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SYNTHESIS, ANTICANCER ACTIVITY AND DOCKING OF SOME SUBSTITUTED BENZOTHIAZOLES AS EGFR TYROSINE KINASE INHIBITORS AND TOPOISOMERASE II INHIBITORS

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ABSTRACT:

Plan: *The present study focuses on the structure based drug design approach for anticancer activity of various substituted benzothiazoles whether as an EGFR TK inhibitor or as topoisomerase inhibitor.*

Preface: *The development of novel therapeutic agents for the treatment of cancer is of vital importance since the currently available chemotherapeutic agents only provide palliative care. Benzothiazoles are multitarget agents with broad spectrum of biological activity. Topoisomerase II inhibitors are in clinical use as anticancer therapy for decades and works by stabilizing the enzyme induced DNA breaks.*

Methodology: *Computer aided drug design brings out the molecular study of the enzymes and the various other targets, it opens the new world of drug discovery into a target specific path of a drug towards disease. The Insilico method mainly includes the target selection, selection of lead and the lead optimization.*

Outcome: *Docking results confirmed the possibility of benzothiazole moiety possessing the anticancer activity. The structure was finally characterized by UV, IR, NMR and Mass spectra. In the present work the tumorigenic cell line activity of the substituted benzothiazole is compared with the two standard drugs Gefitinib and Doxorubicin by an Invitro assay against Dalton's Lymphoma Ascites cell using trypan blue dye and percentage inhibition was calculated.*

1. INTRODUCTION

Cancer is one of major human health problem worldwide. Over the past 30 years, significant progress has been achieved in understanding the molecular basis of cancer. It is a multistep process that requires the accumulation of multiple genetic mutations in a single cell that bestow features characteristics of a neoplastic cell. Growth factors are more sensitive to cancer cell and vice versa. Several methods were developed to inhibit these growth factors and its path of action. Sometimes may be preventing the attachment of growth factors or by inhibiting the signalling pathways.

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Normally Cancer therapy has been focussed mainly by targeting cellular signalling pathways[18] like ERK signalling pathway, RAS signalling pathway, c-MET signalling pathway, apoptosis signalling pathway etc. One of the major breakthroughs in the discovery of anti-cancer drug was the inhibition of signal transduction pathway[14-17]. Receptor tyrosine kinases are cell surface receptor and plays vital role in normal cellular process and there by an important role in the control of cancer.

Over expression of EGFR (Epidermal Growth Factor Receptor) results in cancer. EGFR gene encodes protein containing 1186 amino acid and 621 residues, which compromise the extra cellular domain and binding site for specific ligand amino acid residues, which server binding site for EGFR inhibitors. EGFR functioned by MAPK/ERK (mitogen activated protein kinase / extracellular signal regulated kinases) pathway, a signal transduction pathway that couples intracellular response to binding of growth factors to cell surface receptor. The EGFR inhibitors mainly act on MAPK/ERK pathway. Topoisomerases [31, 34, 35] are nuclear enzymes that unwind or alters the double stranded DNA. These enzymes cuts either the end or a particular end and results in transcription and replication. These enzymes are the targets for anti-cancer drugs. The present work was focused on the structure based drug design approach for the anti-cancer activity of various benzothiazoles, as an EGFR TK inhibitor or as a topoisomerase inhibitor.

2. MATERIALS AND METHODS

2.1.Chemicals and reagents: Chlorosulphonicacid , o-aminothiophenol , p-substitutedbenzaldehyde , N-methyl piperazine , pypiridine , morpholine, sodium sulphate, ethanol , chloroform , hydrochloric acid, Dimethyl sulphoxide.

2.2. Analytical work: The melting points of all the synthesised compounds were determined using melting point apparatus MR-VIS,Visual melting range apparatus, LABINDIA were uncorrected. Reactions were monitered by thin layer chromatography on pre coated silica gel using iodine vapour as visualising agent. The UV spectra were recorded on JASCO V-530 UV/VISspectrometer, IR spectra were recorded on JASCO FTIR-420 series in STIC,cochin and the PMR spectra were recorded on BRUKER FT-NMR 2000MHzin STIC,cochin and the Mass spectra were recorded on shimandzu LCMS-2010EV at SRIPMS,coimbatore.

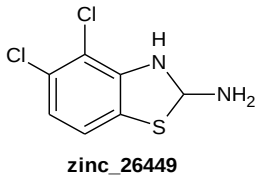
The soft wares and data bases used for the *insilico methods* are iGEMDOCK v.2, ZINC-compound database, Accerlys discovery studio viewer, Molinspiration server, Brookhaven protein data bank Online , SMILES translator, ALOGPS server , AutoDock 4.2 .

2.3. Target Selection.

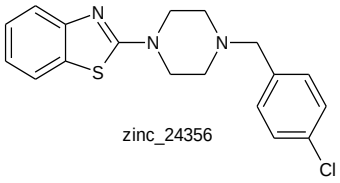
The present study was focused on anti-cancer activity. Drug targets were chosen for the particular activity. Binding site required for exhibiting the anti-cancer activity were obtained from several literature reviews and from the protein data bank. Several targets are available for inhibition of a particular disease .Two targets were selected for the present study are epidermal growth factor receptor (EGFR) and Topoisomerase II. The selected targets were downloaded from Protein Data Bank.

The protein data bank accession code for EGFR is 21TY with a resolution of 3.42 Aº . The 'A' chain of EGFR is constituted by 327 amino acids. The binding site required for the action of EGFR kinase domain is Leu-718-THR 854.The PDB accession code for topoisomerase II is 2RGR with a resolution of 3.3 Aº. The A chain of topoisomerase II is constituted by 759 amino acids and the binding site required for action is Arg 475 –Ser 838.

Virtual screening was carried out using iGEMDOCK and molinspiration server. 1000 compounds were downloaded from Zinc data base and were docked with targets 2ITY and 2RGR separately. Leads were selected based on the binding affinity and their bioactivity score . Some of the structures of the compounds showing highest binding affinity while observing the data base , towards both the targets were as follows :



zinc_26449



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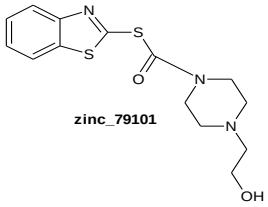
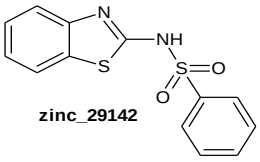
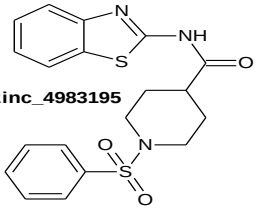
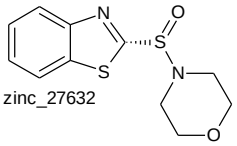


zinc_79101

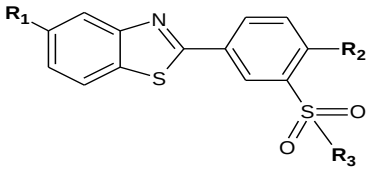


zinc_29142





The lead optimization and toxicity were performed by evaluating the ADME property and drug likeness property .The modified compound on the basis of the lead selected is as follows



Targeted protein were downloaded from Brookhaven Protein Data Bank and docking studies were performed using Auto dock 4.2 version , Acccelrys Discovery Studio , and Cygwin by setting the docking parameters . The snap shots of the docked compounds were downloaded for authenticating and analysing the study data .

Docked result of 30 ligands with two protein targets 2ITY & 2RGR were tabulated in table below. Compounds with lowest binding energy was having the highest binding affinity towards the target 2ITY and 2RGR .

The Binding energies of 30 ligands and 2 standards were tabulated . The results showed that the binding energy of Std -1 docked with 2ITY is -8.53 and the binding energy of Std 2 docked with 2RGR is -8.86. Out of these , the ligands BZ16 ,BZ13 , BZ24, BZ15 , BZ23, BZ4 showed highest binding affinity towards 2ITY and the ligand BZ24 ,BZ6 , BZ8 , BZ13, BZ16, BZ21 showed highest binding affinity towards 2RGR . BZ24 , BZ16 , BZ13 showed highest binding affinity towards both 2ITY and 2RGR.

Table 2: Binding energy values of 30 docked compounds

Sl No:	Compound Code	Binding Energy	
		2ITY	2RGR
1	BZ_1	-8.62	-8.56
2	BZ_4	-8.87	-8.68
3	BZ_5	-8.43	-6.97
4	BZ_6	-8.79	-9.07
5	BZ_7	-7.63	-6.23
6	BZ_8	-8.80	-9.05
7	BZ_9	-8.42	-8.62
8	BZ_{12}	-7.46	-8.33
9	BZ_{13}	-9.18	-9.02
10	BZ_{14}	-7.93	-7.14
11	BZ_{15}	-9.04	-8.69
12	BZ_{16}	-9.50	-8.99
13	BZ_{17}	-7.56	-6.63
14	BZ_{18}	-7.96	-8.26
15	BZ_{20}	-8.79	-6.48
16	BZ_{21}	-7.53	-8.92
17	BZ_{22}	-8.12	-8.52
18	BZ_{23}	-9.03	-8.66
19	BZ_{24}	-9.12	-9.27
20	BZ_{25}	-7.89	-7.78
21	BZ_{26}	-8.32	-7.14
22	BZ_{27}	-8.12	-7.76
23	BZ_{28}	-8.64	-8.46
24	BZ_{29}	-8.12	-6.97
25	BZ_{30}	-8.53	-8.01
26	BZ_{31}	-8.09	-7.96
27	BZ_{32}	-7.86	-7.52
28	BZ_{33}	-7.95	-7.96
29	BZ_{34}	-8.42	-7.43
30	BZ_{35}	-8.01	-8.24

2.4. Invitro Cytotoxicity Assay :

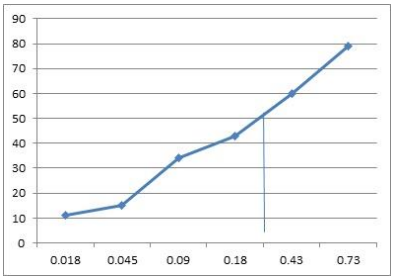
Preliminary invitro cytotoxicity assay was carried out at Amala Cancer Research Centre, Thrissur, by giving sample (dissolving 10 mg of compound in 1 ml DMSO). The Dalton's Lymphoma Ascites Cell tumour cells were aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with normal saline and checked for viability using trypan blue dye exclusion method.

The cell suspension (1×10^6 cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds and the volume was made upto 1 ml using phosphate buffered saline (PBS). Control tube contained only cell suspensions. These assay mixtures were incubated for 3 hour at 37°C and percent of dead cells were evaluated by trypan blue exclusion method.the IC 50 values were calculaed and tabulated in the table .

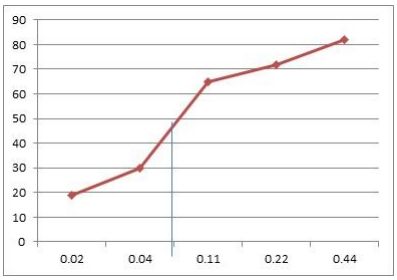
Table.2: The anti-tumour activity screening studies using Dalton's lymphoma ascites

Compound Code		CONCENTRATION (µg/ml)						IC_{50}
		10	20	50	100	200	400	
BZ_6	µM	0.02	0.05	0.13	0.25	0.52	1.03	0.52
	% activity	5	9	22	32	50	71	
BZ_8	µM	0.02	0.05	0.13	0.26	0.53	1.06	0.80
	% activity	0	0	5	16	39	59	
BZ_{13}	µM	0.02	0.05	0.13	0.26	0.53	1.06	0.96
	% activity	0	0	2	10	30	53	
BZ_{16}	µM	0.02	0.05	0.13	0.25	0.51	1.02	0.48
	% activity	6	11	26	34	52	72	
BZ_{24}	µM	0.02	0.05	0.12	0.24	0.49	0.99	0.40
	% activity	7	12	30	41	55	74	
Gefinib	µM	0.02	0.04	0.11	0.22	0.44	0.89	0.08
	% activity	19	30	65	72	82	91	
Doxorubicin	µM	0.02	0.05	0.10	0.18	0.36	0.73	0.30
	% activity	11	15	34	43	60	79	

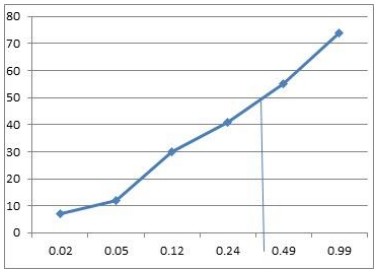
Dose Response Curve for Compounds against *Daltons Lymphoma Ascites Cell*



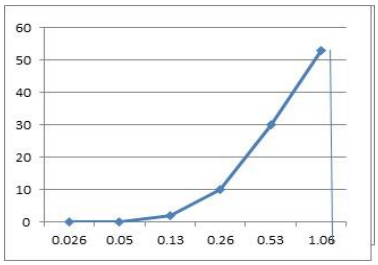
BZ 6



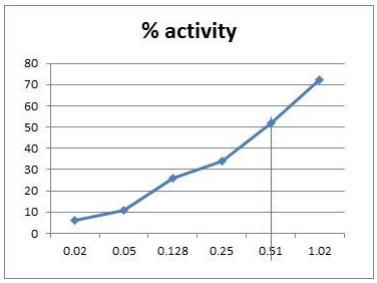
BZ 8



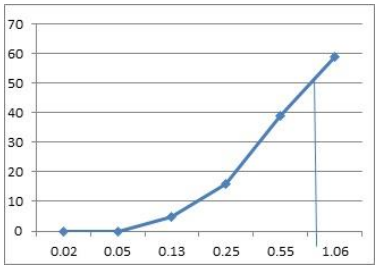
BZ 16



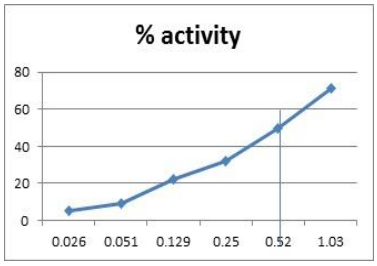
BZ 13



BZ 24



STD -1 (gefinib)



STD- 2 (doxorubicin)

The compounds BZ24 and BZ16 showed potential anticancer activity. Preliminary results showed that they were active as inhibitors of the growth of Daltons lymphoma cells in vitro with IC50 values of 0.4 - 0.48 µM, comparable to gefinib and doxorubicin which were used as reference drugs in this study.

2.5. Synthetic work[19-21]

The two compounds (**BZ 24 and BZ 16**) which show maximum activity during the invitro cytotoxicity assay were selected for synthesis .The purity of the compounds were established by single spot on TLC plates.

Step-1 : Synthesis of5- substituted-2-(4—substituted phenyl)-3-benzothiazole

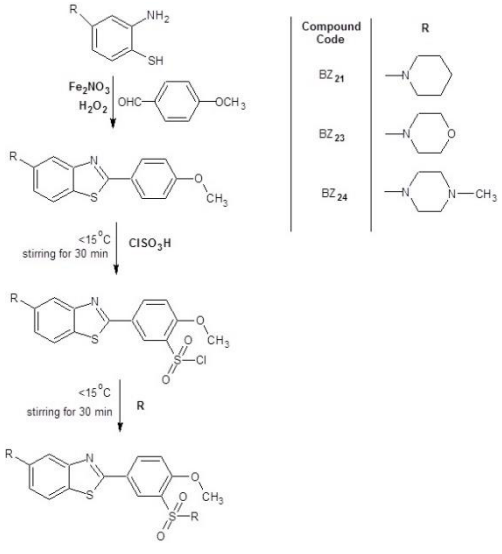
p-substituted benzaldehyde(0.02mol)was treated with 4-substituted o-amino thiophenol 0.02mol ina 250 ml beaker at room temperature.then added 22 ml hydrogen peroxide and 0.8g of ferric nitrate to it.mixture was stirred and vigorous reaction occurred with evolution of heat.compound formed was filtered,dried and recrystallised with ethanol.

Step-2:Synthesis of5-(5-substituted -1,3 benzothiazol-2-yl)-2 substituted benzene sulphonylchloride.

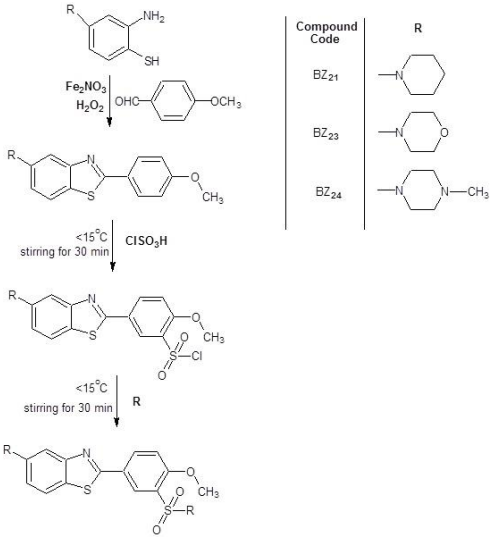
Chloro sulphonic acid 23.2 was taken in a beaker kept in ice bath. Temperature was maintained below 15 C ,4-chloro 2- substituted benzothiazoles was added slowly to the beaker with continous strring using strrer.strring continued for 30 min and temperature was mainteined below 15 C.Thick solution was formed,was as such used in next step.

Step-3: Synthesis of 5- substituted-2(-4 substituted-[4- substituted -1-yl)phenyl 1,3 benzothiazole.

Secondary amines was added drop wise using a dropping funnel with strring to above thicksolution kept in ice bath.strirriong continued for20-30 minutes and temprature maintained below 15º C.The product obtained was recystallised wth mixture of ethanol and chloroform.



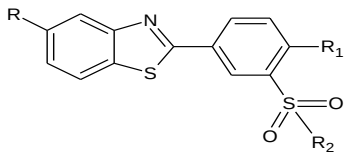
Compound Code	R
BZ_{21}	
BZ_{23}	
BZ_{24}	

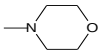
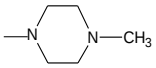
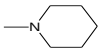
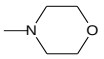
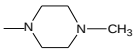
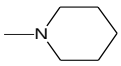
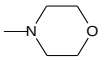
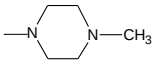


Compound Code	R
BZ_{21}	
BZ_{23}	
BZ_{24}	

R=Cl, R=H

2.6. Physical Characterisation Data:



Code	*R*	*R_1*	*R_2*	*Molecular Formulae*	*Molecular Weight*	*% yield*	*Melting Point °C*	*Rf Value*
BZ_4	-H	$-CH_3$		$C_{19}H_{20}N_2O_2S_2$	372.27	76	139	0.342
BZ_6	-H	$-CH_3$		$C_{18}H_{18}N_2O_3S_2$	374.47	73	142	0.421
BZ_8	-H	$-CH_3$		$C_{19}H_{21}N_3O_2S_2$	387.51	78	127	0.351
BZ_{13}	-H	-OH		$C_{18}H_{18}N_2O_3S_2$	374.47	77	124	0.398
BZ_{14}	-H	-OH		$C_{17}H_{16}N_2O_4S_2$	376.44	80	135	0.452
BZ_{16}	-H	-OH		$C_{18}H_{19}N_3O_3S_2$	389.49	79	125	0.261
BZ_{21}	-CL	OMe		$C_{19}H_{19}ClN_2O_4S_2$	422.94	72	147	0.453
BZ_{23}	-CL	OMe		$C_{18}H_{17}ClN_2O_4S_2$	424.92	75	140	0.396
BZ_{24}	-CL	OMe		$C_{19}H_{20}ClN_3O_3S_2$	455.96	81	132	0.285

2.7. Spectral analysis of compounds:

The structures of the two compounds (**BZ_{24} and BZ_{16}**) were established on the basis of the chemical data, IR, UV, PMR and MASS spectral data. The yields of **BZ_{24}** (5-chloro-2-{4-methoxy-3-[(4-methylpiperazin-1- yl) sulfonyl] phenyl}-1, 3-benzothiazole) and **BZ_{16} (**4-(1, 3-benzothiazol-2-yl)-2-[(4-methylpiperazin-1-yl) sulfonyl] phenol were found to be 81% and 79 % respectively.

Compound: BZ_{24} [$C_{19}H_{20}ClN_3O_3S_2$]**:**

FT IR: 2917.30 cm^{-1} (Aromatic C-H Stretching), 2851.44 cm^{-1} (Aliphatic C-H Stretching), 1083.98 cm^{-1} (C-Cl stretching), 1305.9 cm^{-1} 8 (Asymmetric SO_2 Stretching), 1611.88 cm^{-1} (Aromatic C=N Stretching of Benzothiazole), 621.12 cm^{-1} (Aromatic C-H C-S Stretching of benzothiazole), 1152.76 cm^{-1} (C-O-C stretching).

PMR spectrum: Aromatic proton (6.9 – 8.0 ppm) [no.of protons: 6 nos], hetero cyclic protons 3.9 & 4.8 ppm [no.of protons: 8], aliphatic protons 2.6 ppm [no.of protons: 3], (-OCH_3) aliphatic proton 4.2 ppm [no.of protons: 3].

Mass spectrum: [m/z] 456, 354, 245, 169

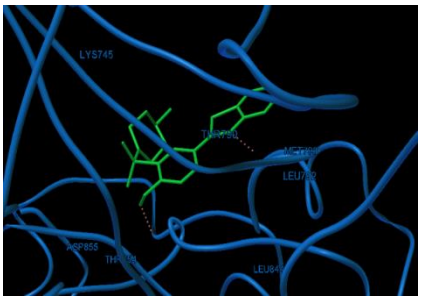
Compound: BZ$_{16}$ **[**$C_{18}H_{19}N_3O_3S_2$]

FT IR: 3428.83 cm^{-1} (Phenolic OH Stretching), 2937.83 cm^{-1} (Aromatic C-H Stretching), 2836.07 cm^{-1} (Aliphatic C-H Stretching), 1600.72 cm^{-1} (Aromatic C=N Stretching of Benzothiazole), 1251.04 cm^{-1} (Asymmetric SO_2 Stretching), 756.8 cm^{-1} (Aromatic C-H bending), 620.33 cm^{-1} Aromatic C-H C-S Stretching of benzothiazole

PMR spectrum: Phenolic OH (10.2 ppm) [no.of protons: 1], aromatic protons (6.9 & 8.5 ppm) [no.of protons: 7], heterocyclic protons 3 & 3.8 ppm [no.of protons: 8], aliphatic proton 2.6 ppm [no.of protons: 3].

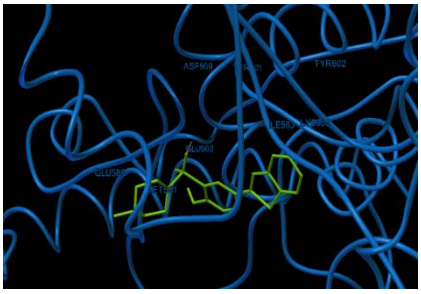
Mass spectrum: [m/z] 390. 306, 211, 135.

Fig.1 Ligands docked to target 2ITY



BZ 16 *BZ 24*

Fig.2 Ligands docked to target 2 RGR



BZ 16 *BZ 24*

3. RESULTS AND DISCUSSION

Nowadays structure based drug design approach proved to be a tool in minimizing the tedious drug discovery process. The docking results obtained confirmed the possibility of benzothiazole moiety possessing the anti-cancer activity. The predicted activity and invitro activity of benzothiazole for topoisomerase II inhibition were easily correlated whereas the action towards EGFR TK could not be established in the study.

In the present work *benzothiazole* has proven as a potent anti-cancer agent, via *topoisomerase II* inhibition. Novel structure based drug design process helped to screen several compounds for the specific activity within in a limited period of time. Present work could be considered as a preliminary study of the titled moiety towards topoisomerase II inhibitory activity and further confirmation studies are warranted in order to further validate several other site specific inhibitory action and enzyme inhibition of these type of compounds.

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