In Sillico Design, Docking, Synthesis, Characterization and EGFR anti cancer Evaluation of Novel 2, 4 Disubstituted Thiazole Derivative

Prabodh V. Sapkale[1]*, Avinash V. Patil[2] and Sandip Patil[3]

[1*]Smt. Sharadchandrika Suresh Patil Institute of Technology (D. Pharmacy), Chopda, Dist. Jalgaon.

[2]Prof. Ravindra Nikam College of Pharmacy, Gondur, Dist. Dhule.

[3]Arts, Commerce and Science College, Chopda, Dist. Jalgaon.

Corresponding Author:

Prabodh V. Sapkale

Ph. D. Research Scholar,

Smt. Sharadchandrika Suresh Patil College of Pharmacy,

Chopda, Dist. Jalgaon

Contact: 9970991877/ 9359744990

Abstract:

Docking studies were performed for the promising compounds to interpret their detected EGFR activities based on their binding interactions with the receptor. Among the series compounds studied, PVS 2.7 exhibited the highest docking score of -7.724 and had a strong affinity towards the target receptor compared to other compounds. All series compounds were synthesized by the conventional economical synthesis method and the reaction was monitored by the TCL method and purify them by column chromatography. Novel 2, 4 disubstituted thiazole derivatives were synthesized to screen their *in vitro* anticancer activity against MCF-7 (Brest Cancer) and A431 (Lung Cancer cell line) cell lines. PVS 2.7 exhibited the best anticancer activity compared to dasatinib. However, PVS 2.10 and PVS 2.4 showed no inhibition against A431 cells, while PVS 2.7 showed no inhibition against MCF-7 cells.

Keywords: Novel thiazole derivatives, anticancer, EGFR Inhibitors, MTT Assay, molecular docking.

Introduction:

Cancer is the uncontrolled growth of cells that have damaged DNA expression. The cancerous cells repeatedly divide, displacing normal tissue. The cancer or neoplasm may be either benign or malignant: a benign cancer stays confined to the tissue of origin, while malignant cancer can spread to other organs. The secondary growths or metastases are a serious complication to any treatment of the cancerous cells[1]. A tumor is any space-filling group of cells that may or may not be cancerous. Benign growths or tumors are usually noted by adding the ending "-oma." For example, adenoma would be a benign growth of the adrenal cortex, a hormone-producing group of cells near the kidney. Malignant tumors are noted by adding "sarcoma" or "carcinoma"[2]. A malignance of the adrenal cortex would be an adenocarcinoma. Bone cancer would be osteosarcoma. Toxicology informs us about cancer on two accounts. First, toxicology research provided insight into the causes of cancer and likelihood of developing cancer. Second, many cancer treatments have serious toxicological side effects. Cancer treatment must often balance the need to kill the cancerous cells and the need to protect healthy cells[3].

Chemicals that induce mutations in the DNA are called mutagens, and when these changes lead to cancer the chemical is called a carcinogen. Not all mutagens are carcinogens, and not all carcinogens are mutagens[4]. In 1946 it was shown that nitrogen mustards (derived from mustard gas first used by the military in 1917 during WWI) could induce mutations in the fruit fly and reduce tumor growth in mice. Genetic toxicology developed ways to test chemical and physical agents for their mutagenic properties, and in the 1970s, Bruce Ames and others developed a cellular-based test for genetic mutations. This test became known as the Ames assay[5]. Sophisticated variations of these tests are now required by many government regulatory agencies to test chemicals for mutagenicity before they are of approved for use[6,7]. Often it is a metabolite (breakdown product) of the compound that causes

cancer, not the original compound[8]. Ideally, a foreign chemical is made less toxic when metabolized, but sometimes a chemical can be made more toxic. This more-toxic chemical can then interact with cellular DNA or proteins and produce malignant cells. This process is called bioactivation[9]. It is also possible for a chemical to encourage bioactivation or to accelerate the development of a cancer. Many variations of the Ames test that include liver cells were developed to simulate the metabolism of the chemical in the liver and determine if bioactivation would result in mutations. Efforts to understand the underlying biology of cancer are ongoing[10]. The genomic sciences are helping to explain why some people are more susceptible to cancer than others. We also know that there are many causes of cancer and that we can reduce the likelihood of developing cancer[11,12].

We are continuously exposed to a wide range of chemical and physical agents, both natural and humangenerated, that may cause cancer. Exposure to sunlight, background radiation, natural and manufactured chemicals, even oxygen can damage our DNA and result in cancer. Because our knowledge is imperfect, there is a great deal of conflicting information on the causes of cancer and what can be done to reduce the risk of developing cancer[13]. And we are just beginning to understand how our individual genetic makeup influences the possibility of developing cancer and other genetic-based disease. Lifestyle choices are the cause of many cancers. Tobacco consumption probably accounts for between 25 to 40% of all cancer deaths. The other major lifestyle choices associated with cancer are alcoholconsumption and diet. Alcohol increases the incidence of liver disease and liver cancer. Diet has a broad range of effects, some good and some not so good. Some cooked meats have a higher concentrations of agents that appear to cause cancer; however, a diet rich in vegetables may reduce the incidence of cancer. High caloric intake and high fat consumption may encourage the onset of cancer from other agents[14]. Numerous organic chemicals are known or likely carcinogens. In the 1930s, benzo(a)pyrene was isolated from coal tar and shown to cause skin cancer.

Further investigation revealed an entire class of carcinogenic compounds called polycyclic aromatic hydrocarbons (PAHs). Shortly after World War II, it was discovered that azo dyes could also cause cancer. In the 1960s, naturally occurring contaminants from a grain fungus (aflatoxin) were found to be a potent liver carcinogen. Inorganic chemicals and fibers are also carcinogenic. Arsenic is the most serious human carcinogen because of exposure from drinking water. Cadmium, chromium, and nickel are all lung carcinogens. The most common lung carcinogen is asbestos, which has unique properties making it ideal for many industrial and even home insulation applications[15]. It was also used in shipyards and in car brake pads. This widespread use resulted in thousands of workers being exposed to asbestos and suffering from a range of lung diseases, including cancer. Asbestos exposure produces a very unique form of lung cancer called mesothelioma. Mesothelioma is caused in part by asbestos fibers inducing a chronic irritation of the lung, resulting in an inflammatory response that ultimately causes some cells to become cancerous. Hormones, which regulate many important bodily functions, are also associated with cancer[16]. One of the first hints of the relationship between hormones and cancers was the observation that nuns had a 8 greater incidence of breast cancer. This was due to the nuns not having children. Since that time there have been numerous studies on the association of birth control, childbirth, and most recently, hormone replacement, with cancer. In males there is ongoing study of hormones and prostate cancer. While it is clear that hormones and cancer are related, the exact characterization of this relationship is still unclear. We are becoming increasingly aware of the importance of diet and nutrition in reducing the risk of cancer. From a toxicological perspective, it is important to reduce exposure to agents that increase the risk of cancer. Cancer, like declining physical and mental ability, is related to old age and may even be a natural consequence of the aging process. However, exposure to cancer-causing agents increases the risk or likelihood of developing cancer[17].

Materials and methods:

Molecular docking study:

The molecular docking Glide module (Schrodinger Inc., USA) has been used for the docking of the compounds against the EGFR Tyrosine Kinase. The X-ray crystallographic structure of EGFR Tyrosine Kinase were retrieved from the protein databank, with accession ID 1M17. The retrieved protein structure was prepared using the "protein preparation wizard" panel. By using prime during the stages of pr-processing, bond ordering was assigned, missing hydrogen was added, disulphide bonds were formed, and missing side chains and loops were modified. In the final refinement stage, the OPLS3 force field has been used to reach complete energetic optimization, with the RMSD of heavy atoms set to 0.3 Å. All the synthesized compound's 3D structures were prepared with the LigPrep panel. The ionization state of each ligand structure was established at a physiological pH of 7.2±0.2. By centralizing the cognate ligand in the crystal structure and using the default box dimension, the active side grid was assigned. Finally, the molecular docking study was carried out using Schrodinger's glide, in which the ready minimum energy 3D structure of the ligands and the receptor grid file were loaded into Maestro's work area and the ligands were docked using extra precision (SP) docking methodology

Synthesis of ethyl 4-methyl-2-phenylthiazole-5-carboxylate.

The solution of benzo thioamide (40 mmol) and ethyl 2-chloro-3-oxo-butanoate (40 mmol) In ethanol (30mL) was refluxed for 3 h. The progress of reaction was monitored by TLC. After completion of the reaction the solvent was distilled on rotary evaporator and residue was dissolved in water. The reaction mixture was neutralized by sodium bicarbonate, extracted by ethyl acetate (3 x 50 mL), the organic was washed with brine, and distilled on rotary evaporator gave crude product. The crude product was purified by the column chromatography using hexane: ethylacetate (9:1) as eluent gave pure product.

Synthesis of 4-methyl-2-phenylthiazole-5-carboxylic acid The solution of ethyl 4-methyl-2-phenylthiazole-5-carboxylate and 10% aq. NaOH was refluxed for 2 h. After completion of the reaction the reaction mass was cooled in ice and acidified by conc. HCl. The solid product

obtained was filtered and washed with water gave 4-methyl-2-phenylthiazole-5-carboxylic acid.

Synthesis of prop-2-yn-1-yl 4-methyl-2-phenylthiazole-5-carboxylate

To the ice-cold solution of 4-methyl-2-phenylthiazole-5-carboxylic acid (10 mmol) in dichloromethane (30 mL) DMAP (20 mmol) was added and the reaction mixture was stirred for 15 min. the clear solution was obtained. To the reaction mixture EDC.HCl (15 mmol) was added and reaction mixture was stirred for 15 min. then propargyl alcohol (15 mmol) was added at 0 °C and reaction mixture was further stirred for overnight. After the completion, the reaction, the reaction mixture was quenched with water and extracted in DCM. Organic layer was washed with brine and dried over sodium sulphate and distilled on rotary evaporator gave crude product. The crude product was purified by column chromatography using hexane: ethylacetate (9:1) as eluent gave pure product.

Synthesis of (1-benzyl-1H-1,2,3-triazol-4-yl)methyl 4-methyl-2-phenylthiazole-5-carboxylate.

The solution of prop-2-yn-1-yl 4-methyl-2-phenylthiazole-5-carboxylate (5 mmol) with substituted benzyl azide (6 mmol), $CuSO_4.5H_2O$ (1 mmol) and sodium ascorbate (1 mmol) in DMF:water (3:1) (4 mL) was stirred at room temperature for 10 h. The progress reaction is monitored by TLC. After completion of the reaction, the reaction mixture was quenched in water and extracted with ethyl acetate. The organic layer was washed with brine, dried by anhydrous sodium sulphate and distilled on rotary evaporator gave crude product. The crude product was purified by column chromatography using hexane:ethylacetate (8:2) as eluent gave pure product.

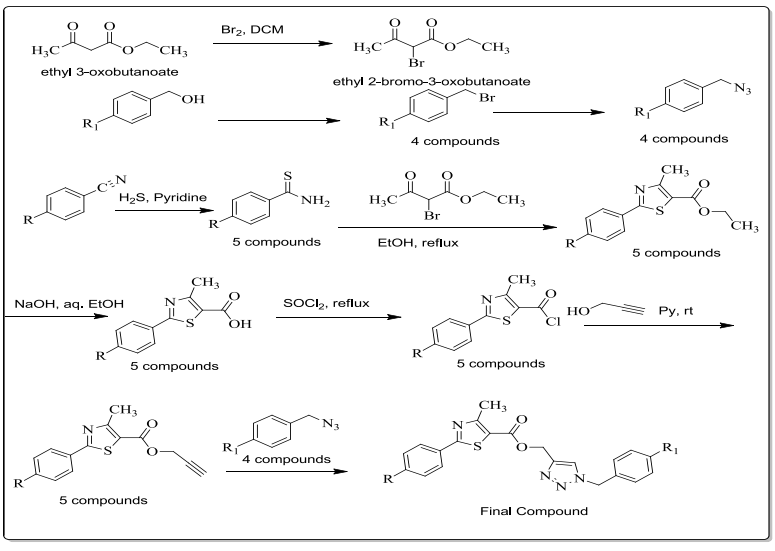
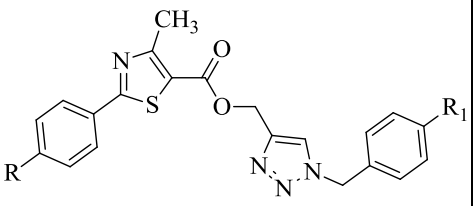


Figure 1: Method for the synthesis of thiazole derivatives

A total of 11 derivatives were synthesised which are summarized below in **table 1.**

Table 1: Novel Thiazole different derivatives

Sr. No.	Compound Code	R Group	Core structure
1.	PVS 2.4	4-chlorobenzyl	
2.	PVS 2.5	4-chlorobenzyl, 4-bromophenyl	
3.	PVS 2.6	4-chlorobenzyl, 4-chlorophenyl	
4.	PVS 2.7	4-fluorobenzyl	
5.	PVS 2.8	4-fluorobenzyl, 4-bromophenyl	
6.	PVS 2.9	4-fluorobenzyl, 4-chlorophenyl	
7.	PVS 2.10	4-methylbenzyl	
8.	PVS 2.11	4-methylbenzyl, 4-bromophenyl	
9.	PVS 2.12	4-methylbenzyl, 4-chlorophenyl	
10.	PVS 2.14	4-fluorobenzyl, 4-fluorophenyl	
11.	PVS 2.16	4-chlorobenzyl, 4-fluorophenyl	

Analysis of the synthesised derivatives:

Melting points were determined by the open tube capillary method and are uncorrected. The progress of reactions was monitored by TLC plates (silica gel G) using mobile phase, Acetone: methanol (8:2), and ethanol: n-hexane (1:5), and the spots were identified by iodine vapours or UV light. IR spectra were recorded on a Schimadzu 8201 PC, FTIR spectrometer (KBr pellets). 1H NMR spectra were recorded on a Bruker AC 400 MHz spectrometer using TMS as an internal standard in DMSO *d6*. Mass spectra were recorded on a Bruker Esquire LCMS using ESI.

Pharmacological Activity:

MTT Assay

Cytotoxicity of the provided samples on MCF-7 (human breast Cell), and A431 (Lung Cancer cell line) was determined by MTT Assay.

1) Cells were incubated at a concentration of 1 × 10_4cells/ml in culture medium for 24 h at 37°C and 5% CO_2.

2) Cells were seeded at a concentration (100µl) 10_4cells/well) in 100µl culture medium and 20, 40, 60, 80, 100µg/ml of Samples into micro plates respectively (tissue culture grade, and 96 wells).

3) Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture.

4) Cell cultures were incubated for 24 h at 37°C and 5% CO_2 in CO_2 incubator.

5) After incubation, the medium was completely removed and Added 20µl of MTT reagent (5mg/min PBS).

6) After addition of MTT, cells incubated for 4 hours at 37oC in CO_2 incubator.

7) Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark colored formazan by viable cells only.

8) After removing the medium completely. Added 200µlof DMSO (kept for 10 min) and incubate at 37^0C (wrapped with aluminum foil).

9) Triplicate samples were analyzed by measuring the absorbance of each sample by a micro plate reader at a wavelength of 550 nm.

Results and Discussions:

Molecular docking study:

The novelty of thiazole derivatives was investigated by searching in Pub Med structural data bank. To explain the observed biological results, molecular docking simulations were performed on PDB ID: 1M17 to determine the binding mode of the novel thiazole derivatives. The H-bond acceptor (HBA) and hydrogen bond donor (HBD) groups in the compounds were optimized for efficient drug-receptor interaction. Dasatinib was used as a standard for novel derivatives.

Among the compounds studied, PVS 2.7 **(Fig no. 1 and table 2)** exhibited the highest docking score of -7.724 and had a strong affinity towards the receptor compared to other compounds.

Table 2: Docking scores of docked molecules with standard Dasatinib

Sr. No.	Compound Code	Docking Score, Receptor (1M17)
1.	**DASATINIB**	**-6.617**
2.	PVS 2.4	-6.146
3.	PVS 2.5	-5.775
4.	PVS 2.6	-6.243
5.	PVS 2.7	-7.724
6.	PVS 2.8	-6.326
7.	PVS 2.9	-7.484
8.	PVS 2.10	-6.056
9.	PVS 2.11	-4.635
10.	PVS 2.12	-5.23
11.	PVS 2.14	-7.718
12.	PVS 2.16	-7.406

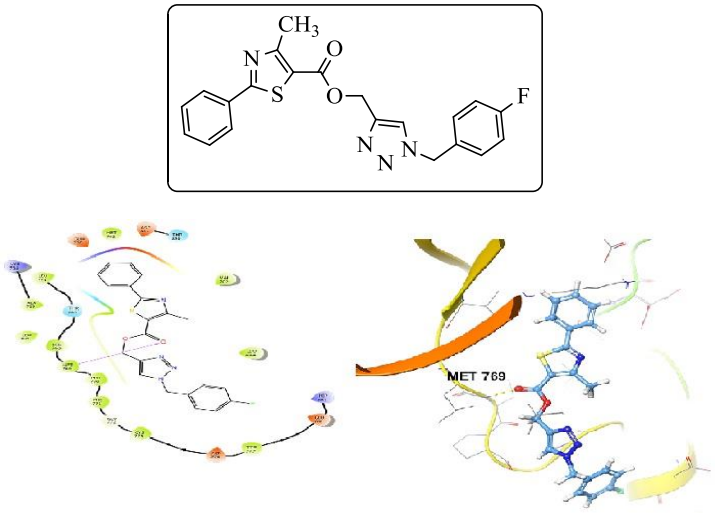


Figure 1: Structure of PVS 2.7 docked molecule; 2D Structure of PVS 2.7 Docked Structure; 3D or Ribbon Structure of PVS 2.7 Docked Structure

Title	Docking Score				Lipnski's Rule					In silico Pharmacokinetic					
Docked molecule	Docking score	Glide evdw	Glide emodel	Glide energy	Molecular Weight	Donor HB	Accpt HB	QPlogPo/w [a]	Rule of Five	QPlogS [b]	QPlogHERG [c]	QPPCaco [d]	QPPMDCK [e]	QPlogKhsa [f]	Percent Human Oral Absorption [g]
PVS 2.4	-6.146	-50.4178	-88.3860	-53.618	424.90	0	4	11.198	0	-9.017	-7.149	4817.189	3493.846	2.319	100
PVS 2.5	-5.775	-48.8931	-61.1843	-51.097	503.80	0	4	16.551	0	-7.319	-10.783	5349.489	5697.746	2.138	100
PVS 2.6	-6.243	-47.5589	-71.1980	-53.299	459.35	4	4	28.589	0	-9.224	-9.481	1147.343	5689.145	1.541	100
PVS 2.7	-7.724	-51.4289	-69.9438	-52.378	408.45	0	4	18.541	0	-6.148	-9.478	5614.134	4178.147	1.571	100
PVS 2.8	-6.326	-52.4928	-78.4872	-49.961	487.34	0	4	19.545	0	-7.219	-8.745	2843.787	3418.213	0.818	100
PVS 2.9	-7.484	-49.1948	-68.4782	-52.093	442.89	0	4	31.785	0	-6.283	-7.061	5019.589	3871.453	1.853	100
PVS 2.10	-6.056	-54.4501	-94.7841	-50.53	404.48	0	4	17.719	0	-14.145	-5.489	4049.486	4089.457	1.589	100
PVS 2.11	-4.635	-39.5949	-54.4578	-46.558	483.38	0	4	18.562	0	-8.417	-6.488	7438.034	3014.452	2.17	100
PVS 2.12	-5.23	-51.8178	-51.4782	-46.505	438.83	0	4	19.315	0	-7.847	-9.182	5038.79	3245.456	2.26	100
PVS 2.14	-7.718	-47.7892	-62.4894	-50.503	426.14	0	4	21.391	0	-8.216	-6.955	6689.789	3256.459	1.15	100
PVS 2.16	-7.406	-53.5948	-90.4782	-52.565	442.89	0	4	20.256	0	-9.478	-7.589	4978.256	3659.145	1.71	100

Table 3: The comparative docking score, Lipnski's Rule parameters, and *In silico* pharmacokinetic parameters of the docked molecules

[a] Predicted octanol/water partition co-efficient log p (acceptable range: −2.0 to 6.5); [b] Predicted aqueous solubility in mol/L (acceptable range: −6.5 to 0.5); [c] Predicted IC_{50} value for blockage of HERG K+ channels (concern below −5.0); [d] Predicted Caco-2 cell permeability in nm/s (acceptable range: < 25 is poor and > 500 is great); [e] Predicted apparent MDCK cell permeability in nm/s; [f] Prediction of binding to human serum albumin; [g] Percentage of human oral absorption (< 25% is poor and > 80% is high)

Chemistry and analysis of synthesised 2, 4 disubstituted thiazole derivatives:

All compounds were synthesized by the traditional synthesis method and the reaction was monitored by the TCL method with an intermediate period also compound was purified by simple solvent crystallization method or column chromatography. The chemical structures of active PVS 2.7 synthesised molecules and standard Dasatinib are presented in **Figure 2.**

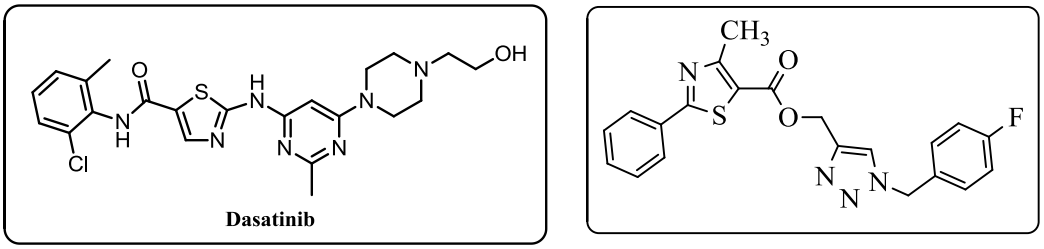


Figure 2: Chemical structures of synthesised molecules and standard Dasatinib

The analysis and purity of the compound were concluded by different analytical methods (NMR, Bruker NMR on 400MHz using $CDCl_3$ as a solvent, Sygenflo Laboratories Llp; Mass by LCMS method; FT-IR by ALR LABS PVT.LTD and HPLC by Chromein Pvt. Ltd.). The percentage yield was calculated in that PVS 2.8 had a greater yield while PVS 2.11 showed a lower yield as shown in table 4.

Table 4: Comparative melting points, %yield, and % purity of the synthesised molecules

Sr. No.	Compound Code	R Group and R_1 Group	Physical Constant (M. P.) (Uncorrected)	% Yield
1	PVS 2.4	4-chlorobenzyl	278^0C-280^0C	89
2	PVS 2.5	4-chlorobenzyl, 4-bromophenyl	190^0C-192^0C	91
3	PVS 2.6	4-chlorobenzyl, 4-chlorophenyl	288^0C-290^0C	78
4	PVS 2.7	4-fluorobenzyl	238^0C-240^0C	72
5	PVS 2.8	4-fluorobenzyl, 4-bromophenyl	260^0C-262^0C	83
6	PVS 2.9	4-fluorobenzyl, 4-chlorophenyl	228^0C-230^0C	71
7	PVS 2.10	4-methylbenzyl	190^0C-192^0C	66
8	PVS 2.11	4-methylbenzyl, 4-bromophenyl	248^0C-250^0C	63

9	PVS 2.12	4-methylbenzyl, 4-chlorophenyl	264^0C-266^0C	78
10	PVS 2.14	4-fluorobenzyl, 4-fluorophenyl	278^0C-280^0C	89
11	PVS 2.16	4-chlorobenzyl, 4-fluorophenyl	214^0C-216^0C	76

The compound PVS 2.4 ((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl 4-methyl-2-phenylthiazole-5-carboxylate) was characterized by ^{1}H NMR, which shows (400 MHz, $CDCl_3$) δ [ppm]: 7.30-7.88 Aromatic (a, b, c, d, e, f) { 10 H }; 5.41 (g) { 2 H }; 5.54 (h) {2H }; 2.74 (i) {2H }. m/z = 425 (M-H) and in IR data it showed the plausible functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1701.52 (C=O, Str., M). 1517.02 (Ar., C=C, Str.), 2952.05 (C-H, Str., med.), 3069.46 (C-H str., aromatic).

PVS 2.5 molecule (1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl 2-(4-bromophenyl)-4-methylthiazole-5-carboxylate, showed ^{1}H NMR of (400 MHz, $CDCl_3$) δ [ppm]: 7.18-7.88 Aromatic (a, b, c, d, e) { 09 H }; 5.41 (f) { 2 H }; 5.49 (g) {2H }; 2.75 (h) {2H }. m/z = 504.8 (M+H). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1653.07 (C=O, Str., M). 1489.07 (Ar., C=C, Str.), 2873.77 (C-H, Str., med.), 2927.82 (C-H str., aromatic).

PVS 2.6 (1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate compound was also characterized by ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 7.24-7.88 Aromatic (a, b, c, d, e) { 09 H }; 5.41 (f) { 2 H }; 5.51 (g) {2H }; 2.75 (h) {2H }. m/z = 459.0 (M+H). The IR data showed the functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1593.43 (C=O, Str., M). 1492.79 (Ar., C=C, Str.), 2953.91 (C-H, Str., med.), 3117.91 (C-H str., aromatic).

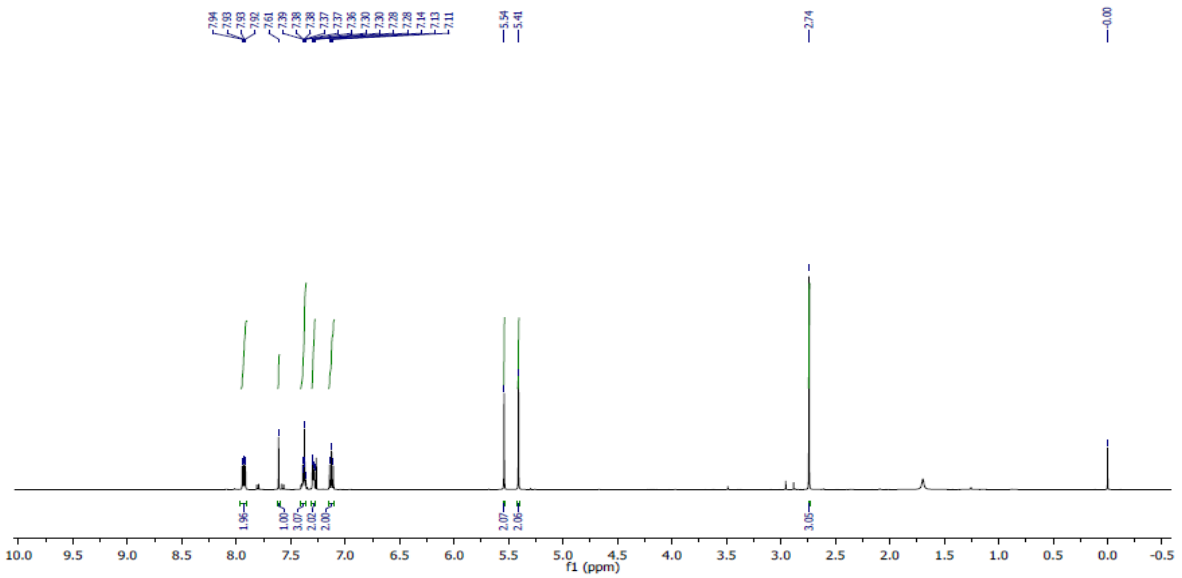


Figure 3: ^{1}H NMR spectra of synthesised PVS 2.7 compound

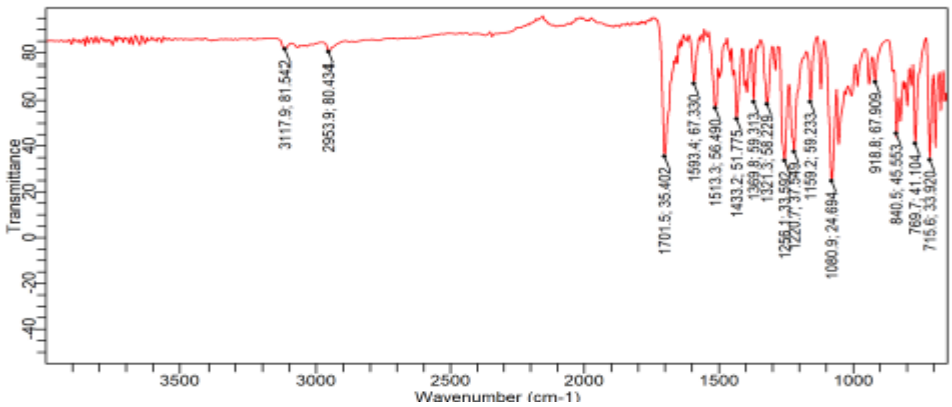


Figure 4: FTIR spectra of synthesized PVS 2.7 compound

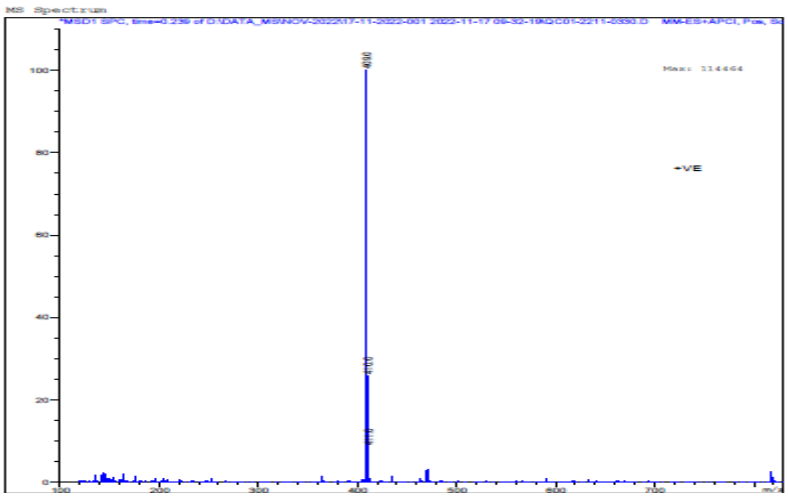


Figure 5: Mass spectra of synthesized PVS 2.7 compound

The compound PVS 2.7 (1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl 4-methyl-2-phenylthiazole-5-carboxylate) was characterized by ^{1}H NMR, and showed peaks at (400 MHz, $CDCl_3$) δ [ppm]: 7.13-7.93 Aromatic (a, b, c, d, e, f) { 09 H }; 5.41 (f) { 2 H }; 5.54 (g) {2H }; 2.74 (h) {2H } while m/z = 409.0 (M+H). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1701.52 (C=O, Str., M). 1593.43 (Ar., C=C, Str.), 2953.91 (C-H, Str., med.), 3117.91 (C-H str., aromatic), 1256.11 (C-F str.)

The compound PVS 2.8 (1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl 2-(4-bromophenyl)-4-methylthiazole-5-carboxylate) showed ^{1}H NMR peaks at (400 MHz, $CDCl_3$) δ [ppm]: 7.11-7.93 Aromatic (a, b, c, d, e, f) { 09 H }; 5.41 (f) { 2 H }; 5.49 (g) {2H }; 2.74 (h) {2H } and m/z = 487.0 (M-H, 1.3%). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1703.39 (C=O, Str., M). 1595.30 (Ar., C=C, Str.), 1256.11 (C-F str.).

The compound PVS 2.9 (1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate) was characterized by ^{1}H NMR, and showed peaks at (400 MHz, $CDCl_3$) δ [ppm]: 7.07-7.88 Aromatic (a, b, c, d, e) { 09 H }; 5.41 (f) { 2 H }; 5.51 (g) {2H }; 2.74 (h) {2H } while m/z = 443.0 (M+H). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1708.98 (C=O, Str., M). 1511.43 (Ar., C=C, Str.), 2924.09 (C-H, Str., med.), 3121.64 (C-H str., aromatic), 1256.11 (C-F str.)

The compound PVS 2.10 (1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl 4-methyl-2-phenylthiazole-5-carboxylate) was characterized by ^{1}H NMR, which shows (400 MHz, $CDCl_3$) δ [ppm]: 7.23-7.83 Aromatic (a, b, c, d, e, f) { 10 H }; 5.41 (f) { 2 H }; 5.54 (g) {2H }; 2.29 (h) {2H } while m/z = 455.0 (M+H). The IR spectra showed a functional group

of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1705.25 (C=O, Str., M). 1513.29 (Ar., C=C, Str.), 2920.36 (C-H, Str., med.), 3082.50 (C-H str., aromatic).

The compound PVS 2.11 (1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl 2-(4-bromophenyl)-4-methylthiazole-5-carboxylate) was characterized by ^{1}H NMR, which shows (400 MHz, $CDCl_3$) δ [ppm]: 7.16-7.84 Aromatic (a, b, c, d, e) { 09 H }; 5.41 (f) { 2 H }; 5.49 (g) {2H }; 2.40 (h) {2H } while m/z = 483.0 (M+H). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1701.52 (C=O, Str., M). 1433.16 (Ar., C=C, Str.), 2916.64 (C-H, Str., med.), 3117.91 (C-H str., aromatic).

The compound PVS 2.12 (1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate) was characterized by ^{1}H NMR, which shows (400 MHz, $CDCl_3$) δ [ppm]: 6.91-7.94 Aromatic (a, b, c, d, e) { 09 H }; 5.40 (f) { 2 H }; 5.47 (g) {2H }; 2.74 (h) {2H } while m/z = 493.0 (M+H). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1703.39 (C=O, Str., M). 1513.29 (Ar., C=C, Str.), 2955.77 (C-H, Str., med.), 3119.78 (C-H str., aromatic).

The compound PVS 2.14 (1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl 2-(4-fluorophenyl)-4-methylthiazole-5-carboxylate) was characterized by ^{1}H NMR, which shows (400 MHz, $CDCl_3$) δ [ppm]: 7.11-7.95 Aromatic (a, b, c, d, e) { 09 H }; 5.41 (f) { 2 H }; 5.51 (g) {2H }; 2.74 (h) {2H } while m/z = 427.0 (M+H). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1708.98 (C=O, Str., M). 1509.57 (Ar., C=C, Str.), 2955.77 (C-H, Str., med.), 3117.91 (C-H str., aromatic).

The compound PVS 2.16 (1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl 2-(4-fluorophenyl)-4-methylthiazole-5-carboxylate) was characterized by ^{1}H NMR, which shows (400 MHz, $CDCl_3$) δ [ppm]: 7.15-7.94 Aromatic (a, b, c, d, e) { 09 H }; 5.41 (f) { 2 H }; 5.51 (g) {2H }; 2.74 (h) {2H } while m/z = 443.0 (M+H). The IR spectra showed a

functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1705.25 (C=O, Str., M). 1511.43 (Ar., C=C, Str.), 2924.09 (C-H, Str., med.), 3119.78 (C-H str., aromatic).

MTT Assay:

MTT assay is one of the most effective method to check the cell viability of cell line, to evaluate safe, and efficient amount of the drug. MTT assay is a colorimetric assay for assessing cytotoxicity or cytostatic activity[18.19].

Cytotoxicity of the provided samples on MCF-7 cell line was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96 well plate for 24 h in RPMI medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO2. Next day cells were treated as per client instructions (different concentration were prepared in complete medium). After incubation for 24 hours, MTT (a final concentration of 0.5 mg/ml) was added to cell culture and further incubated for 2 h. At the end of the experiment, culture supernatant was removed and cell layer with matrix was dissolved in 100 µl DMSO (dimethyl sulfoxide)and read in an Elisa plate reader (iMARK, Biorad, USA) at 540 nm And 660[20].

For A431 (Lung Cancer cell line) following media is used DMEM with high glucose (Cat No-11965-092), FBS (Gibco, Invitrogen) Cat No -10270106 Antibiotic – Antimycotic 100X solution (Thermo fisher Scientific)-Cat No-15240062.

The effect of compounds PVS 2.7, 2.9 on the growth of these cell lines was then assessed. The results showed that MCF-7 cells depleted nearly 90% of the culture medium by 4 days following inoculation, while A431 cells had only consumed 100% of the sugar during the same period. The effect of PVS 2.7 at 100 mcg/ml is presented in **figure 6.**

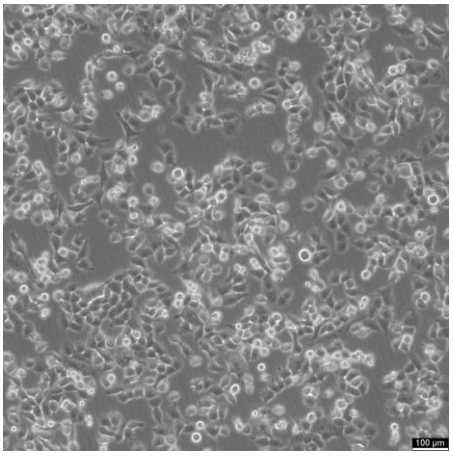


Figure 6: MTT assay observation at 100 mcg/ml concentration of PVS 2.7 molecule

On the other hand, in the presence of compounds PVS 2.10, PVS 2.4 and PVS 2.6 shows the low percent of inhibition and shows the Moderate anticancer activity against the A431 cell. While PVS 2.11, PVS 2.5, PVS 2.14, PVS 2.9 and PVS 2.8 shows greater inhibition and best activity against the same cell line.

Compounds PVS 2.7, PVS 2.9, PVS 2.11, PVS 2.14 shows greater activity against MCF-7 cell while other remain shows moderate and low activity agaist the same cell line.

Conclusion:

These inhibitors were designed to form hydrogen bonds with the protein backbone, while peripheral groups oriented towards two hydrophobic pockets known as binding regions. The binding mode of dasatinib was used as a basis of comparison with compound PVS 2.7, which exhibited the most significant activity in the substituted series. We concluded that replacing the thiazole nitrogen in dasatinib with a C-CN function could result in a novel series of EGFR inhibitors that would not require water bridging, as dasatinib does. This was supported by the cytotoxic effect of PVS 2.7 in the evaluated tumor cell lines. The anticancer activity of the synthesized thiazole derivatives was evaluated against two tumor cell lines, namely MCF-7 and A431. PVS 2.7 exhibited the best anticancer activity compared to dasatinib. However, PVS 2.10, PVS 2.4 and PVS 2.6 showed no inhibition against A431 cells. Overall, these findings suggest that the novel thiazole derivatives could be potential candidates for the development of EGFR inhibitors with improved anticancer activity. The results also highlight the importance of evaluating the anticancer profiles of these compounds on multiple cell lines to assess their broad-spectrum activity.

Conflict of interest: Authors have no conflict of interest.

Acknowledgements:

I am very thankful to my research guide Dr. Avinash V. Patil and Research Center for providing me facilities to do these research work.

References:

1. Baghdiguian S, Martin M, Richard I, Pons F, Astier C, Bourg N, Hay RT, Chemaly R, Halaby G, Loiselet J, Anderson LV, Lopez de Munain A, Fardeau M, Mangeat P, Beckmann JS, Lefranc G (1999) Calpain 3 deficiency is associated with myonuclear apoptosis and profound perturbation of the IkappaB alpha/NFkappaB pathway in limb-girdle muscular dystrophy type 2A. Nat Med 5(5):503–511. doi:10.1038/8385
2. Bamborough P, Morse MA, Ray KP (2010) Targeting IKKbeta for the treatment of rheumatoid arthritis. Drug News Perspect 23(8):483–490. doi:10.1358/dnp.2010.23.8.1447844
3. Cheong JK, Virshup DM (2010) Casein kinase 1: complexity in the family. Int JBiochemCell Biol43(4):465–469. doi:10.1016/j.biocel.2010.12.004
4. Collaborative Computational Project (1994) The CCP4 suite: programs for protein crystallography. Acta Crystallogr D BiolCrystallogr 50(Pt 5):760–763. doi:10.1107/S0907444994003112
5. Criswell LA (2010) Gene discovery in rheumatoid arthritis highlights the CD40/NF-kappaB signaling pathway in disease pathogenesis. Immunol Rev 233(1):55–61. doi:10.1111/j.0105-2896.2009.00862.x
6. Demer L, Tintut Y (2011) The roles of lipid oxidation products and receptor activator of nuclear factor-kappaB signaling in atherosclerotic calcification. Circ Res 108(12):1482–1493. doi:10.1161/CIRCRESAHA.110.234245
7. Elphick LM, Lee SE, Gouverneur V, Mann DJ (2007) Using chemical genetics and ATP analogues to dissect protein kinase function. ACS Chem Biol 2(5):299–314. doi:10.1021/cb700027u
8. Giamas G, Hirner H, Shoshiashvili L, Grothey A, Gessert S, Kuhl M, Henne-Bruns D, Vorgias CE, Knippschild U (2007) Phosphorylation of CK1delta: identification of Ser370 as the major phosphorylation site targeted by PKA in vitro and in vivo. Biochem J 406(3):389–398. doi:10.1042/BJ20070091
9. Gietzen KF, Virshup DM (1999) Identification of inhibitory autophosphorylation sites in casein kinase I epsilon. J Biol Chem to death-receptor mediated apoptosis through multiple mechanisms. Prostate 67(15):1641–1653. doi:10.1002/pros.20653
10. Gil A, Maria Aguilera C, Gil-Campos M, Canete R (2007) Altered signalling and gene expression associated with the immune system and the inflammatory response in obesity. Br J Nutr 98(Suppl 1):S121–S126. doi:10.1017/S0007114507838050

11. Li H, Malhotra S, Kumar A (2008) Nuclear factor-kappa B signaling in skeletal muscle atrophy. J Mol Med (Berl) 86(10):1113–1126.doi:10.1007/s00109-008-0373-8
12. Li X, Su J, Cui X, Li Y, Barochia A, Eichacker PQ (2009) Can we predict the effects of NF-kappaB inhibition in sepsis? Studies with parthenolide and ethyl pyruvate. Expert Opin Investig Drugs 18(8):1047–1060. doi:10.1517/13543780903018880
13. Lin L, Peng SL (2006) Coordination of NF-kappaB and NFAT antagonism by the forkhead transcription factor Foxd1. J Immunol 176(8):4793–4803 pii: 176/8/4793
14. Mashhoon N, DeMaggio AJ, Tereshko V, Bergmeier SC, Egli M, Hoekstra MF, Kuret J (2000) Crystal structure of a conformation-selective casein kinase-1 inhibitor. J Biol Chem 275(26):20052–20060. doi:10.1074/jbc.M001713200
15. Milne DM, Palmer RH, Campbell DG, Meek DW (1992) Phosphorylation of the p53 tumour-suppressor protein at three N-terminal sites by a novel casein kinase I-like enzyme. Oncogene 7(7):1361–1369.
16. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983 Dec 16;65(1-2):55-63. doi: 10.1016/0022-1759(83)90303-4. PMID: 6606682.
17. Basic Biology and Clinical Assessment, Stephen-2005, Pages 343-349.
18 . MTT Assay Protocol for Cell Viability and Proliferation by Sigma pvt.Ltd.
19. Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 65(1-2):55-63.
20. Hansen MB, Nielsen SE, Berg K. 1989. Re-examination and further development of aprecise and rapid dye method for measuring cell growth/cell kill. Journal of Immunological Methods. 119(2):203-210.