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Original Article

IN SILICO DESIGN, DOCKING, SYNTHESIS AND EVALUATION OF THIAZOLE SCHIFF BASES

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ABSTRACT

Objective: The major objective of present study was to synthesize a series of substituted schiff base derivatives of 4-phenyl thiazoles designed as anticancer and anti-inflammatory agents using computational methods.

Methods: The thiazole derivatives for synthesis were selected based on docking studies performed on active site of protein tyrosine kinase (PDB :1T46) for anticancer activity and protein kinase (PDB :3DPK) for anti-inflammatory activity using schrodinger 9.3 software. Molecules with better docking score was subjected to analysis for cytotoxic activity by *in vitro* MTT assay on cervical cancer HeLa cell lines and anti-inflammatory activity.

Results: Among the five thiazole derivatives, CSB2:(4-(4-Methoxyphenyl)-*n*-3-phenylprop-2-en-1-ylidene]-1,3-thiazol-2-amine) was found to have highest docking score and the same exhibited maximum *in vitro* cytotoxic and anti-inflammatory activity.

Conclusion: Thiazole schiff bases derivatives showed good antinflammatory and cytotoxic activity as predicted using molecular docking on respective receptors.

Keywords: 2-amino-4-phenyl thiazole, docking study, protein kinase, 1T46, 3DPK, CSB.

INTRODUCTION

Thiazole derivatives are present in many natural and synthetic products with a wide range of pharmacological activities, such as anticancer, antiviral, antibacterial, antifungal, and anti-inflammatory activities. Among them, 2-aminothiazole derivatives possess an antitumor activity through the inhibition of the kinases[1]. Cinnamaldehyde is an active compound isolated from the stem bark of *Cinnamomum cassia,* a traditional oriental medicinal herb, which has been shown to inhibit tumor cell proliferation[2].

These findings prompted us to investigate a number of newly synthesized thiazole derivatives condensed with cinnamaldehyde to form Schiff bases that have anti-inflammatory and anticancer activity. Much research has been carried out with the aim to discover the therapeutic values of thiazole derivatives which is amongst the most frequently encountered heterocycles in compounds of biological interest, along with many other applications.

Objective of the study include predicting the affinity and the activity of the analogues to the selected protein targets of interest in terms of score through docking using GLIDE 5.8, a ligand binding program provided by SCHRODINGER (9.3) under Maestro. Synthesis of the cinnamaldehyde Schiff bases by condensing 2-amino-4-phenyl thiazole and cinnamaldehyde and in vitro evaluation by different methods. Anti-inflammatory in vitro activity study was done by protein denaturation and proteinase inhibition method[3] and invitro cytotoxicity by MTT assay [4].

MATERIALS AND METHODS

Molecular modeling investigations were carried out using Schrodinger 9.3 software XP Glide 5.8.

Preparation of protein

A typical PDB structure file consists only of heavy atoms and may include a co-crystallized ligand, water molecules, metal., ionsand cofactors. Some structures are multimeric, and may need to be reduced to a single unit. Schrödinger has assembled a set of tools to prepare proteins in a form that is suitable for modelling calculations. The tools are combined in the **Protein Preparation Wizard** under Maestro**.**

Preparation of ligand

The Schrödinger ligand preparation product **LigPrep** is designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules. GLIDE (**Grid-based Ligand Docking with Energetics**) is a ligand binding program provided by Schrödinger that searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. It provides a complete solution for ligand-receptor docking. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ***ligand pose***. The ligand poses that Glide generates pass through a series of hierarchical filters that evaluate the ligand's interaction with the receptor. Finally, the minimized poses are re-scored to generate the **Score** or Glide score is the sum total of various figures generated for each ligand during the docking process.

The best G Score is obtained as the most negative value and the most active ligands in terms of G Score are enlisted in descending order[5].

Docking Studies

Docking studies were carried out using the above mentioned prepared protein (1t46) and (3dpk) and ligands, by employing Glide XP docking program (Schrodinger 9.3) following the reported procedure.

Chemistry

Starting materials, reagents and solvents were purchased from commercial suppliers. All solvents employed were of commercial grade (LR) and used without further purification. Reactions were routinely monitored by thin layer chromatography (TLC) on silica gel G and visualized the products Using Iodine vapour. 1H NMR spectra were determined in DMSO with a Bruker Avance II 400 MHz spectrometer and signals recorded in parts per million (δ) downfield from tetramethylsilane as internal standard, and *J* values are given in Hz. IR spectra were recorded. Mass spectra (ESI) were recorded on Waters Micromass Q-TOF Micro. Melting points were recorded in open capillaries on melting point apparatus and were uncorrected.

General procedure for the synthesis of Substituted 2-amino-4-phenyl thiazole derivatives

Substituted 2-amino 4-phenyl thiazole was prepared by triturating thiourea (0.2 mol) and iodine (0.1) and mixed with substituted

(0.1mol) acetophenone. The mixture was heated on a water bath with occasional stirring for 8 hrs to get the product. The obtained solid was washed with diethyl ether to remove unreacted substituted acetophenone and then with aqueous sodium thiosulfate to remove excess of iodine and then with water. The crude product was dissolved in hot water and filtered to remove the sulphone. Substituted 2-amino-4-phenylthiazole substituted derivatives were precipitated by the addition of ammonia to the above filtrate and recrystallized from ethanol.

Preparation of Schiff bases

A mixture of substituted 2 amino-4-phenyl-1,3-thiazole derivatives (0.01M) and cinnamaldehyde (0.01M) were dissolved in 20-25 ml absolute ethanol and 2, 3 drops of glacial acetic acid were added. The mixture was refluxed for 5 hours. It was then cooled and dilute with ice cold water. The resulting solid was recrystallized from ethanol to yield Schiff bases[6,7].

General procedure for the synthesis of Cinnamaldehyde Schiff bases

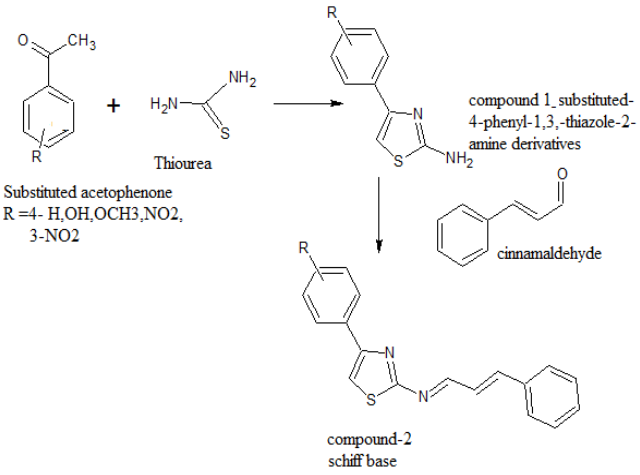
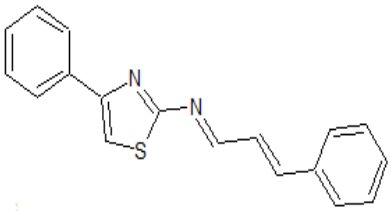
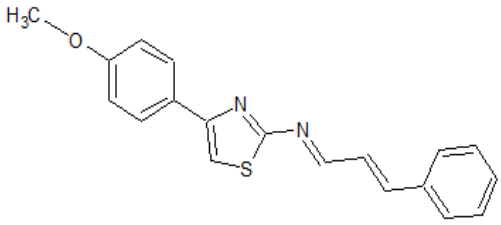
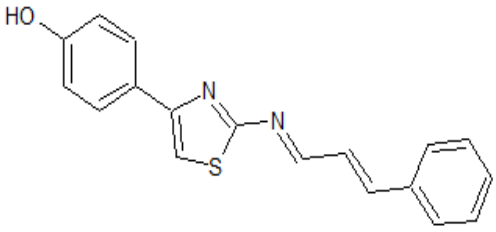
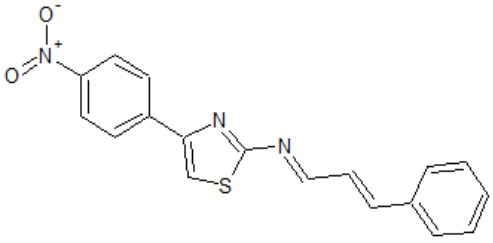
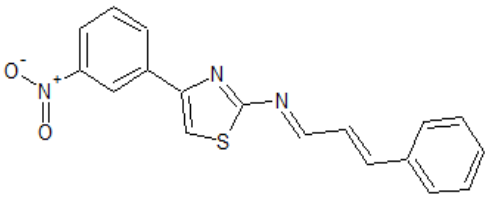


Table 1: Different cinnamaldehyde thiazole Schiff base derivatives

Structure	Name
	CSB1: 4-(Phenyl-*n*-3-phenylprop-2-en-1-ylidene)-1,3-thiazol-2-amine
	CSB2 : 4-(4-Methoxyphenyl)-*n*-3-phenylprop-2-en-1-ylidene]-1,3-thiazol-2-amine
	CSB3: 4-(4-hydroxy phenyl)-*n*-(3-phenylprop-2-en-1-ylidene)-1,3-thiazol-2-amine
	CSB4: 4-(4-Nitrophenyl)-*n*-3-phenylprop-2-en-1-ylidene]-1,3-thiazol-2-amine
	CSB5: 4-(3-Nitrophenyl)-*n*-(3-phenylprop-2-en-1-ylidene)-1,3-thiazol-2-amine

CSB1: 4-(Phenyl-*n*-3-phenylprop-2-en-1-ylidene)-1,3-thiazol-2-amine

Yield: 62.85%, Color: Dark orange, m.p.: 140 ^{0}C, IR (cm^{-1}): 3024, 1620, 1672, 749. ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 9.726 (1H, s,,-**H**C=N-), 7.263-7.611 (11H, m, Ar-**H)**), 8.770-8.851(1H, q, N=CH-C**H**=CH-Ar), 6.704-6.763 (1H, q, N=CH-CH=C**H**-Ar); ESI-MS (*m/z*) =291,177.

CSB2: 4-(4-Methoxyphenyl)-*n*-3-phenylprop-2-en-1-ylidene]-1,3-thiazol-2-amine

Yield: 60.25%, Color: Light red, m.p.: 120 ^{0}C, IR (cm^{-1}): 1245,3022,1624,1670,725.

CSB3: 4-(4-Hydroxy phenyl)-n-[-3-phenylprop-2-en-1-ylidene]-1,3-thiazol-2-amine

Yield: 50.28%, Color: Dark meroon, m.p.: 146 ^{0}C, IR (cm^{-1}): 3345,3018,1621,1682,744.

CSB4: 4-(4-Nitrophenyl)-*n*-3-phenylprop-2-en-1-ylidene]-1,3-thiazol-2-amine

Yield: 46.37%, Color: Dark yellow, m.p.: 132 ^{0}C, IR (cm^{-1}): 1368,3012,1620,1675,742.

CSB5: 4-(3-Nitrophenyl)-*n*-(3-phenylprop-2-en-1-ylidene)-1,3-thiazol-2-amine

Yield: 40.26%, Color: light yellow, m.p.: 138 ^{0}C, IR (cm^{-1}): 1357,3007,1628,1676,739.

RESULTS AND DISCUSSION

Structure based drug design involves detailed knowledge of the binding sites of targets (such as proteins) associated with the disease. A drug's effectiveness depends on structural interaction with the receptor or target molecule. Tyrosine kinases are important mediators of the signalling cascade, determining key roles in diverse biological processes like growth, differentiation, metabolism and apoptosis in response to external and internal stimuli. Recent advances have implicated the role of tyrosine kinases in the patho physiology of cancer. Constitutive oncogenic activation in cancer cells can be blocked by selective tyrosine kinase inhibitors and thus considered as a promising approach for innovative genome based therapeutics. Schiff bases bearing heterocyclic residues possessing excellent biological activity have attracted the attention of many researchers in recent years due to its anticancer, anti-inflammatory and antioxidant properties. In view of these findings, it was considered of interest to undertake the synthesis of thiazole Schiff base derivatives containing 2 amino thiazole moiety, hoping that these compounds might possess significant anticancer, anti-inflammatory and antioxidant activity.

In the present investigation, *in silico* docking studies were performed using the crystal structure of Protein tyrosine kinase (PDB ID: 1T46) to recognize the hypothetical binding mode of the ligands with the receptor in order to design a series of new cinnamaldehyde schiff base derivatives as possible anticancer agents. For anti inflammatory activity protein kinase (PDB ID: 3DPK) was selected. Docking was done by using schrodinger Maestro 9.3 Glide 5.8 XP. The analogues showed good binding interactions with the target site. Thiazole nucleus has a good interaction with lysine 623 amino acid and a strong hydrogen bond interaction with a water molecule. Phenyl alanine 811 has good interaction with benzene in acetophenone portion.

CSB2 was having the highest docking score of **-8.31** kcal/mol shows the maximum activity. The presence of electron donating methoxy group, in its structure may be the major reason behind its good binding interaction as the group formed strong hydrogen bond with amino acid side chain of the receptor as given in fig 1.The hydroxy derivative (CSB3) and the nitro derivative (CSB4) also showed good docking score of **-8.05** and **-8.16** kcal/mol respectively. The least activity was shown by acetophenone (CSB1) and *m*-nitro derivative (CSB5) with docking score of **-7.97** and **-7.79** kcal/mol as given in table 2.

Table 2: Docking results of derivatives (table 1) with anticancer receptor PDB1t46

Target	Receptor PDB ID	Analogue	Docking score
Tyrosine kinase	1T46	CSB1	-7.97693
		CSB2	-8.31384
		CSB3	-8.05424
		CSB4	-8.16742
		CSB5	-7.79737

Table 3: Docking results of derivatives (table 1) with anti-inflammatory receptor 3DPK

Target	Receptor PDB ID	Analogue	Docking score
Protein kinase	3DPK	CSB1	-9.099
		CSB2	-9.264
		CSB3	-7.065
		CSB4	-8.439
		CSB5	-7.985

For anti inflammatory activity there is significant interaction between benzene and alanine 800 of 3DPK receptor and there are strong hydrogen bond interactions as given in Fig 2. Among the 5 derivatives CSB2 was having the highest docking score of **- 9.26** kcal/mol showed the maximum activity.

Then the acetophenone and *p*-nitro derivative showed good interaction with docking score of **-9.09** and **-8.43** kcal/mol. The least activity is shown by hydroxyl and *m*-nitro derivative with score **-7.065** and **-7.985** kcal/mol respectively as explained in table 3.

The designed compounds were found to accommodate the binding pocket of the receptor showing the important interactions with all the crucial amino acid residues. As part of the investigation on the anti-inflammatory activity, ability of sample to inhibit protein denaturation was studied. Since denaturation of proteins is a well documented cause of inflammation the compounds that inhibit protein denaturation has good anti-inflammatory activity. CSB2 (IC_{50} : 81.27 µg/ml) showed the maximum activity compared to the others. CSB1 (IC_{50}: 105.41µg/ml) and CSB4 (IC_{50}: 111.39 µg/ml) showed moderate activity compared to CSB3 and CSB5 that showed the least activity with IC_{50} values 138.23 and 120.33 µg/ml respectively as given in table 4.

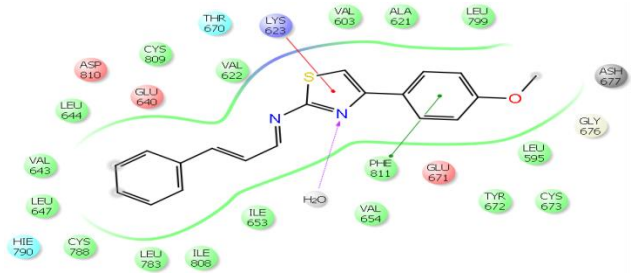


Fig.1: Ligand interaction diagram of CSB2 with PDB ID 1t46

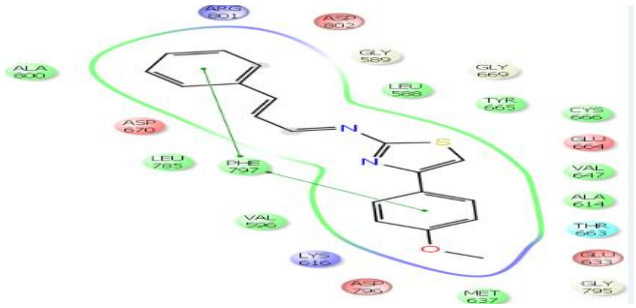


Fig. 2: Ligand interaction diagram of CSB2 with PDB ID 3DPK

It was reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. CSB2 (IC_{50} : 92.87 µg/ml) showed the maximum activity and it may be due to the presence of electon donating *para* methoxy group in the acetophenone portion. CSB1 and CSB4 showed considerable activity with IC_{50} values 116.89 and 132.86 µg/ml respectively.CSB5 (IC_{50} : 148.82 µg/ml) and CSB3 (IC_{50} :132.86 µg/ml) showed minimum activity as given in table 5. MTT assay was performed to determine the *in vitro* cytotoxicity. CSB2 showed maximum cytotoxicity against the cervical carcinoma cell lines which can be correlated with its excellent receptor interaction showed in molecular docking analysis. It showed better activity with an IC_{50} of 29.44 µg/ml. Its increased potency may be due to the presence of the electron donating *p*-methoxy group that may be responsible for better hydrogen bond interaction than others. CSB4 (IC_{50} : 31.74) µg/ml and CSB3 (IC_{50} : 45.69) µg/ml also showed excellent activity. CSB1 and CSB5 showed least activity with IC_{50} values of 78.79 and149.62 µg/ml. From the results given in table 6 it was found that the compounds with electron donating groups at *para* position exhibit excellent cytotoxic activity compared to others.

CONCLUSION

The current research work was focused on the rational approach to design and develop novel thiazole schiff base derivatives.This work has proved the biological action of the synthesized analogues as anticancer and anti inflammatory agents. *In silico* molecular modelling of molecules was done by various softwares like **ACD chemsketch** 12.1, **Molinspiration**, **PASS online** and **Schrodinger** (9.3) software. Fifty derivatives were selected for screening out of which five derivatives were selected for synthesis. The analogue with *para* methoxy group (CSB2) got the maximum docking score for anticancer and anti-inflammatory activity.

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Table 4: Determination of IC_{50} values by protein denaturation method

Sample	IC_{50} (µg/ml)
CSB1	105.41
CSB2	81.27
CSB3	138.23
CSB4	111.39
CSB5	120.33
DICLOFENAC	66.81

Table 5: Determination of IC_{50} values by proteinase inhibition method

Sample	IC_{50} (µg/ml)
CSB1	116.89
CSB2	92.87
CSB3	169.45
CSB4	132.86
CSB5	148.82
DICLOFENAC	69.21

Table 6: Determination of IC_{50} value of the analogues using MTT assay

Sample	IC_{50} (µg/ml)
CSB1	78.79
CSB2	29.44
CSB3	45.69
CSB4	31.74
CSB5	149.62

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