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Synthesis, Docking and Anti-cancerous Activity of Some Novel Thiazole Derivatives of Biological Interest

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ABSTRACT

Objectives: Heterocyclic compounds are enormously widespread in nature and have attracted research interest because of their pharmaceutical and biological properties. Amongst the heterocyclic rings, the thiazoles are the most important building blocks in today's drug discovery and are found to have extensive biological activities against different types of diseases. Many potent anti-cancerous drugs like Tiazofurin are having 1,3 thiazole as an active ring structure and based on this theory, a new series of 2, 4 di substituted 1,3 thiazole derivatives were synthesized. **Methods:** First 2-amino-4-substituted phenyl thiazoles were synthesized by adapting a well-known Hantzsch reaction and subsequently 2-amino substituted derivatives were synthesized using various aryl aldehydes by following established Schiff's reaction. The synthesized compounds were confirmed by TLC, IR, HNMR, CNMR and Mass Spectral Analysis. Then all the synthesized compounds were docked to RAS p21 receptor using PATCH DOCK Software to study their anti-cancerous activity. Then the compounds were screened for cancer cell line studies. **Results:** All the synthesized compounds exhibited some degree of anti-cancerous activity both in docking studies and *in vitro* anti-cancerous cell line studies. **Conclusion:** Amongst all the 16 synthesized, most compounds showed moderate to good anti-cancerous activity and the compounds S3P1c, S3P2c, S3P2d, S3P3a and S3P4d have shown the best activity.

Key words: Thiazole, Hantzsch, Docking, Cancer cell line.

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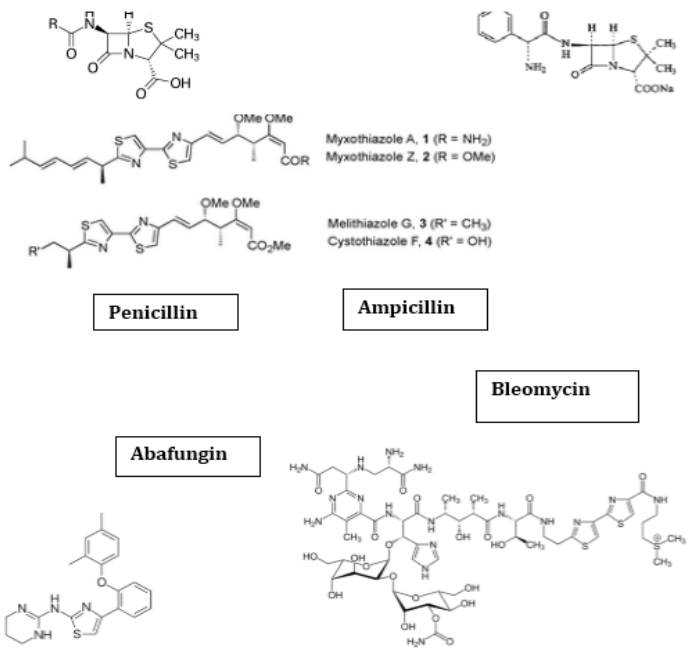
INTRODUCTION

As per statistics heterocyclic compounds with various positional combinations of hetero atoms like nitrogen, sulphur or oxygen constitute 80% of the bio-active chemical entities of plants and animals. Heterocyclic rings have very versatile reactivity due to the electronic distribution in the heterocyclic molecules and they can act as anions or cations depending on the pH of the medium. Their property of high polarity and water solubility leads to their increased bioavailability. 1,3 thiazoles form the considerable group amongst heterocyclic compounds to have wide spread pharmaceutical activity.[1]

The World Drug Index contains well over 100 drugs including a thiazole unit as a core scaffold, a capping fragment or a component in a hybrid system. Furthermore, the thiazole derivatives have been reported to have broad spectrum biological activities as anticancer[2] antifungal, antibacterial, antidiabetic, anticonvulsant, anti-inflammatory, anti HIV, antioxidant, Anti-Alzheimer and antihypertensive agents.[3-5] The major structural feature doing thiazole ring so popular is the nitrogen, which forms a strong complex with its target participating in donor-acceptor type interactions with the substrate. Thiazoles with derivatives of pyridine are important part of heterocyclic chemistry constituting the structure of Vitamin B1 (thiamine) which is of biological and pharmaceutical interest. Thiazole derivatives offer peptidomimetic features and improve compound's solubility and rigidity by maintaining hydrogen bond acceptors. Thiazole-based fragments are more flexible and bond more effectively with hydrogen. The structures of some representative examples of widely used antibiotics like Penicillin, Ampicillin, Myxothiazoles, Melithiazoles, Cystothiazoles, Bleomycins and Abafungin[6] are given below:

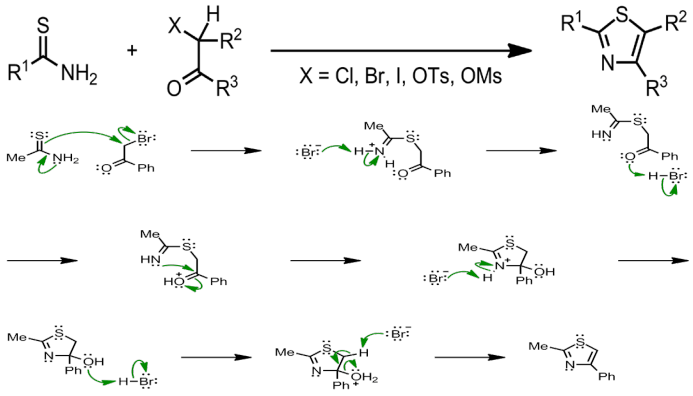


Hantzsch Synthesis of Thiazole Derivatives

In 1888, Hantzsch and Traumann Synthesis derived a unique method of synthesis of 2-aminothiazoles by cyclization of thiourea or thioamides with α-halo ketones and iodine.[7] The advantage is the high yield of

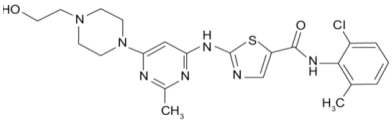
aminothiazoles. It can also be synthesized by various methods such as Gabriel Synthesis where α acyl amino derivative are reacted with Phosphorous pentasulphide and Cook-Heilbron thiazole synthesis which is the synthesis of 5-aminothiazoles by reacting α-aminonitriles or aminocynoacetates with dithioacids, carbon disulphide, carbon oxysulfide, or isothiocynates at room temperature and under mild conditions. The Hantzsch thiazole synthesis was extended to α halo-cyclic ketone, acyclic ketone, polycyclic ketones and aryl ketones with substituted thiourea and iodine. Variation of substituents at the R^1, R^2 and R^3 of the thiazole is introduced by selecting different combinations of starting reagents.

Mechanism of Hantzsch Thiazole Synthesis[8]

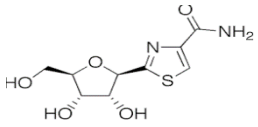


Cancer

Cancer is the most fatal, widespread disease with high rate of mortality in the world today. The high mortality rate of cancer results from uncontrolled multiplication of subtly modified normal human cells. Cancer can be treated by Surgery or Chemotherapy or Radiation therapy or Hormonal therapy or Immunotherapy or Targeted therapy or the combination of one or more above mentioned therapies. The two different types of chemotherapeutic agents are cytostatic and cytotoxic agents. The cytostatic agents have been beneficial in fighting tumors with their ability to arrest the cell growth and multiplication (apoptosis) but do not result in the cell death directly. Eg: Nitric oxide for Breast Cancer, Long chain polyunsaturated fatty acids for malignant epithelium. The Cytotoxic agents destroy or kill the rapidly growing cancer cells, they are classified[9] as 1) Alkylating agents: eg Cisplatin, 2) Antimetabolites: Vincristine, 3) Antibiotics: Bleomycins, 4) Miscellaneous: Hydroxy Urea. Chemotherapy is the only effective therapy for some type of cancers and so there is a raised expectation to develop more potent and selective agents.



Dasatinib[10]



Tiazofurin[11]

The active chemotherapeutic agents Dasatinib and Tiazofurin have 1,3 thiazole ring, which prompted us to synthesize new series of 1,3 thiazole derivatives and evaluate their anticancerous activity.

MATERIALS AND METHODS

Synthetic Method

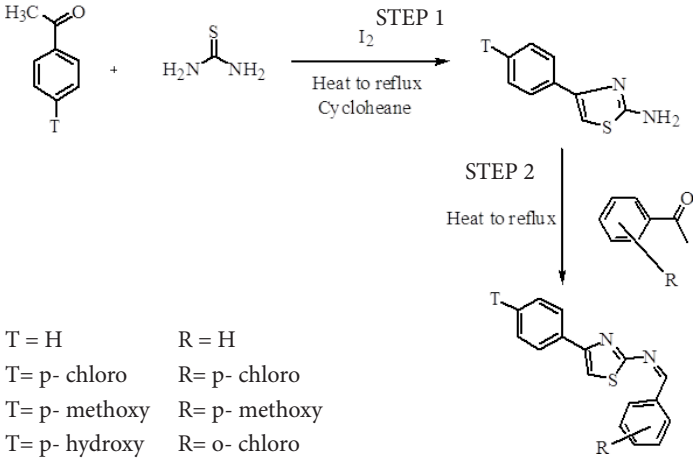
Melting points are uncorrected; the UV spectra were recorded on Shimadzu 1601 spectrometer, IR (KBr) were recorded on Perkin-Elmer FT-IR 1600 spectrometer, ^{1}H NMR spectra were recorded on Brucker AMX 400 using the solvent (CDCl 3 7.26 ppm and 77.0 ppm, DMSO-d6 2.49 ppm and 39.7 ppm) and TMS used as an internal standard. Low-resolution MS data were obtained using ESI and high-resolution spectra were recorded on QSTARXL hybrid MS/MS system Elemental analyses were within ± 0.4% of their calculated values. For Molecular Docking, the ligand structures are built by using builder in Molinspiration Cheminformatics and converted to 3D with Corina 3D. The synthesized molecules (ligands) were docked into the active site using Molecular docking software PATCH Dock with default parameters. The precise location of the binding site and the potentiality of the ligand to bind to the active site were determined using an automated docking software, molegro virtual docker 2008, version 3.2.1 (MolegroApS, Aarhus, Denmark, http://molegro.com).

Procedure: Step 1 Hantzsch Reaction: A mixture consisting of 0.1mole of ketone, 0.2 mole of thiourea and 0.1 mole of Iodine were heated overnight on the steam bath. This crude reaction mixture was cooled and extracted with ether to remove unreacted ketone and iodine. This residue was then dissolved in boiling water and filtered to remove sulphur. Then the solution was cooled somewhat and made basic with ammonium hydroxide. The 2-amino, 4-substituted phenyl thiazole, which separated, was recrystallized from water and alcohol. The four different parent compounds synthesized from following four different ketones a) p-methoxy acetophenone b) acetophenone c) p-chloro acetophenone d) p-hydroxy acetophenone.

Step 2: Schiff's Reaction: Further the above synthesized compounds was subjected to schiff's reaction with substituted Aryl aldehydes and corresponding 2-amino substituted derivatives were synthesized.

A mixture of Substituted aldehydes (0.004 mol) and 2- amino substituted thiazole (products from step1) (0.004 mol) in ethanol (10 ml) were added in microwave. The contents were subjected to microwave irradiation at 200 W for about 30 sec–2 min. After the completion of the reaction, solid product was obtained in reaction mixture which was filtered and recrystallized with methanol. The scheme of the reaction is shown below and scheme of synthesis is shown in Table 1. The Molecular mass, IR and NMR spectral data of the synthesized compounds is recorded in Table 2.

Scheme of Reaction.



T = H	R = H
T= p- chloro	R= p- chloro
T= p- methoxy	R= p- methoxy
T= p- hydroxy	R= o- chloro

Path 1 para-methoxyacetophenone + o chloro Benzaldehyde
+ Benzaldehyde
+ p methoxy Benzaldehyde (Anisaldehyde)
+ p chloro Benzaldehyde

Path 2 acetophenone + o chloro Benzaldehyde
+ Benzaldehyde
+ p methoxy Benzaldehyde (Anisaldehyde)

+ p chloro Benzaldehyde

Path 3 para-hydroxyacetophenone + o chloro Benzaldehyde
+ Benzaldehyde
+ p methoxy Benzaldehyde (Anisaldehyde)
+ p chloro Benzaldehyde

Path 4 para-chloroacetophenone + o chloro Benzaldehyde
+ Benzaldehyde
+ p methoxy Benzaldehyde (Anisaldehyde)
+ p chloro Benzaldehyde

Anti-cancerous *in vitro* activity- Method

MCF cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in their respective media viz., MEM/DMEM-HG/Ham's F-12 supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 well microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

In all the cell lines, the monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/ml using respective media viz., MEM/DMEM-HG/Ham's F-12 containing 10% BS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, monolayer washed once with medium and 100 µl of different test concentrations of test substances were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 72 h in 5% CO_2 atmosphere and microscopic examination was carried out and observations were noted every 24 h interval.

MTT assay

After 72 h incubation, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The mean values of all the compounds is given in Table 3. The percentage growth inhibition was calculated mathematically and concentration of test substances needed to inhibit cell growth by 50% (CTC50) values was generated from the dose-response curves for each cell line (Table 4).

Molecular Docking Method

Molecular docking studies were carried out by docking the synthesized compounds on RAS p21. RAS p21 is a protein which inactivates RAS from its active GTP- bound form to inactive GDP-bound form thereby allowing control of cellular proliferation and differentiation. A frequent oncogenic mutation of H-ras, N-ras, or K-ras genes is seen in different types of cancerous tumours. The members of the Ras GTPase family are crucial players in many signaling networks in the pathways linked to the functional controls of cell cycle progression, growth, migration, cytoskeletal changes, apoptosis and senescence. The refined crystal structure of the triphosphate conformation of H-Ras p21 (5P21) was retrieved from Protein Data Bank and the protein structure was corrected by using Protonate 3D and energy minimization was done. The ligands structures are built using MOLINSPIRATION and CORINA 3D software. The active site of protein was predicted by using site finder with default settings, dummies were assigned. The constructed structures of the ligands were docked into the active site using Molecular docking software PATCH Dock with default parameters. Molecular docking of the molecules revealed the atomic contact energy (ACE) and the amino acid binding residues that are as depicted in Table 5. The precise location of the binding site and the potentiality of the ligand to bind to the active site were determined using automated docking software, that is based on guided differential evolution and a force filed based screening function. With the help of clustering methods, the possible binding conformations and orientations were determined. The enzyme was visualized using the sequence option. The binding site was calculated within a spacing range so that the binding site was well into the grid and interactions were analysed using detailed energy estimates. The PATCH Dock software was utilized to identify Atomic contact energy, hydrogen bonds and hydrophobic interactions between residues at the active site and the ligand. The corresponding results were tabulated in the Table 5. As per the docking results, the compounds that showed maximum affinity to the receptor and shown the best anticancerous activity in the ascending order are as follows: S3P2c, S3P2d, S3P4d, S3P1c, S3P3a and S3P2c.The images of the docking of the compounds with the Biomarker Ras p21 is depicted in Figure 1.

RESULTS

The IUPAC name, molecular mass, NMR and IR data of the synthesized compounds is given in the Table 2. When the IR spectras of the synthesized compounds were analyzed, it was seen that a distinct peak at 2575 -2295cm^{-1} which represents the formation of thiazole ring and completion of step 1 of the synthesis. A peak at 2278 -2279cm^{-1} was seen in all the compounds distinct from that of the parent compound. This peak is due to the imine group –C=N- which represents the completion of step 2 synthesis. When the NMR spectras of the synthesized were analyzed, it was observed generally that the aromatic hydrogens ortho to the electron withdrawing substitutes like chloro showed desheilding effect and are shifted to downfield.

Mean values of percentage inhibitions and graphs of percentage inhibition Vs concentrations

The percentage inhibition of the cellular growth at different concentrations is tabulated and the graph is also plotted. The CTC_{50} which is the cytotoxic concentration at which 50% of the cancer cells die after exposure to the synthesized compounds is calculated. Lower the $CTC_{50,}$ better the anti -cancerous activity. Based on this it was decided that S3P2c, S3P2d, S3P1c, S3P4d and S3P3a were the most active anti cancerous compounds.

Results of Docking

A three dimensional structures of the synthesized molecules was developed and docked with the RAS p21.

Human Protein receptor (from PDB). During the docking procedure, only the best fit active site pocket with respect to the ligands is selected. Then respective Atomic Contact Energy and active protein site (amino acid residues) and the interactive hydrogen bonds is tabulated in Table 5. The best docked conformation of the receptor and the ligand is depicted in Figure 1. Lower the atomic contact energy, better the ligand binding and is eligible to be a promising candidate for anticancerous activity.

Table 1: **Scheme of Synthesis.**

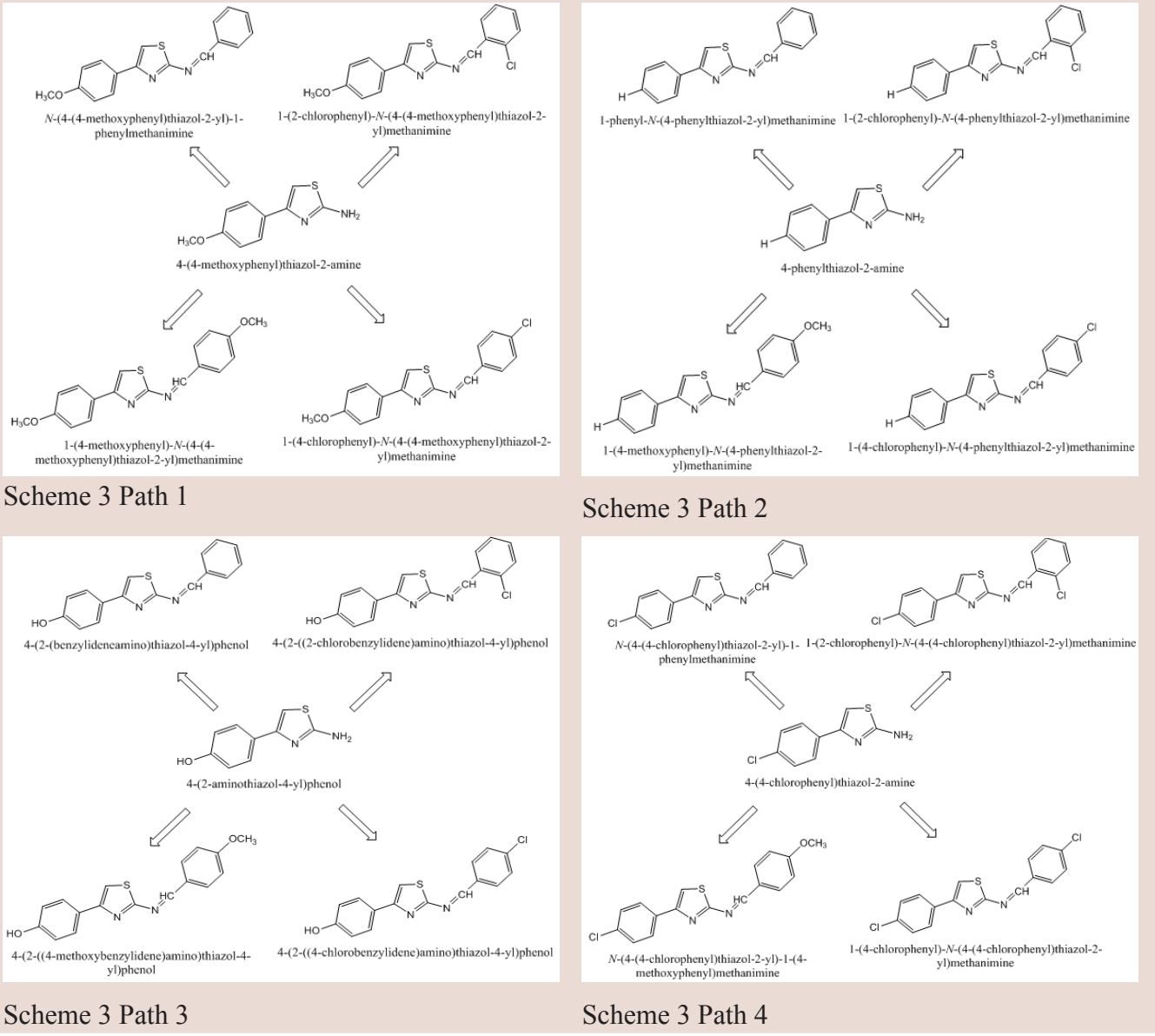
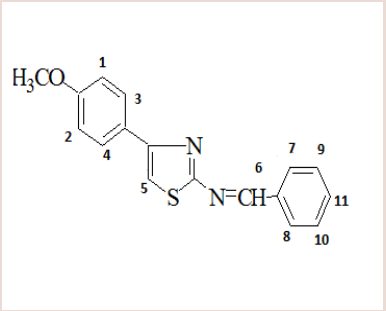
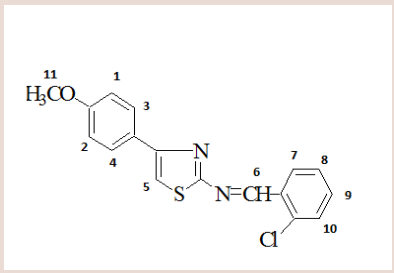


Table 2: **Spectral Data of the synthesized compounds.**



	Chemical / IUPAC Name
S3P1a	Benzylidene-[4-(4-methoxy-phenyl)-thiazol-2-yl]-amine
Molecular Mass 295.18	
HNMR data (ppm)	Infrared Spectroscopy Values
H1,H2: δ6.57 (m),	C-H (str): 2991.26cm^{-1}
H3 and H4: δ7.28 (m)	H=C (str): 3035.65cm^{-1}
H5: δ7.57 (m)	C=N (str): 2278.16cm^{-1}
H7 and H8: δ7.96 (s),	C-O (Str): 1182.26cm^{-1}
H9, H10 and H11: δ 7.28	C-S (Str): 2591.26cm^{-1}
H10 and H14: δ 7.28	
H11 and H13: δ 6.85, H16: δ 3.68	

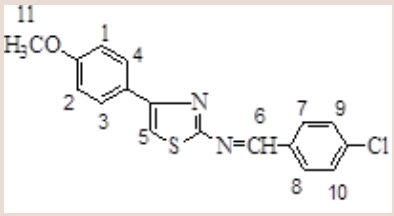


S3P1b
Molecular Mass 308.07

HNMR data (ppm)
H1 and H2: δ6.83 (m)
H3 and H4: δ7.57 (m)
H5: δ7.26 (s), H6: δ7.81 (s)
H7 : δ 7.85, H8 : δ 7.64(m)
H9: δ 7.57, H10: δ 6.16 (m)
H11 (OCH_3): δ 3.69 (s)

Chemical / IUPAC Name
2-Chloro-benzylidene)-
(4-phenyl-thiazol-2-yl)-amine

Infrared Spectroscopy Values
C-H (str): 2998.26cm^{-1}
H=C (str): 3045.65cm^{-1}
C-O (Str): 1178.18cm^{-1}
C-S (Str): 2576.06cm^{-1}
C=N (str): 2288.16cm^{-1}



S3P1c
Molecular Mass 329.05

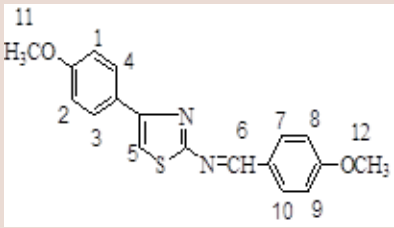
HNMR data (ppm)

H1 and H2: δ6.78 (m)
H3 and H4: δ7.18 (m)
H5: δ7.09 (s), H6: δ7.57 (s)
H7 and H8: δ 7.48(m),
H9 and H10: δ6.85 (m),
H11: δ 3.68 (s)

Chemical / IUPAC Name
(4-Chloro-benzylidene)-[4-(4-methoxy-phenyl)-thiazol-2-yl]-amine

Infrared Spectroscopy Values

C-H (str): 2988.06cm^{-1}
H=C (str): 3055.54cm^{-1}
C-O (Str): 1168.18cm^{-1}
C-S (Str): 2586.06cm^{-1}
C=N (str): 2278.16cm^{-1}

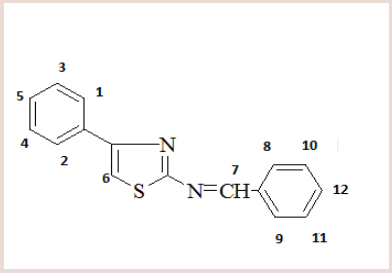


S3P1d
Molecular Mass 295.31

HNMR data (ppm)
H1 and H2: δ6.83 (m)
H3 and H4: δ7.27 (m)
H5: δ7.19 (s), H6: δ7.67 (s)
H7 and H10: δ 7.56(m),
H8 andH9: δ6.78 (m),
H11 and H12: δ 3.68 (s)

Chemical / IUPAC Name
(4-Methoxy-benzylidene)-(4-phenyl-thiazol-2-yl)-amine

Infrared Spectroscopy Values
C-H (str): 2998.26cm^{-1}
H=C (str): 3045.65cm^{-1}
C-O (Str): 1178.18cm^{-1}
C-S (Str): 2576.06cm^{-1}
C=N (str): 2278.16cm^{-1}

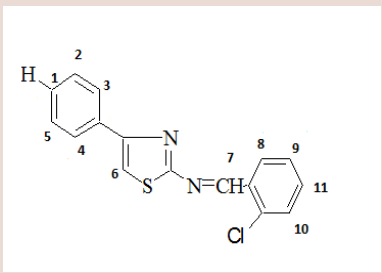


S3P2a
Molecular Mass 308.07

HNMR data (ppm)
H1 and H2: δ6.85 (m)
H3, H4 and H5: δ7.32 (m)
H6: δ7.34 (s), H7: δ7.67 (m)
H8,H9: δ 7.68
H10, H11, H12: δ 7.78(m),

Chemical / IUPAC Name
Benzylidene-(4-phenyl-thiazol-2-yl)-amine

Infrared Spectroscopy Values
C-H (str): 2978.16cm^{-1}
H=C (str): 3035.24cm^{-1}
C-S (Str): 2571.16cm^{-1}
C=N (str): 2278.16cm^{-1}

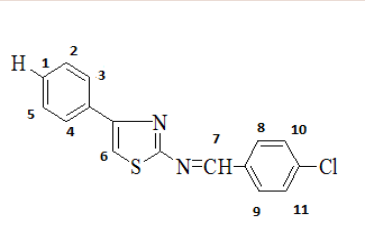


S3P2b
Molecular Mass 308.07

HNMR data (ppm)
H1, H2 and H5: δ6.81 (m)
H3 and H4: δ7.21 (m)
H6: δ7.29 (s), H7: δ7.77 (s)
H8: δ 7.68 (d), H9: δ 7.48(m),
H11: δ 7.48(m),
H10: δ6.24 (d),

Chemical / IUPAC Name
(2-Chloro-benzylidene)-(4-phenyl-thiazol-2-yl)-amine

Infrared Spectroscopy Values
C-H (str): 2978.16cm^{-1}
H=C (str): 3035.24cm^{-1}
C-NO_2 (str): 1368.16cm^{-1}
C-O (Str): 1168.12cm^{-1}
C-S (Str): 2571.16cm^{-1}
C=N (str): 2278.16cm^{-1}

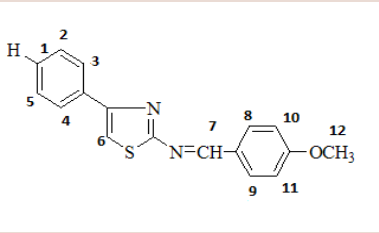


S3P2c
Molecular Mass 299.05

Chemical / IUPAC Name
(4-Chloro-benzylidene)-[4-(4-methoxy-phenyl)-thiazol-2-yl]-amine

HNMR data (ppm)
H1, H2 and H5: δ6.78 (m)
H4 and H5: δ7.18 (m)
H6: δ7.09 (s), H7: δ7.57 (s)
H8 and H9: δ 7.48(m),
H10 and H11: δ6.85 (m),

Infrared Spectroscopy Values
C-H (str): 2968.16cm^{-1}
H=C (str): 3025.24cm^{-1}
C-O (Str): 1188.12cm^{-1}
C-S (Str): 2581.16cm^{-1}
C=N (str): 2278.16cm^{-1}

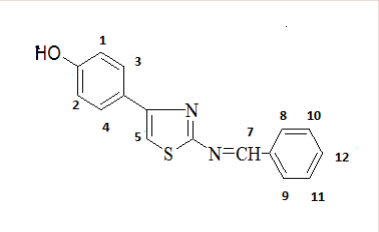


S3P2d
Molecular Mass 295.31

Chemical / IUPAC Name
(4-Methoxy-benzylidene)-(4-phenyl-thiazol-2-yl)-amine

HNMR data (ppm)
H1,H2 and H5: δ6.83 (m)
H3 and H4: δ7.27 (m)
H6: δ7.19 (s), H6: δ7.67 (s)
H8 and H9: δ 7.56(m),
H10 and H11: δ6.78 (m),
H12: δ 3.68 (s)

Infrared Spectroscopy Values
C-H (str): 2998.26cm^{-1}
H=C (str): 3045.65cm^{-1}
C-O (Str): 1178.18cm^{-1}
C-S (Str): 2576.06cm^{-1}
C=N (str): 2278.16cm^{-1}

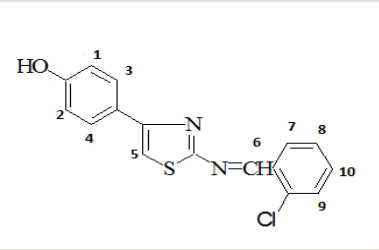


S3P3a
Molecular Mass 281.05

Chemical / IUPAC Name
4-[2-(Benzylidene-amino)-thiazol-4-yl]-phenol

HNMR data (ppm)
H1 and H2: δ6.75 (m)
H3 and H4: δ7.28 (m)
H5: δ7.42 (s), H7: δ7.71 (m)
H8 andH9: δ 7.71
H10, H11 and H12 - δ 7.89(m),

Infrared Spectroscopy Values
C-H (str): 2981.18cm^{-1}
H-O (str): 3535.24cm^{-1}
H=C (str): 3039.14cm^{-1}
C-S (Str): 2581.16cm^{-1}
C=N (str): 2278.16cm^{-1}

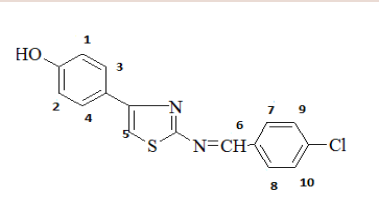


S3P3b
Molecular Mass 324.25

Chemical / IUPAC Name
4-{2-[(2-Chloro-benzylidene)-amino]-thiazol-4-yl}-phenol

HNMR data (ppm)
H1 and H2: δ6.85 (m)
H3 and H4: δ7.37 (m)
H5: δ7.52 (s), H6: δ7.81 (m)
H9: δ 6.64
H7, H8 and H10 - δ 7.76(m),

Infrared Spectroscopy Values
C-H (str): 2981.18cm^{-1}
H-O (str): 3535.24cm^{-1}
C=N (str): 2278.16cm^{-1}
H=C (str): 3039.14cm^{-1}
C-S (Str): 2581.16cm^{-1}

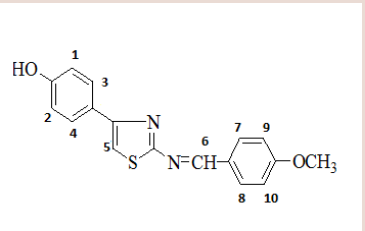


S3P3c
Molecular Mass 315.02

Chemical / IUPAC Name
4-{2-[(4-Chloro-benzylidene)-amino]-thiazol-4-yl}-phenol

HNMR data (ppm)
H1 and H2: δ6.82 (m)
H3 and H4: δ7.38 (m)
H5: δ7.68 (s), H6: δ7.79 (m)
H7 and H8: δ 7.66 (m)
H9 and H10: δ 6.14 (m)

Infrared Spectroscopy Values
C-H (str): 2990.10cm^{-1}
H-O (str): 3532.41cm^{-1}
H=C (str): 3039.25cm^{-1}
C-S (Str): 2580.16cm^{-1}
C=N (str): 2278.16cm^{-1}

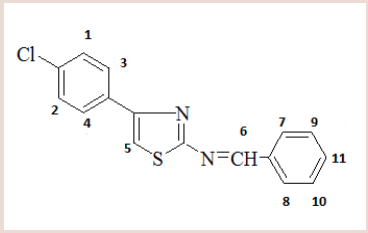


S3P3d
Molecular Mass 311.05

Chemical / IUPAC Name
4-{2-[(4-Methoxy-benzylidene)-amino]-thiazol-4-yl}-phenol

HNMR data (ppm)
H1 and H3: δ6.74 (m)
H4 and H6: δ7.41 (m)
H7: δ7.62 (s), H9: δ7.71 (m)
H10, H11: δ 7.64 (m),
H12andH13: δ 7.74, H14 - δ 3.73(S),

Infrared Spectroscopy Values
C-H (str): 2991.18cm^{-1}
H-O (str): 3530.64cm^{-1}
H=C (str): 3041.15cm^{-1}
C-S (Str): 2581.16cm^{-1}
C=N (str): 2278.16cm^{-1}

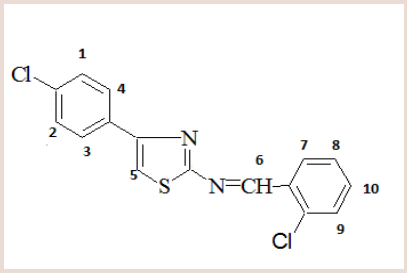


S3P4a
Molecular Mass 299.05

HNMR data (ppm)
H1 and H2: δ6.76 (m)
H3 and H4: δ7.72 (m)
H5: δ7.58 (s), H6: δ7.76 (m)
H7, H8: δ 7.59 (d),
H9, H10 and H11: δ7.75(m)

Chemical / IUPAC Name
Benzylidene-[4-(4-chloro-phenyl)-thiazol-2-yl]-amine

Infrared Spectroscopy Values
C-H (str): 2990.79cm^{-1}
C=N (str): 2279.08cm^{-1}
H=C (str): 3041.12cm^{-1}
C-S (Str): 2581.22cm^{-1}
C=N (str): 2278.16cm^{-1}

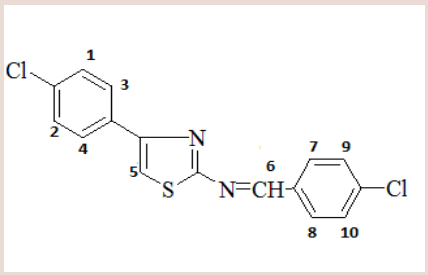


S3P4b
Molecular Mass 343.02

HNMR data (ppm)
H1 and H3: δ6.81 (m)
H4 and H6: δ7.62 (m)
H7: δ7.61 (s), H9: δ7.81 (m)
H10: δ 7.59 (d),
H12, H13 and H14: δ 7.87(m)

Chemical / IUPAC Name
(2-Chloro-benzylidene)-
[4-(4-chloro-phenyl)-thiazol-2-yl]-amine

Infrared Spectroscopy Values
C-H (str): 2991.02cm^{-1}
C=N (str): 2278.08cm^{-1}
H=C (str): 3041.92cm^{-1}
C-S (Str): 2580.02cm^{-1}

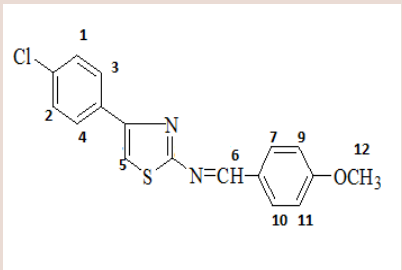


S3P4c
Molecular Mass 332.01

HNMR data (ppm)
H1 and H3: δ6.86 (m)
H4 and H6: δ7.56 (m)
H7: δ7.68 (s), H9,H10: δ7.81 (m)
H12 and H13: δ 7.78(m)

Chemical / IUPAC Name
(4-Chloro-benzylidene)-[4-(4-chloro-phenyl)-thiazol-2-yl]-amine

Infrared Spectroscopy Values
C-H (str): 2995.20cm^{-1}
C=N (str): 2278.11cm^{-1}
H=C (str): 3040.19cm^{-1}
C-S (Str): 2582.10cm^{-1}



S3P4d
Molecular Mass 329.80

HNMR data (ppm)
H1 and H2: δ6.86 (m)
H3 and H4: δ7.56 (m)
H5: δ7.68 (s), H6: δ7.81 (m)
H7, H10: δ 7.61 (m)
H9 and H11: δ 7.78

Chemical / IUPAC Name
(4-Chloro-benzylidene)-[4-(4-chloro-phenyl)-thiazol-2-yl]-amine

Infrared Spectroscopy Values
C-H (str): 2995.20cm^{-1}
C=N (str): 2278.11cm^{-1}
H=C (str): 3040.19cm^{-1}
C-S (Str): 2582.10cm^{-1}

Table 3(a): Cytotoxic activity of S3P1a to S3P1d and S3P2a to S3P2d.

con in µM	S3P1a	S3P1b	S3P1c	S3P1d	S3P2a	S3P2b	S3P2c	S3P2d
5	13.63±0.14	7.7±0.36	29.94±0.27	15.59±0.14	14.53±0.33	18.97±0.50	36.41±0.25	34.9±0.25
10	14.53±0.38	9.6±0.26	40.93±0.22	16.22±0.38	16.29±0.24	33.09±0.41	46.38±0.20	45.11±0.21
20	19.87±0.32	16.5±0.40	55.45±0.99	20.96±0.67	22.68±0.37	42.56±0.38	59.56±0.90	58.6±0.92
30	26.45±0.5	22.03±1.01	58.3±0.37	28.65±0.10	27.8±0.93	51.55±0.14	62.66±0.12	61.25±0.34
40	38.22±0.32	38.63±0.21	67.76±0.29	46.96±0.27	43.17±0.19	61.02±0.11	73.3±0.27	70.05±0.5
50	51.19±1.02	49.9±0.1	69.97±0.35	61.5±0.43	53.61±0.09	69.47±0.19	84.15±0.28	76.69±0.28
100	65.76±0.05	62.2±0.1	75.76±0.31	67.57±0.05	65±0.09	72.48±0.31	93.92±0.21	82.46±0.16

Table 3(b): Cytotoxic activity of S3P3a to S3P3d and S3P4a to S3P4d.

con in µM	S3P3a	S3P3b	S3P3c	S3P3d	S3P4a	S3P4b	S3P4c	S3P4d
5	25.18±0.29	12.09±0.34	17.73±0.09	17.07±0.28	10.38±0.35	16.47±0.33	21.2±0.6	30.53±0.3
10	36.92±0.24	13.9±0.25	21.87±0.74	18.03±0.38	12.23±0.26	18.19±0.24	39.12±0.64	41.42±0.2
20	52.42±1.06	20.47±0.38	28.95±0.74	25.52±0.16	18.93±0.39	24.43±0.36	45.21±0.19	55.82±1.0
30	55.47±0.39	25.74±0.96	46.9±0.23	35.26±0.29	24.3±0.98	29.44±0.51	53.45±0.41	60.89±0.1
40	65.58±0.84	41.55±0.20	56.01±0.27	52.51±0.14	40.42±0.20	44.46±0.19	62.77±0.78	68.03±0.3
50	73.21±0.33	52.28±0.1	63.1±0.23	63.28±0.38	51.35±0.1	54.66±0.09	67.75±0.28	71.76±0.3
100	75.32±0.19	64±0.1	68.2±0.05	66.9±0.23	63.3±0.1	65.79±0.09	70.55±0.28	77.08±0.2

Table 4: The CTC_{50} in µM values of all synthesized compounds.

S3P1a	65.13416537	S3P2a	62.85939968	S3P3a	34.96904025	S3P4a	66.7788162
S3P1b	69.13867488	S3P2b	41.60060976	S3P3b	65.20689655	S3P4b	61.06687898
S3P1c	32.26229508	S3P2c	22.75324675	S3P3c	50.44207317	S3P4c	40.18181818
S3P1d	58.11487482	S3P2d	26.34556575	S3P3d	54.79638009	S3P4d	30.77170418

Table 5: ACE values, details of hydrogen bonds and amino acid residues on the docked domain.

Sl no	Name of Biomarkers	Compound Code		Details of Hydrogen Bonds	Atomic Contact Energy	Amino acid residues on the docked domains
1		S3P1a	06	3.96, 5.08, 4.62, 4.09, 2.5, 1.62	-121.86	Tyr 32, Pro 34, Gly 60, Gln 61, Arg 68, Asp 92, Gln 95, Tyr 96
2		S3P1b	07	0.45, -2.5, 1.71, 2.27, 2.45, 1.04, 1.22	-103.74	Gly 60, Glu 62, Arg 68, Asp 92, Gln 95, Glu 98
3		S3P1c	05	-0.74,-2.5,-2.5,0.56, 13.02	-173.07	Ile 36, Pro 34, Tyr 32, Glu 63, Gln 61, Gly 13
4		S3P1d	04	-2.5, 7.9, 1.12, -2.5	-140.88	Lys 5, Glu 37, Asp 54, Leu 56, Met 67, Gln 70, Tyr 70, Thr 74
5		S3P2a	05	-1.46,-1.01, 3.02, 0.84, -0.904	-121.60	Gly 60, Gln 61, Arg 68, Asp 92, Gln 95, Tys 96, Gln 99
6		S3P2b	01	-2.5	-160.47	Tyr 32, Pro 34, Gln 61, Glu63
7		S3P2c	03	-1.99,-2.2, -2.5	-249.05	Gly 12, Gly 13, Glu 31, Tyr 32 Asp 33, Pro 34, Gln 61
8		S3P2d	02	-2.5, 0.51	-196.31	Gly 12, Tyr 32 Asp 33, Pro 34 Gln 61
9		S3P3a	03	1.24, -1.15,-2.5	-162.71	Tyr 32, Pro 34 Gln 61, Glu 63
10		S3P3b	10	-0.02, -0.54, -0.86, 5.59,9.65,-0.14,-2.5, -2.5,-1.46	-109.00	Gly 60, Gln 61, Glu 62, Arg 68, Gln 95, Tyr 96
11		S3P3c	05	-2.5, 5.25, 4.98,1.86, -0.57	-158.97	Gln 131, Arg 135, Tyr 141
12		S3P3d	05	-1.13, -2.5, -1.84, 10.72, -2.22	-137.16	Lys 5, Glu 37, Asp 54 , Leu 54, Tyr 64, Met 67, Gln 70, Tyr 71, Thr 74
13	RAS p21	S3P4a	01	-1.34	-105.79	Lys 5, Glu 37, Asp 54, Leu 56, Tyr 64, Gln 70
14		S3P4b	06	-0.35,-2.12,-0.16,-1.99, -4.15, -2.5	-132.21	Ala 11, Gly 60, Gln 61, Glu 62, Arg 68, Asp 92, Gln 95, Tyr 96 Gln 99
15		S3P4c	06	-0.79,-2.5,1.09,-1.19 -2.5, -1.82	-158.74	Gln 70, Thr 74 Lys 5, Tyr 71, Glu 37 Tyr 64, Met 67
16		S3P4d	02	-2.5, -2.5	-192.62	Gly 13, Tyr 32 Pro 34, ile 36 Gln 61, Glu 63

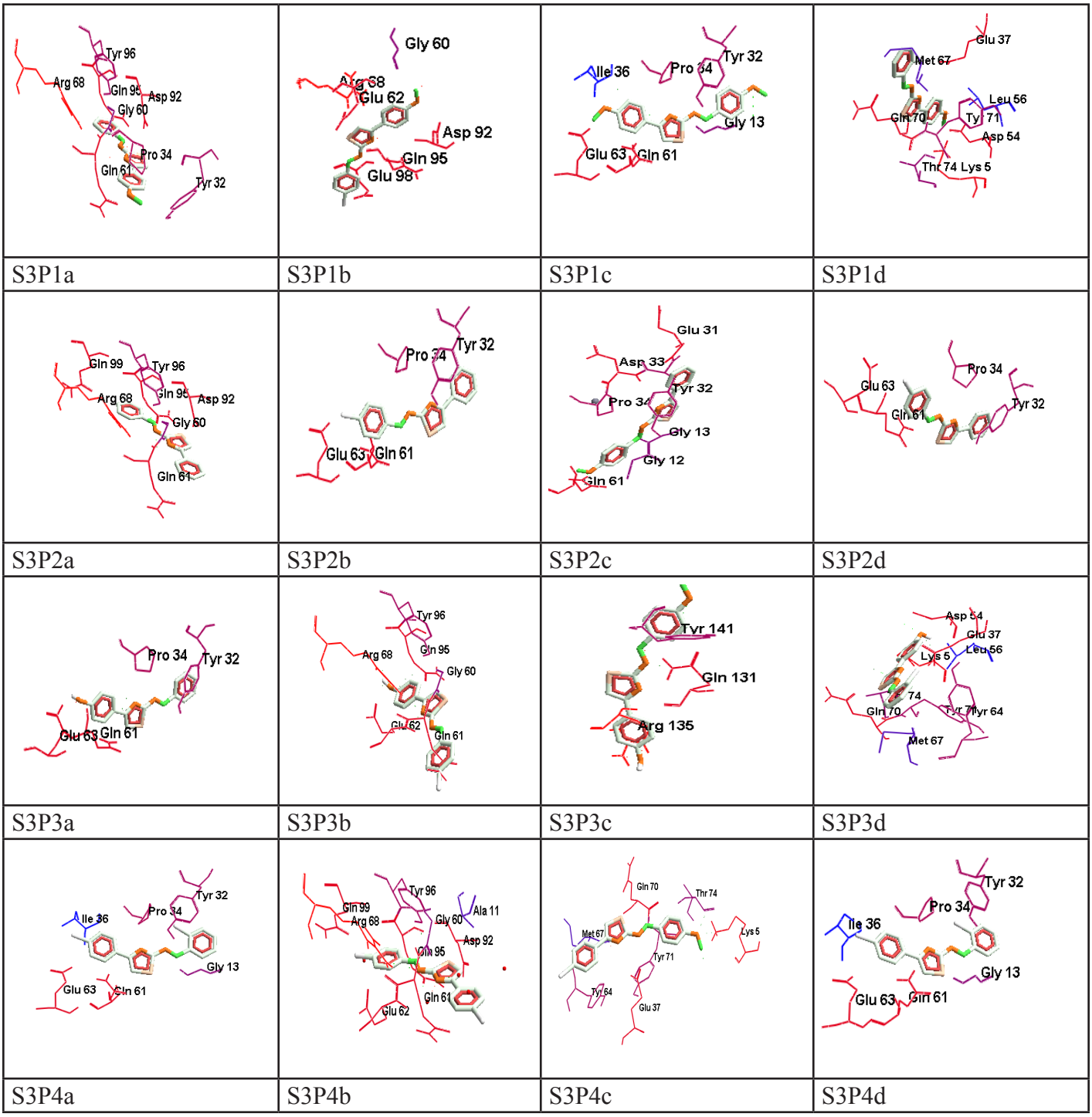


Figure 1: Docking pictures of the compounds with the Ras p21 Biomarker.

DISCUSSION

Amongst the wide range of small-ring heterocycles explored, rings including nitrogen and sulfur have been under investigation for a long time on account of their synthetic diversity and therapeutic relevance. The thiazoles have been identified as the most privileged candidates in drug discovery because of their diverse pharmaceutical activity. The thiazole derivatives are known to have potent anti-cancerous activity. As per the chemistry of the 1,3 thiazoles is biologically most active which is proved by Tiazofurin, Thiazole Netropsin and Bleomycin. Epithalones is a recent class of natural products which have been reported to exhibit extraordinarily potent cytotoxicity in a broad range of human cancer cell lines, The newer anti-neoplastic antibiotics such as Siomycine A, Thiostreptone, Nosiheptide, Sporangiomycin and Thiopeptine are also 1,3 thiazoles. 2, 4-disubstituted 1,3 thiazole is proved to be potent antineoplastic agents in the Structure Activity Related studies and they show desired pharmacological actions because of their relative stability, enhanced lipid solubility and also hydrophilicity. They are proved to have better ADME properties. 2 amino derivatives of thiazole is proved to be a potent anticancerous drug.[12] In the recent similar studies it is

seen that 2,4,5 tri substituted thiazole is proven to have anti-cancerous activity.[13] There are a variety of mechanisms for the antitumor action of thiazole and fused thiazole derivatives, acting on cancer biotargets, such as tumor necrosis factor TNF- α inosine monophosphate dehydrogenase (IMPDH) and apoptosis inducers.

Based on the above studies, sixteen derivatives of 2, 4-disubstituted 1,3 thiazole were synthesized and were screened for *in vitro* anti-cancerous activity on breast cell line and found that all the derivatives showed moderate to good inhibition of cell proliferation following MTT Assay. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium yellow coloured salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. Hence it is a colorimetric assay. The number of cells was found to be proportional to the extent of formazan production by the cells used. Therefore this assay is used for assessing cell viability.

The synthesized compounds were docked with Ras p21 protein receptor. In the docking studies the optimum Atomic Contact Energy of ligand-receptor binding is usually between -100 to -250 scores. If the ACE score is less than -100, then it is not sufficiently bound to exhibit the expected pharmacological activity and if it is higher than -250, then the binding is so strong that the dissociation of the ligand from the receptor becomes difficult and leads to the toxicity.

CONCLUSION

A series of sixteen compounds were synthesized by following the standardized and established procedures. Molecular docking studies were also carried out for all the new compounds with Ras p21 which is a protein involved in control of proliferation of cancerous cells. All the compounds were screened for *in-vivo* anti cancerous activity on the breast cell lines and most of the synthesized compounds have shown moderate to good anti cancerous activity. Amongst all the 16 compounds, S3P1c, S3P2c, S3P2d, S3P3a and S3P4d have shown the best activity. When analysed, the presence of methoxy group and chloro substitutions have influenced the increase in the activity.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

TLC: Thin Layer Chromatography; **MP:** Melting Point; **R Values:** Gas constant; **IR:** Infra-red; **UV:** Ultraviolet; **NMR:** Nuclear Magnetic Resonance; **MS:** Mass Spectroscopic; **CHN:** Carbon hydrogen and nitrogen; **FTIR:** Fourier-transform infrared spectroscopy; **KBr:** Potassium bromide; **H1NMR:** Hydrogen-1 NMR; **CdCl:** Cadmium Chloride; **DMSO:** Dimethyl Sulfoxide; **DB:** Protein DATA Bank; **ACE:** Atomic Contact Energy, viz- that is to say.

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