharmacological activities including antitumor (5), anti-microbial (6), etc.

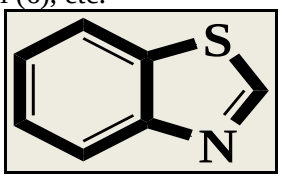

Figure 1: Benzthiazole moiety.

2. ADME and Toxicity prediction studies:

In-silico model to estimate oral bioavailability can be used early in the drug discovery process to select the most promising analogue for further Insilico studies, and later on to identify potential molecules for additional clinical development (7). Three benzthiazole derivatives were gone through pre-software studies, drug-likeness, and solubility parameters of drugs and lipophilic character by ALOGPS 2.1 programme and the prediction of ADME was computed from SwissADME software (8). The toxicity studies were carried out online by ProTox II software (9).

3. Molecular Docking Studies:

The ligands 3a-c were investigated for molecular docking against Structure of HSP90 with small molecule inhibitor bound. The protein data bank (PDB) provided the X-ray crystallographic structure of HSP-90 (PDB code: 2XJX) (10). The ligands (3a-c) saved as mole file and the docking was carried out in accordance with the procedure outlined elsewhere (11).

4. Benzthiazole Scaffold and its substituent's: (Table 1)

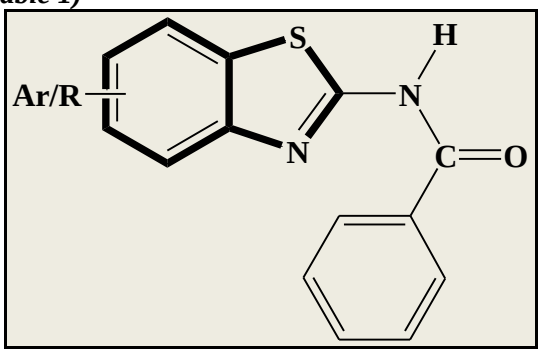

Figure 2: Scaffold of Benzthiazole

Table 1: Detail of Benzthiazole analogues, substituent's and their IUPAC names.

Compounds	Ar/R	IUPAC Name of benzthiazole
3a	7-chloro-4-hydroxy-	N-(7-chloro-4-hydroxybenzothiazol-2-yl)benzamide
3b	H-	N-(benzothiazol-2-yl)benzamide
3c	4,6-dimethyl-	N-(4,6-dimethylbenzothiazol-2-yl)benzamide

5. Result and discussion:

5.1ADME and toxicity prediction for Benzthiazole analogues:

The prediction of ADME was computed from SwissADME software and their results are given in **Table 2** (8). Calculated Partition Coefficients and solubility of benzthiazole analogues investigated are as per shown in **Table 3,** whereas biological activity prediction of the benzthiazole analogues by PASS ONLINE software as discussed in **Table 4.** Apart from all the prediction's the toxicity prediction was also studied for the Benzthiazole (3a-c) and their results are given in the **Table 5**. All the compounds were predicted to be class IV compounds in terms of toxicity and poses slight carcinogenicity.

Table 2: ADME studies calculated for good oral response of title BENZTHIAZOLE Compounds:

Compound	Volume (A^3)	TPSA (A^2)	NROTB	HBA (<10)	HBD (<5)	Log *P* (iLOGP)(≤5)	MW (<500)	Lipinski's Violations(≤1)
3a	238.94	90.46	3	3	2	2.27	304.75	0
3b	217.38	70.23	3	2	1	2.06	254.31	0
3c	230.92	70.23	3	2	1	2.17	288.75	0

Table 3: Calculated Partition Coefficients and solubility of BENZTHIAZOLE analogues investigated.

S. No.	Compound	ALOGPs	XLOGP2
1.	3a	2.27	3.82
2.	3b	2.06	3.10
3.	3c	2.55	4.28

Table 4: Biological activity prediction of the BENZTHIAZOLE compounds by PASS ONLINE software:

Compound	Pa	Pi	Biological profile
3a	0,718	0,007	Neurodegenerative diseases treatment
	0,579	0,003	Proto-oncogene tyrosine-protein kinase Fgr inhibitor
3b	0,799	0,019	Mucomembranous protector
	0,758	0,020	Taurine dehydrogenase inhibitor
	0,730	0,004	Transcription factor STAT inhibitor
3c	0,766	0,029	Mucomembranous protector
	0,690	0,004	Transcription factor STAT3 inhibitor
	0,646	0,004	Transcription factor inhibitor

Table 5: Calculated Toxicity Risk Assessment of the BENZTHIAZOLE investigated by ProTox II Property Explorer.

Compound	Carcinogenicity	Mutagenicity	Cytotoxicity	LD50 (mg/Kg)
3a	-	-	-	1000
3b	+	-	-	1000
3c	-	-	-	1000

(-) INACTIVE, (+) LOW RISK, (++) MEDIUM RISK, (+++) HIGH RISK

5.2Molecular Docking Studies of Benzthiazole Analogues:

We conducted molecular docking experiments for the ligands (3a-c) against Heat Shock Protein HSP-90 (ALPHA) (PDB code: 2XJX) and their results are shown in **Table6.**

Table 6: The molecular docking results of oxadiazole analogues against Heat Shock Protein HSP-90 (ALPHA) (PDB code: 2XJX).

Compound	Docking score	Glide e model	Types of interactions
3a	-6.771	-63.081	H bonding (THR184 and GLY97)
3b	-6.593	-56.081	H bonding (THR184)
3c	-6.481	-53.919	H bonding (THR184)
Onalespib (XJX)	-7.993	-89.853	H bonding (THR184) and salt bridge (ASP54)

• OBSERVATIONS:

The docking was completed in accordance with the procedure with ligands stored as mol files. Molecular docking experiments revealed a variety of interactions, including halogen bonds, π-π stacking, and Hydrogen bonds. The findings of these investigations are as shown in **Table 6.**

- The native ligand **Onalespib (XJX)** was re docked and with docking score = −7.993 kcal/mol, and shown H-bond with the amino acid (Thr184), also there is a salt bridge between nitrogen and amino acid (Asp54) showing strong bonding as shown in Figure 6.3.
- The ligands 3a to 3c demonstrated an efficient binding within the binding site of HSP-90 with docking scores ranging from −6.481 to −7.771 kcal/mol with no any significant interaction with any residues.
- The ligand **3a** (docking score = −6.771 kcal/mol), demonstrated two types of interactions within the binding site of HSP-90 with an H-bond with the amino acid residue (Thr184) and (Gly97), while ligands 3b (docking score = −6.593 kcal/mol), and 3c (docking score = −6.481 kcal/mol) demonstrated similar types of interactions within the binding site of HSP-90 with an H-

bond with the amino acid residue (Thr184) (with carbonyl oxygen of amide group).

- The binding pattern of native ligand Onalespib (XJX) and our screened derivatives (3a-c) shown good binding within the binding pockets of HSP-90 through similar type of H-bond with the key essential amino acid (Thr184), analogue **3a** has stronger H-bond with the amino acid (Thr184 and Gly97). The native ligand Onalespib (XJX) has shown binding energy of −7.993 kcal/mol, where as the screened derivatives **3a** shown nearly same binding energies of −6.771 kcal/mol respectively. From all these studies we conclude that an analogue 3a is an excellent lead for the discovery of promising anticancer agents through the suppression of HSP-90.

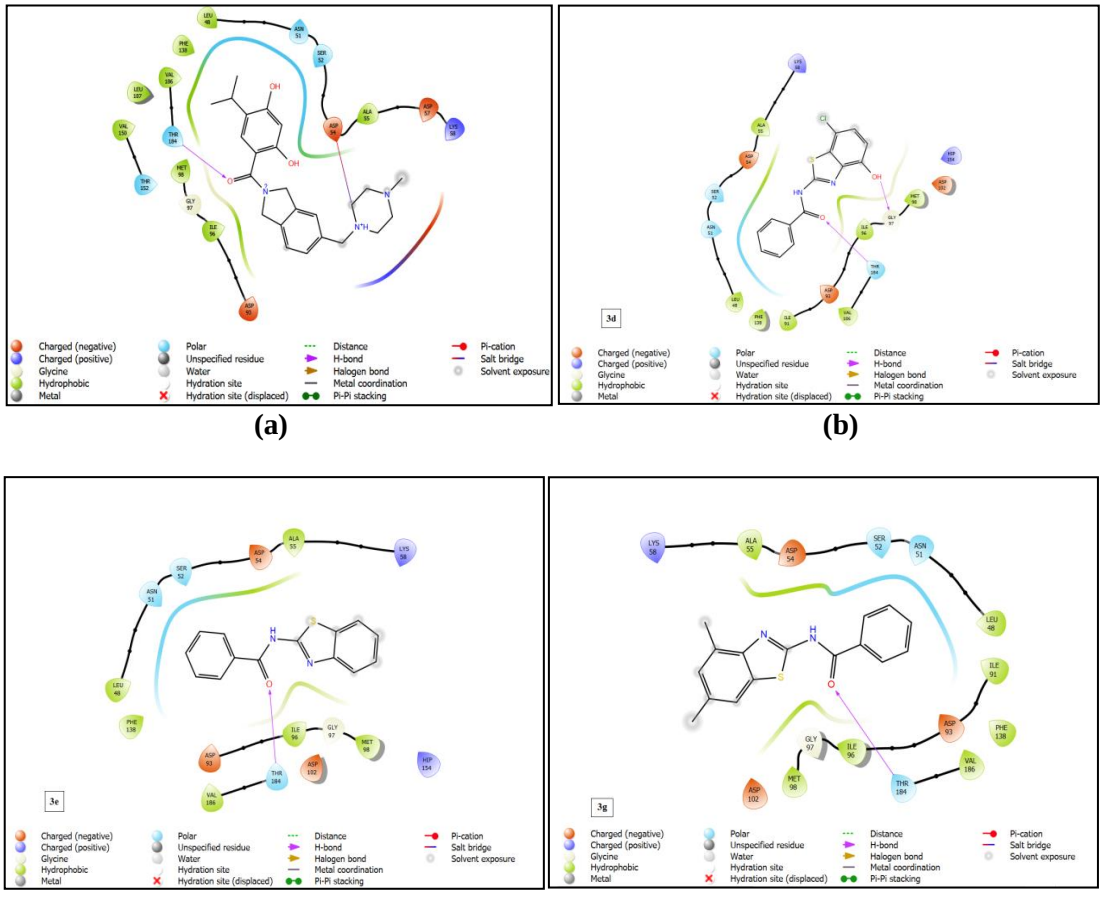

Figure 6.3: The 2D Interactions of the native ligand (a) **Onalespib** (XJX), (b) 3a, (c) 3b and (d) 3c within the binding site of HSP-90.

6. Conclusion:

A series of eight new benzthiazole analogues (3a-c) is prepared. The ADME and toxicity studies were performed. All three analogues followed Lipinski's rule of five with low toxicity. Docking scores for the benzthiazole ligands ranged from −6.425 to −7.822 kcal/mol. The binding pattern of native ligand **Onalespib (XJX)** and derivatives **(3a-c)** shown good binding with the binding pockets of **HSP-90** through similar type of H-bond with amino acid **(Thr184),** one of our derivative **3a** have stronger H-bond with the key essential amino acid (**Thr184**and **Gly97)**. The native ligand **Onalespib (XJX)** has shown binding energy of **-7.993,** whereas derivatives **3a** shown nearly same binding energies of **-6.771** kcal/mol. From all these studies we conclude that the screened analogues **3a** have promising anticancer activity through the suppression of **HSP-90.**

The current study on the anticancer activity of benzthiazole and their in-silico studies may add therapeutic value to benzthiazole. Also, the reported analogues can be modified further to increase their anticancer potentials.

Ethics Approval and Consent to Participate

Not applicable

Human and Animal rights

Not applicable

Consent for Publication

Not applicable

Conflicts of interest

The author confirms that this article content has no conflicts of interest.

Acknowledgements

I consider myself most lucky to work under the guidance of Dr. Vandana Sharma. I take this opportunity to express my heartfelt gratitude to my reverend guide. I am very much grateful to her for her invaluable guidance and everlasting encouragement throughout my course.

The people holding the management of Arya College of pharmacy, Kukas, Jaipur, Rajasthan, India is acknowledged for providing research facilities and help in carrying out my research work.

Reference:

1. Prakash N, Devangi P (2010) Drug Discovery. J Antivir Antiretrovir 2:063-068.
2. Sathishkumar K, Chaturvedi M, Das P, Stephen S, Mathur P. Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. Indian J Med Res. 2022 Dec 13.
3. Punniyakoti Veeraveedu Thanikachalam, Rahul Kumar Maurya, Vishali Garg, Vikramdeep Monga, 'An insight into the medicinal perspective of synthetic analogs of indole: A review', European Journal of Medicinal Chemistry, Volume 180, 2019, Pages 562-612, ISSN 0223-5234.
4. Kakkar, S., Narasimhan, B. A comprehensive review on biological activities of oxazole derivatives. BMC Chemistry 13, 16 (2019).
5. Cai J, Sun M, Wu X, Chen J, Wang P, Zong X, Ji M. Design and synthesis of novel 4-benzothiazole amino quinazolines Dasatinib derivatives as potential anti-tumor agents. Eur J Med Chem. 2013 May;63:702-12.
6. Padalkar, Vikas & Gupta, Vinod & Phatangare, Kiran & Patil, Vikas & Umape, Prashant & Sekar, Nethi. (2014). Synthesis of novel dipodal-benzimidazole, benzoxazole and benzothiazole from cyanuric chloride: Structural, photophysical and antimicrobial studies. Journal of Saudi Chemical Society.
7. Hou,T.; Wang,J.; Zhang,W.;Xu,X. ADME evaluation in drug discovery.6.Can oral bioavailability in humans be effectively predicted by simple molecular property-based rules. *J. Chem. Inf. Model*., 2007, 47, 460-463.
8. Swiss ADME: http://www.swissadme.ch/ (retrieved on 21st January 2023)
9. ProTox II: https://tox-new.charite.de/protox_II/index.php?site=compound_input (retrieved on 25th January 2023)
10. X-ray crystallographic structure of Heat Shock Protein HSP-90 (ALPHA): https://www.rcsb.org/structure/2XJX (Retrieved on 28th January 2023).
11. Woodhead AJ, Angove H, Carr MG, Chessari G, Congreve M, Coyle JE, Cosme J, Graham B, Day PJ, Downham R, Fazal L, Feltell R, Figueroa E, Rees DC, Rich S, Smith DM, Trewartha G, Vinkovic M, Williams B, Woolford AJ. Discovery of (2,4-dihydroxy-5-isopropylphenyl)-[5-(4-methylpiperazin-1-ylmethyl)-1,3-dihydroisoindol-2-yl]methanone (AT13387), a novel inhibitor of the molecular chaperone Hsp90 by fragment based drug design. J Med Chem. 2010 Aug 26;53(16):5956-69.