Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.14.029

Design and synthesis of 2, 4 disubstituted thiazole derivatives as a potential anticancer agent: Molecular docking, synthesis, analysis, and biological activity study

Prabodh V. Sapkale[1]*, Avinash V. Patil[2], Sandeep A. 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, Commerece and Science College, Chopda, Dist. Jalgaon

***Corresponding author:** Prabodh V. Sapkale, Smt. Sharadchandrika Suresh Patil Institute of Technology (D. Pharmacy), Chopda, Dist. Jalgaon.

Submitted: 20 March 2023; Accepted: 17 April 2023; Published: 28 May 2023

ABSTRACT

Molecular docking studies were performed for the promising compounds to interpret their detected enzymatic activities based on their binding interactions with the receptor. Among the compounds studied, PVS 03 exhibited the highest docking score of -7.811 and had a strong affinity towards the receptor compared to other compounds. It also formed a hydrophobic bond with C10, C17, C18, and C14, which is essential for anticancer activity. All compounds were synthesized by the traditional synthesis method and the reaction was monitored by the TCL method. The percentage yield was calculated in that PVS 01 had a greater yield while PVS 04 showed a lower yield as shown in table 4, also % purity was carried out by the HPLC method in that PVS 10 have a greater purity while PVS 04 have less purity in the series of novel thiazole derivatives. Novel 2, 4 disubstituted thiazole derivatives were synthesized to screen their in vitro anticancer activity against Hep-G2 and MDAMB-231 cell lines. PVS 03 exhibited the best anticancer activity compared to dasatinib. However, PVS 06 and 07 showed no inhibition against MDAMB-231 cells, whilePVS01 to PVS 10 showed no inhibition against Hep-G2 cells. These previous encouraging results of biological evaluation of the newly synthesized thiazole could recommend an excellent framework for the detection of new potent antitumor leads. Overall, these findings suggest that the novel thiazole derivatives could be potential candidates for the development of EGFR inhibitors with improved anticancer activity.

Keywords: *thiazole derivatives, anticancer, EGFR inhibitors, molecular docking, Lipinski's Rule*

INTRODUCTION

Cancer is a complex disease that arises from abnormal cell growth and division. It is a major public health concern worldwide and one of the leading causes of death. According to the World Health Organization, cancer is responsible for approximately 9.6 million deaths annually. [1] The development of targeted therapies that specifically inhibit the signaling pathways involved in cancer growth and proliferation has been a major focus of cancer research in recent years. [2] One approach has been to target mutant or aberrantly expressed oncogenic growth factor receptors and non-receptor tyrosine kinases, which play a key role in these signaling pathways. EGFR (epidermal growth factor receptor) is a protein that is found on the surface of cells in the body.

It plays a role in the growth and division of cells, and its overexpression has been associated with the development and progression of several types of cancer. [3] EGFR tyrosine kinase inhibitors (TKIs) are a class of drugs that target the EGFR protein and inhibit its activity. EGFR TKIs have been approved for the treatment of various types of cancer, including non-small cell lung cancer (NSCLC), pancreatic cancer, and head and neck cancer. [4] While they have shown promising results, not all patients respond to EGFR TKIs and resistance can develop over time. [5] Ongoing research is focused on identifying new treatment strategies to overcome resistance and improve outcomes for cancer patients.

Heterocyclic compounds are a diverse class of molecules widely found in nature and crucial for various biological processes. [6] Among the heterocyclic molecules, those containing the thiazole moiety demonstrate a broad range of biological activities. [7] The synthesis of the basic thiazole nucleus is well-established, and literature on the involved reactions can guide the creation of derivatives. [8] Thiazoles and their derivatives are fundamental components of many biochemical processes and play important roles in drug development. [9] Thiazoles are organic compounds related to azoles, characterized by a shared thiazole functional group. [10] Thiazole was first described by Hantzsch and Waber in 1887. Popp confirmed its structure in 1889. [11, 12] Thiazole-containing molecules that contain a thiazole amine-moiety exhibit compelling anticancer activities that vary depending on the substitution pattern at the thiazole ring. [13] Thiazole derivatives have been extensively studied for their pharmacological applications, particularly in the development of drugs with anticancer properties. This unique scaffold is useful for several natural, non-natural, and semi-synthetic drugs due to its ability to interact with biological targets. [14] Tiazofurin is a thiazole-containing drug that has been shown to have anticancer properties by inhibiting the enzyme inosine monophosphate dehydrogenase (IMPDH). [15] Dasatinib is another thiazole-containing drug that has been approved for the treatment of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL). [16] Dabrafenib is a thiazole-containing drug that is used to treat patients with metastatic melanoma that has a specific genetic mutation (BRAF V600E or V600K). [17] Overall, thiazole derivatives have shown great promise in the development of drugs with anticancer properties. Ongoing research in this area may lead to the discovery of even more effective treatments for various types of cancer.

Molecular docking is a computational technique that has become increasingly important in recent years for drug discovery and design. To improve the efficiency of virtual screening, sequential filters can be applied to narrow down the number of compounds that are selected for further biological evaluation. These filters may include criteria such as drug-likeness, pharmacokinetic properties, and predicted toxicity. [18] Overall, molecular docking and virtual screening approaches are powerful tools that can help to accelerate the drug discovery process by identifying potential drug candidates with a high binding affinity and selectivity for a target protein or enzyme. The virtual screening protocol used in this study is based on the application of sequential filters to select a restricted number of compounds to be submitted for biological evaluation. In the present study, structure-based virtual screening approaches have been used. [19] In detail, (i) a library of EGFR tyrosine kinase was made based upon literature search (ii) the binding mode of all retrieved compounds was evaluated by molecular docking, using the 3D structure of EGFR (PDB ID: 1M17); (iii) The next filter was Lipinski's rule of five to evaluate drug-likeness, which becomes an essential tool to facilitate drug discovery. (iv) Further we checked the novelty of compounds in terms of Tyrosine Kinase Antagonists using SciFinder (v) finally; the virtually screened hits were synthesized and evaluated for their inhibitory. [20]

This study focuses on the design and synthesis of 2,4 disubstituted thiazole derivatives with potential anticancer properties. Building on previous research in this area, our goal was to identify biologically active targets for the treatment of cancer. The synthesized prototypes were evaluated through *in vitro* antitumor screening against two cancer cell lines: HepG-2 and MDAMB-231. The most active derivatives were then assessed for their inhibitory activity against the EGFR kinase. In addition, molecular docking studies were conducted to gain insights into the binding modes of the most potent compounds within the active site of the EGFR kinase.

Overall, this study provides a comprehensive analysis of the potential anticancer properties of thiazole derivatives and sheds light on their mechanism of action through molecular docking studies. The results of this research may help to inform the development of more effective treatments for cancer.

MATERIALS AND METHODS

Experiments

Structure-based virtual screening was conducted using a graphical user interface SP-docking mode of the program Maestro 9. The protein structure of EGFR-TK (PDB ID: 1M17) was obtained from the RCSB Protein Data Bank (PDB). [21] The protein was optimized for docking from its raw state employing protein preparation wizard with OPLS 2005 force field for minimization Receptor gird generation was accomplished using Glide. Further, we analyzed the compounds for Advance Lipinski's rule of five to evaluate drug-likeness using QikProp. [22]

Molecular docking study

The molecular docking tool, GLIDE was used for ligand docking studies into the EGFR-TK pocket. The crystal structure of EGFR was obtained from the protein data bank, PDB ID: 1M17. The protein preparation was carried out using 'protein preparation wizard' in Maestro 9.0 in two steps, preparation, and refinement. Grids were generated centering on Dasatinib. The ligands were developed using a maestro build panel and prepared by the Ligprep 2.2 module that produces the low-energy conformer of ligands using the OPLS 2005 force field. The low energy conformation of the ligands was selected and docked into the grid generated from protein structures using standard precision (SP) docking mode.

Advance Lipinski's Rule for Drug Likeliness

Physically significant descriptors and pharmaceutically relevant properties such as molecular weight, log p, H-bond donors, and H-bond acceptors of all the synthesized compounds, according to Advance Lipinski's rule of five. Hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) groups in the compound optimize the drug-receptor interaction. Advance Lipinski's rule of five is a rule of thumb to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would most likely make it an orally active drug in humans. The rule describes the delicate balance between the molecular properties of a compound, which directly influence its pharmacodynamics and pharmacokinetics, and ultimately affect their ADME in the human body like a drug. In general, these parameters allow us to ascertain poor oral absorption or membrane permeability that occurs when the evaluated molecules present values higher than five H-bond donors (HBD), 10 H-bond acceptors (HBA), molecular weight (MW) > 500 Da and LogP (cLogP) > 5 (Lipinski's 'rule-of-five'). We also evaluated the number of violations of Advance Lipinski's rule of five. Compounds that satisfy these rules are considered drug-like. Compounds with fewer (and preferably no) violations of these rules are more likely to be orally available.

Synthesis of 2, 4 disubstituted thiazole derivatives

Ammonium thiocyanate (2) (0.071 mol, 1 Eq.) was taken in acetone in a 250 ml round bottom flask. To it, corresponding aroyl chloride (1) (0.071 mol, 1 Eq.) was added. The reaction mixture was then refluxed for 5 minutes. The formation of aroyl isothiocyanate (3) was identified by yellow coloration. The precipitate of ammonium chloride was then filtered and washed with acetone to remove aroyl isothiocyanate (yellow liquid) completely. To a stirred solution of aroyl isothiocyanate (3) taken in acetone was cooled to 0^0 C and added a secondary amine adduct (4) (0.071 mol, 1 Eq.) dropwise while maintaining the temperature between 0–5^0 C. After completion of the addition of secondary amine adducts (4) reaction was maintained between 0-5^0 C until the product (5) precipitated out. The product was washed with cold acetone and dried under a vacuum. Benzamidine derivatives (0.6288 moles, 2 eq.) were taken in acetonitrile in a 250 ml round bottom flask. To it, corresponding triethyl amine was added, and stirred the reaction for 10 to 15 min. Then dichloro acetone (0.3144 moles, 1eq.) was added to reflux the reaction mixture for 3-4 hours. The completion of the reaction was observed by the TLC. When both the reactants reacted with each other shown by TLC the reaction was completed. After completion of the reaction cold water was added to the reaction

mixture the product was precipitate out. After that product was filtered out. For the purification of the compound, column chromatography was carried out.

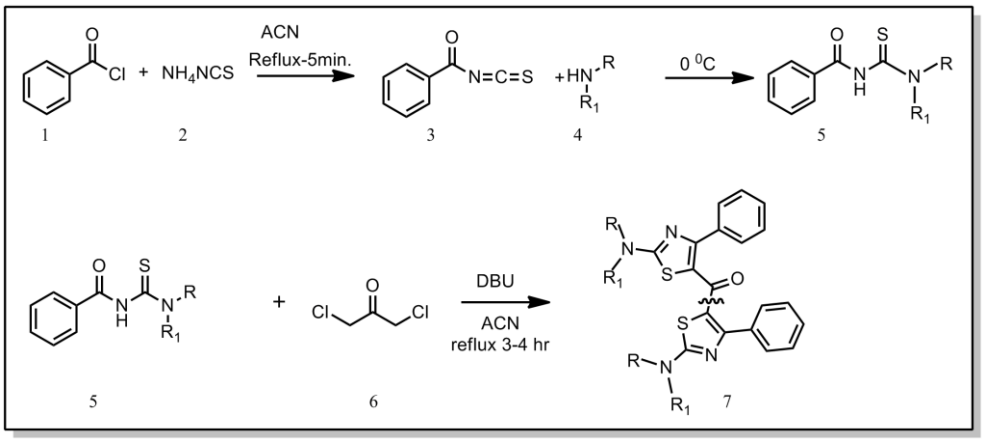
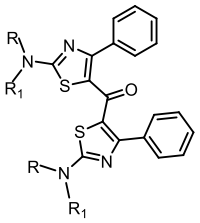


FIGURE 1: General method for the synthesis of thiazole derivatives

A total of 10 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 1	N-methyl-1-phenylmethanamine	
2.	PVS2	Dibutyl amine	
3.	PVS3	2-azanediyldiethanol	
4.	PVS4	dipropylamine	
5.	PVS5	dimethylamine	
6.	PVS6	diphenylamine	
7.	PVS7	N-ethylpropan-2-amine	
8.	PVS8	N-methylpropan-2-amine	
9.	PVS9	N-ethylaniline	
10.	PVS10	N-methylaniline	

Analysis of the synthesised derivatives

Melting points were determined by the open tube capillary method and are uncorrected. The purity of the compounds was checked by elemental analysis and the progress of reactions was monitored by TLC plates (silica gel G) using mobile phase, chloroform: methanol (9:1), and acetone: n-hexane (8:2), 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 300 MHz spectrometer using TMS as an internal standard in DMSO *d6*. Mass spectra were recorded on a Bruker Esquire LCMS using ESI and elemental analyses were performed on Perkin-Elmer 2400 Elemental Analyzer.

Pharmacological Activity

MTT Assay

Cytotoxicity of the provided samples on MDAMB-231 and HepG2 (Lungs) cell line was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96 well plates for 24 h in DMEM medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO_2. The next day cells were treated as per the client's instructions (different concentrations were prepared in a complete medium). After incubation for 24 hours, MTT (a final

concentration of 0.5 mg/ml) was added to the cell culture and further incubated for 2 h. At the end of the experiment, the culture supernatant was removed and the 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. [23]

RESULTS AND DISCUSSIONS

Molecular docking study

Molecular docking is a computational technique that predicts the binding modes and affinities of small molecules with their target macromolecules, making it valuable in drug discovery and development. It can guide the design and optimization of drug candidates, explore the structure-activity relationship of lead compounds, reduce the cost and time of experimental methods, and identify new therapeutic targets and drug leads. [24] In the context of the study mentioned, molecular docking simulations were used to rationalize the observed biological results of the novel thiazole derivatives, which could guide the design and development of more potent and selective anticancer agents.

The novelty of thiazole derivatives was investigated by searching in sci-finder and 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 03 **(Fig no. 1 and table 2)** exhibited the highest docking score of -7.811 and had a strong affinity towards the receptor compared to other compounds. It also formed a hydrophobic bond with C10, C17, C18, and C14, which is essential for anticancer activity.

TABLE 2: Docking scores of docked molecules with standard Dasatinib

Sr. No.	Compound Code	Docking Score, Receptor (1M17)
1.	**DASATINIB**	**-6.617**
2.	PVS3	-7.811
3.	PVS5	-5.986
4.	PVS2	-5.299
5.	PVS4	-5.108
6.	PVS9	-4.532
7.	PVS10	-4.134
8.	PVS1	-3.134
9.	PVS5	-5.986
10.	PVS6	-5.944
11.	PVS7	-5.278
12.	PVS8	-5.215

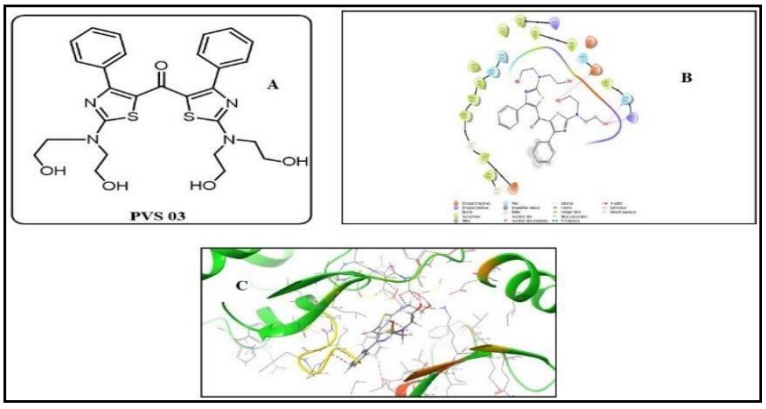


FIGURE 1: (A): Structure of PVS03 docked molecule; (B): 2D Structure of PVS 03 Docked Structure; (C): 3D or Ribbon Structure of PVS 03 Docked Structure

Advance Lipinski's rule of five

Optimizing the pharmacokinetic properties of drug candidates is a complex task that often involves modifying the molecular features responsible for binding affinity and specificity, such as hydrogen bonding. Unfortunately, many drug candidates have failed during clinical trials due to issues related to their ADME properties, which encompass absorption, distribution, metabolism, and excretion. [25] To mitigate these risks, researchers typically assess various physicochemical descriptors and pharmaceutical properties, such as molecular weight, log P, the number of hydrogen bond donors, and the number of hydrogen bond acceptors. These assessments are performed in accordance with Advanced Lipinski's Rule of Five, which helps ensure that drug candidates possess desirable pharmaceutical properties **[26]**. The comparative docking score, Lipnski's Rule parameters, and *In silico* pharmacokinetic parameters are presented in **table 3.** Considering all these parameters it was observed that PVS03 docked molecule had the highest docking score, excellent Lipnski's parameters, and in silico pharmacokinetic parameters.

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

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 01	-3.134	-51.4879	-80.2259	-58.7942	586.768	0	6	11.098	0	-10.075	-8.919	5177.799	4289.307	1.913	100
PVS 02	-5.299	-50.7841	-66.1413	-51.8628	602.895	0	6	6.951	0	-8.379	-7.243	4249.079	3297.043	2.093	100
PVS 03	-7.811	-47.5589	-65.1008	-50.3867	554.678	4	12.8	21.159	0	-10.204	-7.481	37.335	18.582	-0.171	100
PVS 04	-5.108	-48.2663	-67.4794	-51.4997	546.787	0	6	8.101	0	-5.538	-6.069	4664.234	3561.737	1.571	100
PVS 05	-5.986	-52.2549	-73.2867	-55.2305	434.573	0	6	9.505	0	-8.039	-7.023	4343.557	3606.713	0.818	100
PVS 06	-5.944	-39.0988	-75.5086	-52.8391	682.856	0	6	13.785	0	-7.143	-11.06	4918.269	3553.163	2.653	70.133
PVS 07	-5.278	-56.405	-90.5082	-61.2401	518.734	0	6	7.777	0	-12.537	-5.341	3940.864	3044.949	1.19	100
PVS 08	-5.215	-35.5844	-52.1614	-37.2154	490.68	0	6	8.262	0	-6.527	-5.433	3574.088	2992.972	1.07	100
PVS 09	-4.532	-38.0783	-52.3018	-38.2258	586.768	0	6	10.634	0	-6.487	-8.082	3834.76	3393.846	1.816	100
PVS 10	-4.134	-40.5892	-72.8594	-37.2258	558.715	0	6	11.39	0	-9.126	-8.559	3566.726	3146.269	1.676	100

[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 03 synthesised molecules and standard Dasatinib are presented in **Figure 2.**

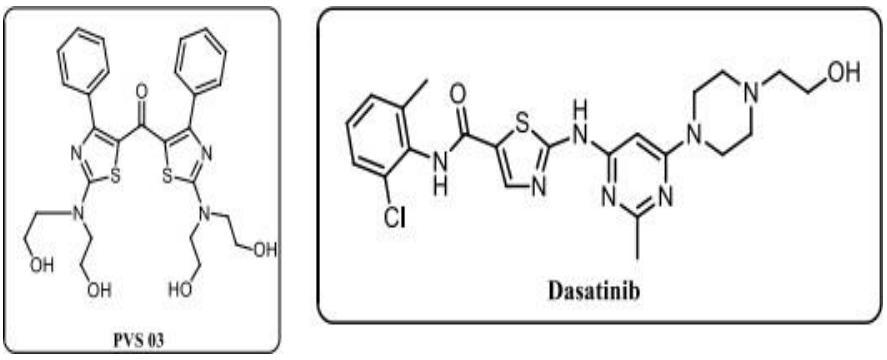


FIGURE 2: Chemical structures of all 10 synthesised molecules and standard Dasatinib

The analysis and purity of the compound were concluded by different analytical methods (NMR, Bruker NMR on 4000MHz using CDCl3 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 01 had a greater yield while PVS 04 showed a lower yield as shown in table 4, also % purity was carried out by the HPLC method in that PVS 10 have a greater purity while PVS 04 have less purity in the series of novel thiazole derivatives.

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

Sr. No.	Compound Code	R Group	Physical Constant (M. P.) (Uncorrected)	% Yield	% Purity
1	**PVS 1**	N-methyl-1-phenylmethanamine	212^0C-214^0C	95	99.04
2	PVS2	Dibutyl amine	180^0C-182^0C	88	95.28
3	PVS3	2,2'-azanediyldiethanol	166^0C-168^0C	65	96.14
4	PVS4	Diisopropylamine	254^0C-256^0C	48	90.10
5	PVS5	Dimethylamine	206^0C-208^0C	84	98.89
6	PVS6	Diphenylamine	248^0C-250^0C	78	91.94
7	PVS7	N-ethylpropan-2-amine	170^0C-172^0C	56	98.07
8	PVS8	N-methylpropan-2-amine	286^0C-288^0C	68	98.80
9	PVS9	N-ethylaniline	196^0C-198^0C	71	93.20
10	PVS10	N-methylaniline	188^0C-190^0C	92	99.21

The compound PVS 1 was characterized by ^{1}H NMR, which shows (400 MHz, $CDCl_3$) δ [ppm]: 7.23-7.79 Aromatic (c, d, e) { 20 H }; 3.4-3.74 (a, a) { 4 H }; 4.71 (b, b) {2.68 H }. m/z = 586 (M-H) and in IR data it showed the plausible functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1616.06 (C=O, Str., M). 1520.6 (Ar., C=C, Str.), 2848.35 (C-H, Str., med.), 3059.51 (C-H str., aromatic).

^{1}H NMR, of PVS2 molecule, showed (400 MHz, $CDCl_3$) δ [ppm]: 7.41-7.79 Aromatic (c, d, e) { 10 H }; 3.4-3.74 (a, a) { 8 H }; 1.02-1.49 (b, b) { 16H }. m/z = 603.2 (M+H). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1658.70 (C=O, Str., M). 1480.6 (Ar., C=C, Str.), 2701.35 (C-H, Str., med.), 3080.18 (C-H str., aromatic).

PVS 3 compound was also characterized by ^{1}H NMR (Figure 3) showing (400 MHz, $CDCl_3$) δ [ppm]: 7.41-7.79 Aromatic (c, d, e) {10 H}; 3.4-3.74 (a, a) { 8 H }; 1.02-1.49 (b, b) { 16H }. The IR data (Figure 4) showed the functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1578.90 (C=O, Str., M). 1448.70 (Ar., C=C, Str.), 2695.40 (C-H, Str., med.), 3045.88 (C-H str., aromatic). m/z = 510.8 (M+H, 1.5%) (Figure 5).

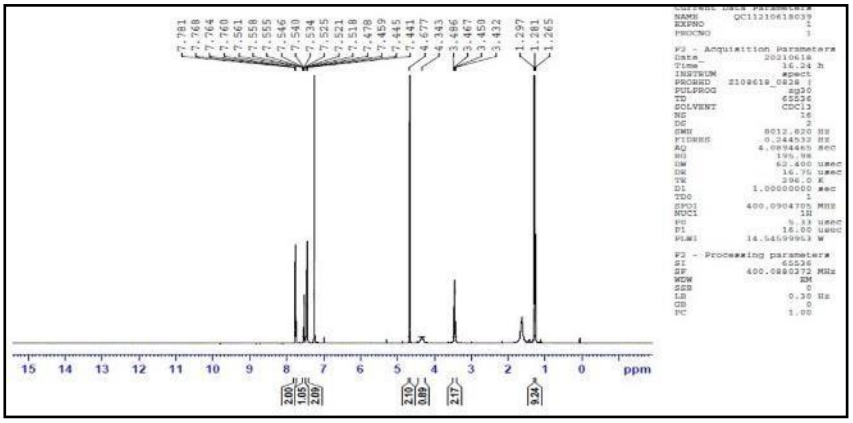


FIGURE 3: ^{1}H NMR spectra of synthesised PVS 3 compound

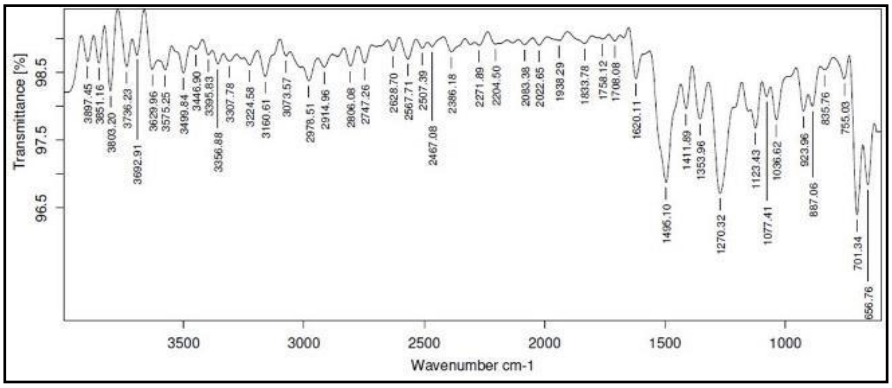


FIGURE 4: FTIR spectra of synthesized PVS 3 compound

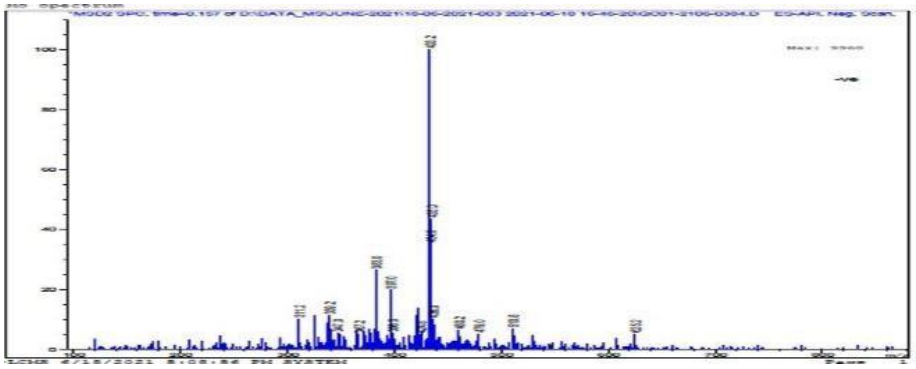


FIGURE 5: Mass spectra of synthesized PVS 3 compound

The compound PVS 4 was characterized by ^{1}H NMR, and showed peaks at (300 MHz, $CDCl_3$) δ [ppm]: 7.13-7.49 Aromatic (c, d, e) { 20 H }; 3.76-4.008 (a, a) { 4 H }; 1.31-.1.56 (b, b) {4H } while m/z = 547.2 (M+H). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1616.06 (C=O, Str., M). 1520.6 (Ar., C=C, Str.), 2848.35 (C-H, Str., med.), 3059.51 (C-H str., aromatic).

The compound PVS 5 showed ^{1}H NMR peaks at (300 MHz, $CDCl_3$) δ [ppm]: 7.41-7.79 Aromatic (c, d, e) { 10 H }; 3.28-3.46 (a, a) { 8 H }; 1.02-1.49 (b, b) { 11H } and m/z = 411.0 (M-H, 1.3%). The IR spectra showed a functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1678.10 (C=O, Str., M). 1558.70 (Ar., C=C, Str.), 2715.80 (C-H, Str., med.), 3406.41 (C-H str., aromatic).

The compound PVS 6 showed ^{1}H NMR, peaks at (300 MHz, $CDCl_3$) δ [ppm]: 7.203-7.240 Aromatic (c, d, e) { 08 H }; 7.285-7.309 (a, a) { 8 H }; 7.330-7.377 (b, b) { 4H }. m/z = 683.2 (M+H). The IR data showed the functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$) at 1769.21 (C=O, Str., M). 1571.28 (Ar., C=C, Str.), 2411.49 (C-H, Str., med.), 3489.37 (C-H str., aromatic).

The compound PVS 7 was characterized by ^{1}H NMR, which shows (300 MHz, $CDCl_3$) δ [ppm]: 7.08-7.29 Aromatic (c, d, e) { 10 H }; 3.3-3.5 (a, a) { 4 H }; 1.16-1.30 (b, b) { 6H }. m/z = 500.8 (M-H). In IR data which shows the plausible functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$): 1649.14 (C=O, Str., M). 1543.19 (Ar., C=C, Str.), 2698.80 (C-H, Str., med.), 3389.29 (C-H str., aromatic).

The compound PVS 8 was characterized by ^{1}H NMR, which shows (300 MHz, $CDCl_3$) δ [ppm]: 7.07-7.19 Aromatic (c, d, e) { 10 H }; 3.16-3.34 (a, a) { 8 H }; 0.08-1.56 (b, b) { 12H }. m/z = 499.9 (M-H, 9.1%). In IR data showing the plausible functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$): 1578.22 (C=O, Str., M). 1610.50 (Ar., C=C, Str.), 2685.40 (C-H, Str., med.), 3510.34 (C-H str., aromatic).

The compound PVS 9 was characterized by ^{1}H NMR, which shows (300 MHz, $CDCl_3$) δ [ppm]: 7.16-7.48 Aromatic (c, d, e) { 20 H }; 3.90-4.06

(a, a) { 4 H }; 1.19-1.56 (b, b) { 6H }. m/z = 587.2 (M+H). In IR data which shows the plausible functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$): 1653.70 (C=O, Str., M). 1579.42 (Ar., C=C, Str.), 2768.88 (C-H, Str., med.), 3478.53 (C-H str., aromatic).

The compound PVS 10 was characterized by ^{1}H NMR, which shows (300 MHz, $CDCl_3$) δ [ppm]: 7.17-7.46 Aromatic (c, d, e) { 20 H }; 3.47-3.57 (a, a) { 6 H };. m/z = 559.2 (M+H) in IR data it shows the plausible functional group of the synthesized thiazole derivatives I.R. (KBr, cm $^{-1}$): 1620.60 (C=O, Str., M). 1540.20 (Ar., C=C, Str.), 2748.60 (C-H, Str., med.), 3459.59 (C-H str., aromatic).

MTT Assay

The MTT assay is a commonly used technique for measuring cell viability and proliferation. In this study, the assay was performed using RPMI medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO2. The initial experiments were carried out with two different cell lines, namely MDAMB-231 human breast cells and HepG2 human lung cells. The cells were allowed to proliferate for 24 hours, and then MTT was added at a final concentration of 0.5 mg/ml. The cultures were further incubated for 2 hours to allow for the conversion of MTT into formazan.

The effect of compounds PVS 03, 05, 08, 09, and 10 on the growth of these cell lines was then assessed. The results showed that MDAMB-231 cells depleted nearly 90% of the culture medium by 4 days following inoculation, while HepG2 cells had only consumed 15% of the sugar during the same period. The effect of PVS 03 at 250 mcg/ml is presented in **figure 6.**

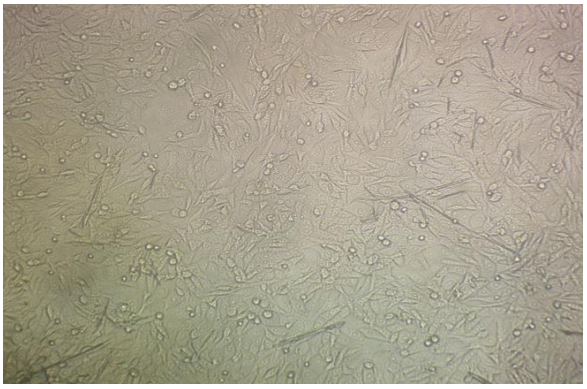


FIGURE 6: MTT assay observation at 250 mcg/ml concentration of PVS 03 molecule

This finding indicated that MDAMB-231 cells proliferate faster than HepG2 cells. Moreover, the decrease in medium FBS concentration in MDAMB-231 cell cultures was accompanied by a marked decrease in MTT-specific activity. On the other hand, in the presence of compounds PVS 06, 07, and 02, the specific activity of MTT in both MDAMB-231 and HepG2 cells remained nearly constant during the 4-day growth period, indicating no inhibition. These contrasting results prompted the researchers to conduct further investigations using a more diverse range of tumor cell lines. It is essential to assess the effects of compounds on multiple cell lines to determine their broad-spectrum activity and potential as therapeutics. Therefore, the researchers carried out similar experiments using various other tumor cell lines. The results of these experiments would help in evaluating the efficacy of the compounds against different types of tumors and aid in the development of novel cancer therapies.

CONCLUSION

In this study, we synthesized a novel series of 2,4-disubstituted thiazole derivative compounds designed to act as inhibitors of EGFR. These inhibitors were designed to form hydrogen bonds with the protein backbone, while peripheral groups oriented towards two hydrophobic pockets known as binding region-I and binding region-II. The binding mode of dasatinib was used as a basis of comparison with compound PVS 03, 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 03 in the evaluated tumor cell lines. The anticancer activity of the synthesized thiazole derivatives was evaluated against two tumor cell lines, namely Hep-G2 and MDAMB-231. PVS 03 exhibited the best anticancer activity compared to dasatinib. However, PVS 06 and 07 showed no inhibition against MDAMB-231 cells, while PVS 01 to PVS 10 showed no inhibition against Hep-G2 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 beloved research guide Dr. Avinash V. Patil and Dr. T. A. Deshmukh (Prinicpal, Arunamai College of Pharmacy, Mamurabad, Dist. Jalgaon) also KBC, North Maharashtra University, Jalgaon for giving me an opportunity for VCRMS Project Research grant.

REFERENCES

1. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer. Biological research. 2017;50:1-23.
2. Cheng F, Guo D. MET in glioma: signaling pathways and targeted therapies. Journal of Experimental & Clinical Cancer Research. 2019;38:1-3.
3. London M, Gallo E. Epidermal growth factor receptor (EGFR) involvement in epithelial-derived cancers and its current antibody-based immunotherapies. Cell biology international. 2020;44(6):1267-1282.
4. Shah RR, Shah DR. Safety and tolerability of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors in oncology. Drug safety. 2019;42(2):181-198.
5. Rebuzzi SE, Alfieri R, La Monica S, Minari R, Petronini PG, Tiseo M. Combination of EGFR-TKIs and chemotherapy in advanced EGFR mutated NSCLC: Review of the literature and future perspectives. Critical Reviews in Oncology/Hematology. 2020;146:102820.
6. Arora P, Arora V, Lamba HS, Wadhwa D. Importance of heterocyclic chemistry: a review. International Journal of Pharmaceutical Sciences and Research. 2012;3(9):2947-2954.
7. Graham R. Lawton, Haitao Ji, Pavel Martáse,Linda J. Roman,and Richard B. Silverman,Synthesis and enzymatic evaluation of 2- and 4-aminothiazole-based inhibitors of neuronal nitric oxide synthase, Beilstein Journal of Organic Chemistry. 2009; 5-28
8. Burkhart BJ, Schwalen CJ, Mann G, Naismith JH, Mitchell DA. YcaO-dependent posttranslational amide activation: biosynthesis, structure, and function. Chemical reviews. 2017;117(8):5389-456.
9. SR Pattan, Synthesis and Biological Evaluation of Some Substituted Amino Thiazole Derivatives, Asian Journal of Research in Chemistry. 2009; 22: 196-201.
10. Grybaitė B, Vaickelionienė R, Stasevych M, Komarovska, Porokhnyavets O, Kantminienė K, Novikov V, Mickevičius V. Synthesis and antimicrobial activity of novel thiazoles with reactive functional groups. ChemistrySelect. 2019;4(23):6965-6970.
11. Chang-Ling Liu, Zheng-Ming Li, Synthesis and biological activity of novel 2- methyl-4-trifluoromethyl-thiazole-5-carboxamide derivatives, Journal of Fluorine Chemistry 125;2004:1287–1290.
12. A.Th. Chaviara, CopperII Schiff base coordination compounds of dien with heterocyclic aldehydes and 2-amino-5-methyl-thiazole: synthesis, characterization, antiproliferative and antibacterial studies. Crystal structure of CudienOOCl2, Journal of Inorganic Biochemistry. 98; 2004:1271–1283.
13. Gülhan Turan-Zitouni, Synthesis of some 2-[benzazole-2-ylthioacetylamino]thiazole derivatives and their antimicrobial activity and toxicity, European Journal of Medicinal Chemistry 39;2003:267–272.
14. Petrou A, Fesatidou M, Geronikaki A. Thiazole ring—A biologically active scaffold. Molecules. 2021;26(11):3166.
15. Kaur H, Goyal A. A review on thiazole as anticancer agents. International Journal of Pharmaceutical Drug Analysis. 2018;6:509-522.
16. Niu ZX, Wang YT, Zhang SN, Li Y, Chen XB, Wang SQ, Liu HM. Application and synthesis of thiazole ring in clinically approved drugs. European Journal of Medicinal Chemistry. 2023:115172.
17. Sharma PC, Bansal KK, Sharma A, Sharma D, Deep A. Thiazole-containing compounds as

therapeutic targets for cancer therapy. European journal of medicinal chemistry. 2020 ;188:112016.
18. Kimber TB, Chen Y, Volkamer A. Deep learning in virtual screening: recent applications and developments. International Journal of Molecular Sciences. 2021;22(9):4435.
19. Lee K, Jeong KW, Lee Y, Song JY, Kim MS, Lee GS, Kim Y. Pharmacophore modeling and virtual screening studies for new VEGFR-2 kinase inhibitors. European journal of medicinal chemistry. 2010;45(11):5420-5427.
20. Singh N, Tiwari S, Srivastava KK, Siddiqi MI. Identification of novel inhibitors of Mycobacterium tuberculosis PknG using pharmacophore based virtual screening, docking, molecular dynamics simulation, and their biological evaluation. Journal of chemical information and modeling. 2015;55(6):1120-1129.
21. Gabr MT, El-Gohary NS, El-Bendary ER, El-Kerdawy MM, Ni N. Synthesis, in vitro antitumor activity and molecular modeling studies of a new series of benzothiazole Schiff bases. Chinese Chemical Letters. 2016;27(3):380-386.
22. A. Verma, Synthesis, characterization and biological evaluation of mutual prodrug of indomethacin and aminothiazole with reduced ulcerogenicity. AAPS. 2009;21-25.
23. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunological Methods. 1983 ;65(1-2):55-63.
24. Yuriev E, Holien J, Ramsland PA. Improvements, trends, and new ideas in molecular docking: 2012–2013 in review. Journal of Molecular Recognition. 2015;28(10):581-604.
25. Kar S, Leszczynski J. Open access in silico tools to predict the ADMET profiling of drug candidates. Expert opinion on drug discovery. 2020;15(12):1473-1487.
26. Chagas CM, Moss S, Alisaraie L. Drug metabolites and their effects on the development of adverse reactions: Revisiting Lipinski's Rule of Five. International journal of pharmaceutics. 2018;549(1-2):133-149.