Modeling, Simulation, Docking Studies of Tyrosine Kinase Involved in Leukemia and Microwave Synthesis of Quinazoline Derivatives

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Abstract: *Acute Mylenoid Leukemia is a type of cancer which affects blood and bone marrow and is characterized by an abnormal proliferation of white blood cells. A number of genes are involved in regulating heamatopoiesis, and thereby affecting the susceptibility to Leukemia.One among them Tyrosine kinase which plays an important role in cell proliferation and differentiation. In this work three dimensional model of Tyrosine kinase was generated using 1Z3S as a template with the help of Modeller7v7. With the aid of the molecular mechanics and molecular dynamics methods, the final model is obtained and is further assessed by Procheck and Verify 3D graph programs, which showed that the final refined model is reliable. After energy minimization the three dimensional structure of Tyrosine was compared with template. Molecular docking of 6-thiogunaosine (6-TG) analogs was performed on the ATR1 model and selective inhibitor was selected based on the docking results. The docking results showed that the three residues in the Tyrosine kinase (GSN235, AsN257, and ILE289) were essential for making hydrogen bond with the analogues. The data presented here strongly indicate that the interactions of these four residues are necessary for a stronger binding of the Tyrosine kinase with synthesized molecules. Also, the study proposed analogues were an effecctive inhibitors by the comparison of docking energy. Synthesis is of Structure Of 3'-(4-Oxo-2-Phenylquinazolin-3(4h)-Yl)-4'h-Spiro[Indole-3,2'-[1,3]Thiazolidine]-2,4'(1h)-Dione derivatives were not possible because of stearic hindrance by bulky groups present on the nitrogen atoms.*

Keywords: *Acute mylenoid Leukemia, Tyrosine Kinase, Modelling, Molecular Dynamics, Docking studies, stearic hindrance.*

I. Introduction

Tyrosine Kinase is one of the most mutated gene in the human leukemia, is a class III receptor tyrosine kinase (RTK) protein that is an important regulatory gene involved in normal heamatopoiesis (Gillaland DG, Griffin JD 2002 and Small D. 2006). RTK also includes C-KIT, C-FMS, platelet derived growth factor (PDGF) and membrane bound receptor with intrinsic tyrosine kinase domain. FLT3 is a share common structure with class III RTKs consisting of 5 extracellular immunoglobulin domain, single transmembrane domain, a cytoplasmic juxtramembrane region, and highly conserved a cytoplasmic kinase domain interrupted by a kinase insert. Tyrosine kinase recepotor exists as monomeric, unphosphorylated state with an inactive kinase moiety in the unstimulated state. After the interaction of the receptor with FLT ligand (FL), receptor undergoes conformational change, which results in the unfolding and the exposure of the dimerization domain, allowing receptor-receptor dimerization.The receptor dimerization is important to the activation of the tyrosine kinase enzyme which leads to phosphorylation of various sites in the intracellular domain. In the Intracellular domain, the activated receptor along with many proteins in the cytoplasm forms a complex of protein-protein interactions. FL activates the different downstream targets, which include proteins in the activators of transcription (STAT), signal transducers, mitogen activated protein (MAP) kinase, and AKT pathways that are involved in the regulating, proliferation, differentiation and cell survival. In vitro studies have shown that the activated Tyrosine Kinase controls downstream signaling pathways which results in continuous cellular proliferation and resistance to apoptotic cell death. Activation of Tyrosine kinase occurs through two main mechanisms: coexpression of FL, which leads to activation of autocrine, paracrine, or intracrine signaling and via conferring ligand independence, mutation of the FLT3 gene itself (Abu-Duhier FM et al 2001, Drexler HG (1996), Meierhoff G et al 1995, Nakao M et al 1996). These mutations are internal tandem duplications of the juxtramembrane domain (ITD), or point mutations of the second tyrosine kinase domain (TKD) and point mutations of the juxtramembrane domain. Constitutively activated FLT3 contributes to the leukemic phenotype

in transgenic murine model systems (Grundler R et al 2005, Li L et al 2008). FLT3 primarily expressed in early lymphoid progenitors and myeloid plays an important role in proliferation and differentiation. FLT3 expression has been described in lymphoheamtopoitic organs such as the liver, spleen, thymus and placenta. The majority of human acute leukemias, including 100% of B-cell lineage acute lymphoblastic leukemias (ALL), 27% of T-lineage ALL, and 89% of acute myelogenous leukemias (AML) overexpress Tyrosine Kinase (Brig F et al 1992, Carow CE et al 1996). ITD mutations are found in 3% of patients with myelodysplastic syndromes (MDS) (Shih LY et al 2004), and up to 15% and 25% of pediatric and adult AML patients, respectively. In both pediatric and adult AML patients, the presence of an ITD mutation is associated with a significantly higher relapse rate and worse overall survival (Klo H et al 1999 and Meshinchi S et al 2003).

II. Methods

3D model building

The initial model of Human Tyrosine Kinase protein was built by using homology-modeling methods and the MODELLER software; a program for comparative protein structure modeling optimally satisfying spatial restraints derived from the alignment and expressed as probability density functions (pdfs) for the features restrained. The pdfs restrain C^α-C^α distances, main-chain N-O distances, main-chain and side-chain dihedral angles. The 3D model of a protein is obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as a combination of pdfs restraining individual spatial features of the whole molecule. The optimization procedure is a variable target function method that applies the conjugate gradients algorithm to positions of all non-hydrogen atoms.

Domain Identification and Template Search

The query sequence from *Homo sapiens* was submitted to domain fishing server for Human Tyrosine Kinase protein prediction. The predicted domain *was* searched to find out the related protein structure to be used as a template by the BLAST (Basic Local Alignment Search Tool) (Altschul, S. F et al 1997 and, Altschul et al 1990) program against PDB (Protein Data bank). Sequence that showed maximum identity with high score and less e-value was aligned and was used as a reference structure to build a 3D model for Tyrosine Kinase protein. The co-ordinates for the structurally conserved regions (SCRs) for Tyrosine Kinase protein were assigned from the template using multiple sequence alignment, based on the Needleman-Wunsch algorithm (Needleman, S. B. and Wunsch, C. D. 1970).

Molecular Dynamics

The structure having the least modeller objective function, obtained from the modeller was improved by molecular dynamics and equilibration methods using NAMD 2.5 software (Kale L et al 1999) using CHARMM27 force field for lipids and proteins (Schlenkrich, M et al 1996) along with the TIP3P model for water (Jorgensen, W. L et al 1983). The energy of the structure was minimized with 1, 00,000 steps. A cutoff of 12 Å (switching function starting at 10 Å) for van der Waals interactions was assumed. No periodic boundary conditions were included in this study. An integration time step of 2 fs was used, permitting a multiple time-stepping algorithm (Grubmuller, H et al 1991) to be employed in which interactions involving covalent bonds were computed every time step, short-range nonbonded interactions were computed every two time steps, and long-range electrostatic forces were computed every four time steps. The pair list of the nonbonded interaction was recalculated every ten time steps with a pair list distance of 13.5 Å. The short-range nonbonded interactions were defined as van der Waals and electrostatics interactions between particles within 12 Å. A smoothing function was employed for the van der Waals interactions at a distance of 10 Å. CHARMM27 [force-field parameters were used in all simulations in this study. The equilibrated system was simulated for 1 ps with a 500 kcal/mol/Å2 restraint on the protein backbone under 1 atm constant pressure and 310 K constant temperature (NPT) and the Langevin damping coefficient was set to 5 ps unless otherwise stated. With no harmonic constraints, the simulations ran for 30 ns in the NPT ensemble using Langevin dynamics at a temperature of 300 K. Pressure was maintained at 1 atm using the Langevin piston method with a piston period of 100 fs, a damping time constant of 50 fs, and a piston temperature of 300 K. Non-bonded interactions were smoothly switched off from 10 to 12 A°. The list of non-bonded interactions was truncated at 14 A°.

Structure Validation

Finally, the structure having the least energy with low RMSD (Root Mean Square Deviation) was used for further studies. In this step, the quality of the initial model was improved.The final structure obtained was analyzed by Ramachandran's map using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) (Brunger, A et al 1992) and environment profile using ERRAT graph (Structure Evaluation server) (Laskoswki et al 1993). This model was used for the identification of active site and for docking of the substrate with the enzyme.

Binding-site analysis

The Bindig-site of Tyrosine Kinase was identified using CASTP server. A new program, CAST, for automatically locating and measuring protein binding pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CAST identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings. When the search is complete, the largest site is automatically displayed on the structure. The results can be used to guide the protein –ligand docking experiment**.**

Molecular docking

30 analogs were built and optimized with Chemsketch software. Ligands were docked with model Tyrosine Kinase by GOLD software. GOLD is an implementation of multi conformer docking, which implies conformational search of the ligand is carried out. Thereafter all relevant low-energy conformations are rigidly placed in the binding site. The GOLD process uses a series of shape-based filters and default scoring function (based on Gaussian shape fitting) (Diaz et al., 2004).

III. Results And Discussion

Homology Modelling of Tyrosine Kinase protein Domain

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure.In the results of BLAST search against PDB, only the 1RJB which has a high level of sequence identity with the Human Tyrosine Kinase protein domain. Structurally conserved regions (SCRs) for the model and the template were determined by superimposition of the two structures and multiple sequence alignment.
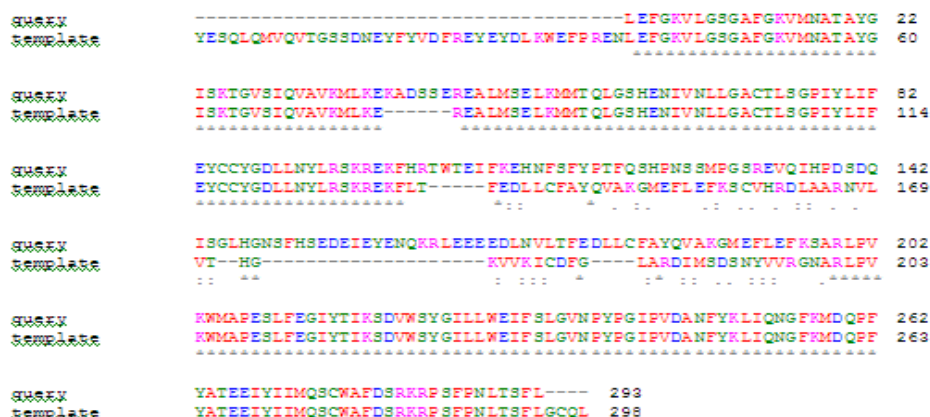
```
query        ------------------------------------LEFGKVLGSGAFGKVMNATAYG  22
template     YESQLQMVQVTGSSDNEYFYVDFREYEYDLKWEFPRENLEFGKVLGSGAFGKVMNATAYG  60
                                                 **********************

query        ISKTGVSIQVAVKMLKEKADSSEREALMSELKMMTQLGSHENIVNLLGACTLSGPIYLIF  82
template     ISKTGVSIQVAVKMLKE------REALMSELKMMTQLGSHENIVNLLGACTLSGPIYLIF  114
             *****************      **************************************

query        EYCCYGDLLNYLRSKREKFHRTWTEIFKEHNFSFYPTFQSHPNSSMPGSREVQIHPDSDQ  142
template     EYCCYGDLLNYLRSKREKFLT-----FEDLLCFAYQVAKGMEFLEFKSCVHRDLAARNVL  169
             ******************* :    *        :   .: :: ..  ::  .

query        ISGLHGNSFHSEDEIEYENQKRLEEEEDLNVLTFEDLLCFAYQVAKGMEFLEFKSARLPV  202
template     VT--HG------------------KVVKICDFG----LARDIMSDSNYVVRGNARLPV  203
             .: :   **                 : :::  *     :* :: .. :::   .*****

query        KWMAPESLFEGIYTIKSDVWSYGILLWEIFSLGVNPYPGIPVDANFYKLIQNGFKMDQPF  262
template     KWMAPESLFEGIYTIKSDVWSYGILLWEIFSLGVNPYPGIPVDANFYKLIQNGFKMDQPF  263
             ***********************************************************

query        YATEEIYIIMQSCWAFDSRKRPSFPNLTSFL----  293
template     YATEEIYIIMQSCWAFDSRKRPSFPNLTSFLGCQL  298
```

Figure 1: Alignment of Tyrosine kinase with 1Z3S

In the following study, we have chosen 1Z3S as a reference structure for modeling Human Tyrosine Kinase protein domain. Coordinates from the reference protein (1Z3S) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. In the modeller we will get a 20 PDB out of which we select a least energy. The energy unit will be in kilo joule. All side chains of the model protein were set by rotamers. The final stable structure of the Human Tyrosine Kinase protein obtained is shown in Figure 2.
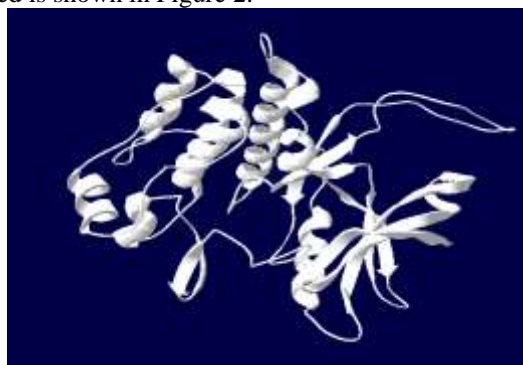

Figure 2: Modeller result

The structure having the least energy with low RMSD (Root Mean Square Deviation) which was obtained by the NAMD is in Figure 2.
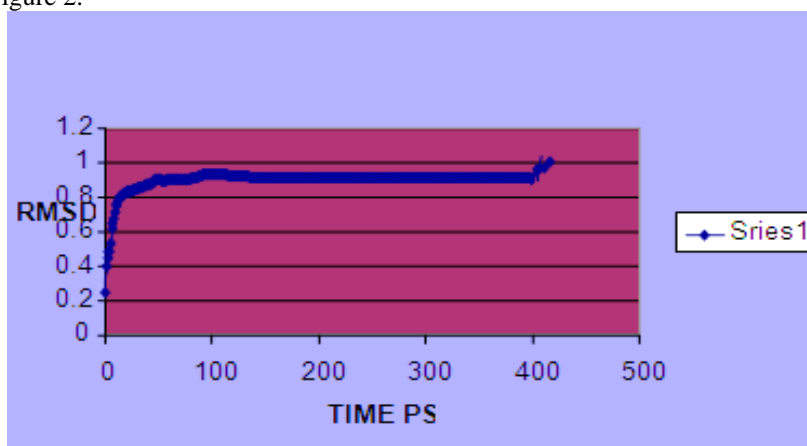

Figure 3: Calculated RMSD graph of molecular dynamics simulations of Human Tyrosine Kinase protein using NAMD software. Time (Ps) was taken in X-axis and RMSD in Y-axis

By the help of SPDBV it is evident that Human Tyrosine Kinase protein domain has 9 helices and 10 sheets. The final structure was further checked by verify3D graph and the results have been shown in Figure 4.
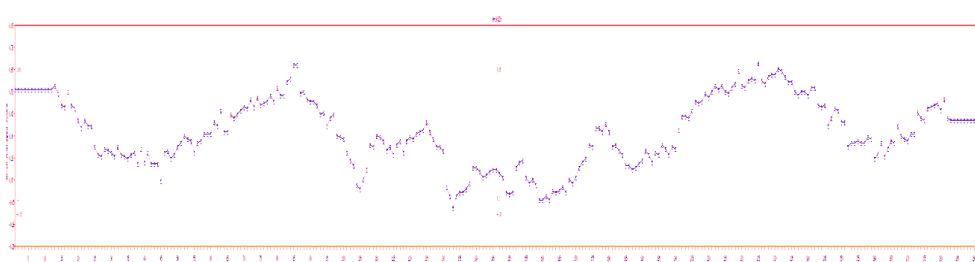

Figure 4: The 3D profiles verified results of Human Tyrosine Kinase Protein model; overall quality score indicates residues are reasonably folded.

Validation of Human Tyrosine Kinase protein Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program. The Ψ and θ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table I. The RMSD (Root Mean Square deviation) deviation for covalent bonds and covalent angles relative to the standard dictionary of Human Tyrosine KINASE protein was -4.27 and -0.85 Å. Altogether 100 % of the residues of Human Tyrosine protein was in favored and allowed regions. The overall PROCHECK G-factor of Human Tyrosine Kinase protein was – 2.32 and verify3D environment profile was good.
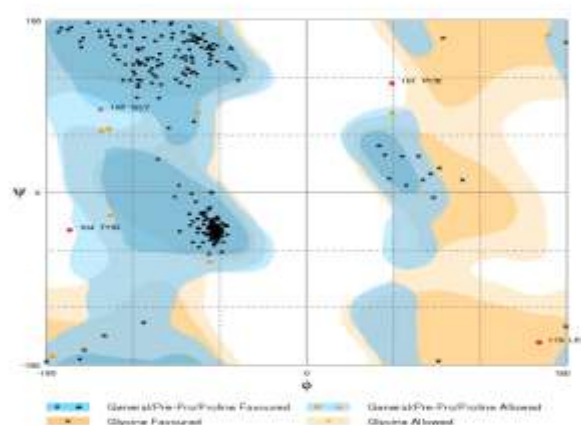

Figure 5: Ramachandran plot

Table I: % of residue falling in the core region of the Ramachandran's plot

% of residue in most favored regions	90.0
% of residue in the additionally allowed zones	8.9
% of residue in the generously regions	1.1
% of residue in disallowed regions	0.0
% of non-glycine and non-proline residues	100.0

Superimposition of 1Z3S with Human Tyrosine kinase protein domain

The structural superimposition of 1Z3S template and Human Tyrosine Kinase protein is shown in Figure 6. The weighted root mean square deviation of trace between the template and final refined models 0.42A°. This final refined model was used for the identification of active site and for docking of the substrate with the domain Human Tyrosine kinase protein.
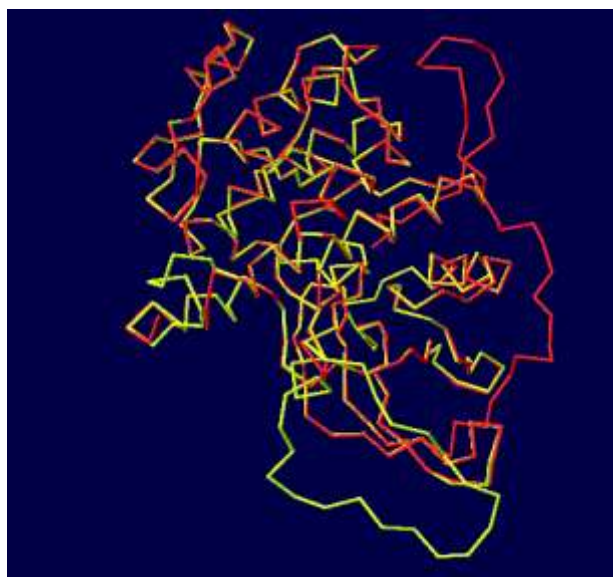

Figure 6: superimposition of Cα trace of Human Tyrosine kinase protein (red colour) and 1Z3S (represented in yellow color).

Activesite Identification

Active site is obtained using CASTp server, and the location of the site in the 3D structure of Tyrosine Kinase is shown in Fig.7. In fact from the sequence alignment of Tyrosine Kinase with 1Z3S, we know that the residues GLY-21, LEU-22, SER_23, LEU-24, GLU-25, ARG-216, VAL-217, and HSD-220 are conserved. By considering the experimental fact that the active site of 1Z3S includes all the residues mentioned above, and on other hand, the shape of the site in Tyrosine kinase is similar to that of 1z3s binding site. T Thus in this study the binding site is chosen to dock the analouges.
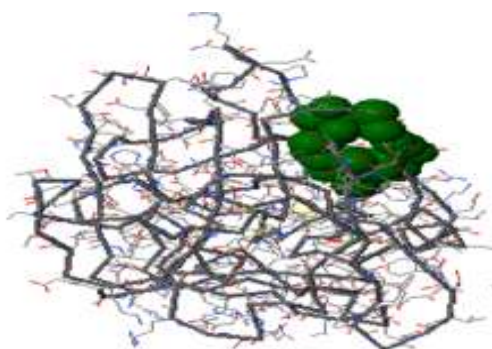

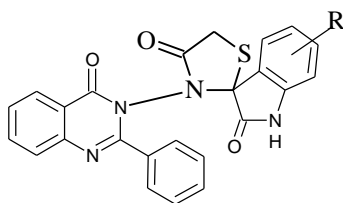**Figure-7:** Identification Of Active Site

Figure-8: STRUCTURE OF 3'-(4-OXO-2-PHENYLQUINAZOLIN-3(4H)-YL)-4'H-SPIRO[INDOLE-3,2'-[1,3]THIAZOLIDINE]-2,4'(1H)-DIONE

Docking study

In the presence of inhibitory results of analogues, new structural features and fictionalization requirements were proposed for the basic scaffold that could increase affinity with Tyrosine Kinase. Among these requirements, the modifications of the group in the structure by a more polar group were expected to increase the activity. These interesting results prompted us to prepare analogues. Here we developed different analogues by replacing with more polar groups than already existing groups, with little change in the properties of analogues represented in Table 1. These were used for docking studies to identify better drug derivative. Docking of inhibitors given in Table 1 (see supplementary material) with Tyrosine Kinase was performed using GOLD 3.0.1, which is based on genetic algorithm (CCDC). The docking procedure includes several steps. First, the protein-ligand complex is generated using the GOLD package without constraints between the ligand and the specific amino acids of the pocket. The algorithm exhaustively searches the entire rotational and translational space of the ligand with respect to the receptors. The flexibility of the ligand is given by dihedral angle variations. The various solutions evaluated by a score, which is equivalent to the absolute value of the total energy of the ligand in the protein environment. Thus docking with the program GOLD version 3.0.1 was employed to locate the appropriate binding orientation and conformation of compounds with Tyrosine Kinase.
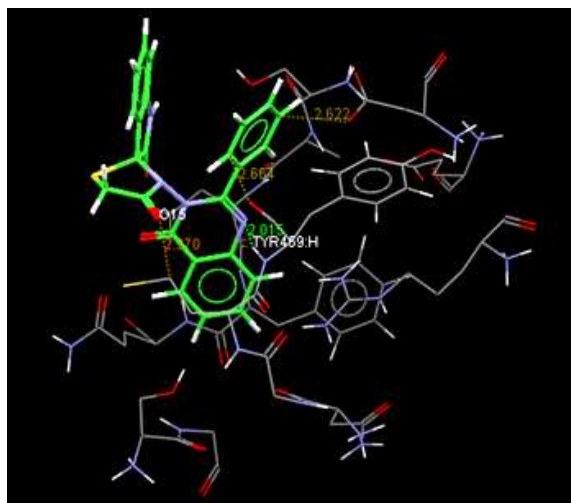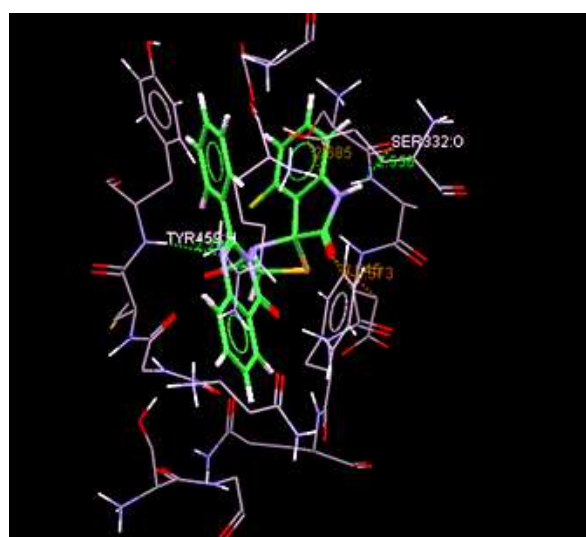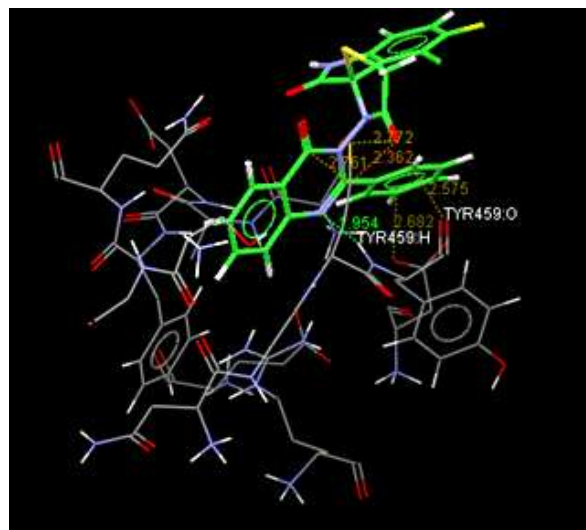
Table 1: Docking scores

Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)	Ligand name
39.54	0.36	31.83	0.00	-4.59	mol1
39.89	0.44	31.67	0.00	-4.10	mol10
40.27	1.32	31.21	0.00	-3.97	mol11
33.16	0.34	28.47	0.00	-6.34	mol12
43.08	0.28	34.46	0.00	-4.57	mol13
35.81	2.12	29.57	0.00	-6.97	mol14
33.75	1.65	28.96	0.00	-7.73	mol15
38.55	1.66	30.02	0.00	-4.39	mol16
39.92	1.78	30.10	0.00	-3.24	mol17
38.55	0.44	30.87	0.00	-4.34	mol18
39.61	1.53	32.93	0.00	-7.19	mol19
41.03	2.44	30.58	0.00	-3.46	mol2
39.77	2.40	30.06	0.00	-3.97	mol20

-25.64	0.32	31.32	0.00	-69.03	mol21
32.22	0.29	34.41	0.00	-15.38	mol22
34.33	0.44	30.30	0.00	-7.76	mol23
42.09	2.00	37.60	0.00	-11.61	mol24
36.50	0.38	33.82	0.00	-10.38	mol25
18.70	2.13	29.43	0.00	-23.90	mol26
41.13	2.00	38.29	0.00	-13.52	mol27
37.12	0.94	32.79	0.00	-8.91	mol28
32.34	1.15	30.03	0.00	-10.10	mol29
39.89	2.03	30.84	0.00	-4.55	mol3
40.54	0.55	36.31	0.00	-9.94	mol30
40.48	2.04	30.81	0.00	-3.92	mol4
36.25	0.37	35.42	0.00	-12.81	mol5
31.12	0.29	29.34	0.00	-9.51	mol6
39.55	1.50	30.93	0.00	-4.47	mol7
38.43	0.54	30.67	0.00	-4.28	mol8
39.20	1.67	31.39	0.00	-5.63	mol9

Basic Guidelines for Microwave Organic Chemistry Applications.
Introduction:
The main advantages of microwave assisted organic synthesis are:
a.   Faster reaction: the microwave can use higher temperatures than conventional heating system, and consequently the reactions are completed in few minutes instead of hours
b.   Better yield and higher purity: less formation of side product are observed using microwave irradiation, and the product is recovered in higher yield. Consequently, also the purification step is faster and easier.
c.   Easy scale-up: its technology and large range of reactor vessels, allows scale-up from few milliliters to one liter without changing reaction parameters.
d.   Reproducibility: the patented microwave diffuser for homogeneous microwave irradiation inside the cavity and precise control of reaction parameters, such as temperature, pressure and power, always reproduces the same reaction conditions. It is very simple to save and use an optimized synthesis method.
e.   Easy to use: all the reactors and software are very easy to use and all reactions can be easily moved from conventional to microwave heating.

How To Convert A Conventional Reaction Into A Microwave Reaction
When the reaction is performed the first time under microwave irradiation, run the reaction in small scale, slowly increasing the temperature.
The parameters that are needed to be defined are: -
   • Solvent
   • Temperature-time
   • Vessel
   • Microwave program
Solvent The same solvent that is usually used with conventional heating chemistry can also be used with microwave heating. Solvents interact differently with microwaves, depending on their polarity. Polar solvents (alcohols, DMF, water, ketone, acid) couple well with microwaves and reach high temperatures in a short time. Non-polar solvents (toluene, chloroform, hexane) are transparent to microwaves. Therefore, two situations are possible: 1) non polar solvent, but polar reagents or at least one polar reagent: the reaction mixture is heated by microwave. 2) non polar reaction mixture (both solvent and reagents). (Weflon has to be added in order to heat the mixture.

Temperature-Time
   a) If the reaction has already been performed with conventional heating, take in consideration the standard reaction temperature and time. Based on these two parameters, consider the Arrhenius equation, e.g.

how the time decreases when the temperature increases. This law defines that every ten degrees that the temperature increases, the time of the reaction is halved.

For example, if a reaction is run in EtOH at 80°C for 8 hours and the Arrhenius law is applied, the time is reduced in accordance to the table below

Temperature (°C)	Time
80	8 h
90	4 h
100	2 h
110	60 min
120	30 min
130	15 min
140	8 min
150	4 min

This simple procedure can be applied to all the reactions. b) If the reaction has never been performed before with conventional or microwave heating, fix the temperature at 30-40°C higher than the boiling point of the solvent, and run the reaction for 10 minutes. Then check the obtained reaction mixture

Vessel

All reactors that work with the Micro synthesizer have different:

a) volume limit b) temperature limit c) pressure limit When the target temperature is fixed. Check which vapor pressure is developed from the solvent at the chosen temperature. Based on this value, and on the volume that is needed, decide the appropriate reactor vessel.
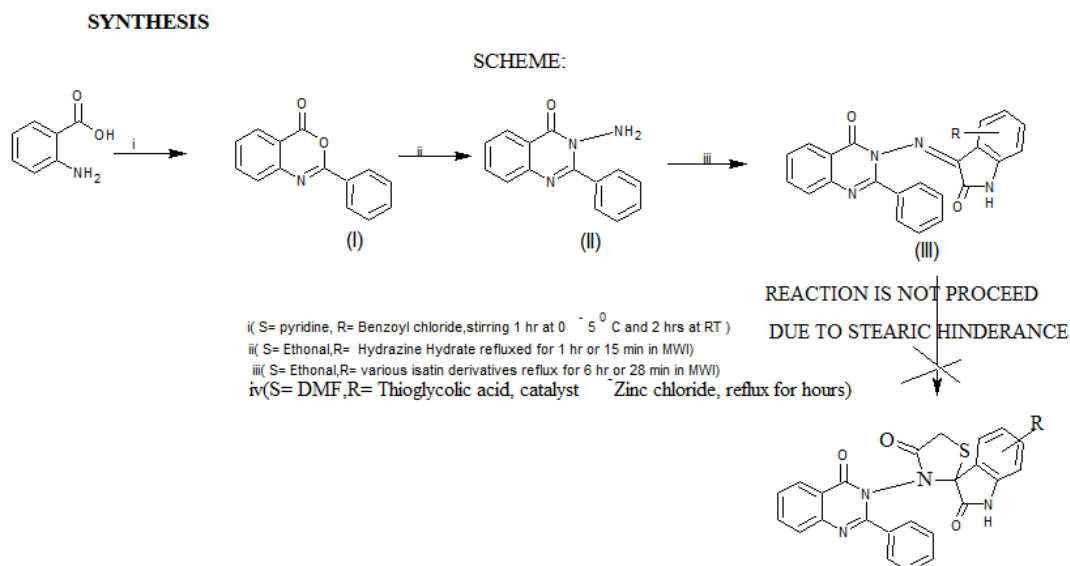
How To Optimize The Reaction Conditions

After the first run of the reactions, there could be four different cases:

1) The reaction is complete (the starting material is not present any more): transfer the mixture in proper glassware and proceed with the usual work-up of the reaction.
2) The reaction starts to work but is not complete (some starting material is still present): - extend the reaction time - increase the temperature (not over the temperature and pressure limit of the vessel).
3) The reaction doesn't work at all: - extend the time - increase the temperature - use more equivalent of one of the starting material or of the catalyst
4) Decomposition of the reagents: - use lower temperature - use short reaction time Note: always remember to check the temperature and pressure limit of the vessel before increasing the temperature.

SYNTHESIS

Synthetic method development trials was found that not possible of making the thiozolidine moiety on 3-{[(3E)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]amino}-2-phenylquinazolin-4(3H)-one derivatives with thioglycolic acid.

SYNTHESIS

SCHEME:

i(S= pyridine, R= Benzoyl chloride, stirring 1 hr at 0 ̄5°C and 2 hrs at RT)
ii(S= Ethonal, R= Hydrazine Hydrate refluxed for 1 hr or 15 min in MWI)
iii(S= Ethonal, R= various isatin derivatives reflux for 6 hr or 28 min in MWI)
iv(S= DMF, R= Thioglycolic acid, catalyst ̄Zinc chloride, reflux for hours)

REACTION IS NOT PROCEED
DUE TO STEARIC HINDERANCE

Experimental Proceedure:
Step –I-(2-phenyl-4*H*-3,1-benzoxazin-4-one)
In to 250 ml of conical flask 1.37 gram of anthranilic acid was dissolved in the10ml of pyridine then add 1.4 ml of benzoyl chloride as drop wise while the mixture is stirring and maintain the temperature at 5 degree Celsius for 60 min with continues stirring.
After that stir the reaction mixture for 120 min at room temperature, a solid precipitate will be form, and then precipitate was collected by filtration. Recrystalization by the help of ethanol.
Percentage of yield is 87%
MeltingPoint:122-125ºC
Boiling Point: 190-193 ºC .
Step-II-(3-amino-2-phenylquinazolin-4(3*H*)-one).
0.01 mole (**2-phenyl-4*H*-3,1-benzoxazin-4-one**) of sample was taken in ethanol. To this equimolar quantity of hydrazine hydrate was added and refluxed for 15 minutes under microwave synthesizer or one hour reflux. Then the reaction was cooled. Product so formed as precipitate by filteration, dried and recrystallised using ethanol. Purity of the compound was checked by TLC using hexane and ethyl acetate solvent system in 8:2 ratio. Melting point was found to be 179-182 degree celcius.
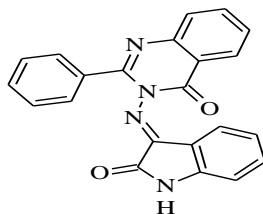Percentage of yield is 92%
 Step-III:
3-{[(3*E*)-2-OXO-1,2-DIHYDRO-3*H*-INDOL-3-YLIDENE]AMINO}-2-PHENYLQUINAZOLIN-4(3*H*)-ONE
0.01 mole of 3-amino-2-phenylquinazolin-4(3*H*)-one dissolved in ethanol and 0.01 mole of different isatin derivative each time in to the round bottom flask and reflux under microwave synthesizer for 28 minutes or 6 hours for conventional reflux. After completion of the reaction kept a side for cooling the reaction, then forms precipitate which we collect by filtration. Then dry the compound and recrystalized with alcohol.
Percentage of yield is 89%

3-{[(3*E*)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]amino}-2-phenylquinazolin-4(3*H*)-one

Step IV:
0.001 mole of 3-{[(3*E*)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]amino}-2-phenylquinazolin-4(3*H*)-one soluble in 10 ml of DMF / T.E.A / Ethanol / Methanol /THF/ toluene/ pyridine, then 0.001 mole of thioglycolic acid is added, to this solvent added catalytic amount of anhydrous zinc chloride. refluxed for hours together by changing the concentrations. Reaction is not proceeded why because stearic hindrance which is having on both sides of nitrogen atom.

IV. Conclusion

HUMAN Tyrosine Kinase Protein is one of the important proteins in Blood cancer. In this work, we have constructed a 3D model of Human Tyrosine Kinase protein domain, from human using the MODELLER software and obtained a refined model after energy minimization. The final refined model was further assessed by ERRAT & PROCHECK program, and the results show that this model is reliable. The interaction between the domain and the inhibitors proposed in this study are useful for understanding the potential mechanism of domain and the inhibitor binding. The hydrogen bonds play important role for the structure and function of biological molecule in this study, and we found that GSN235, AsN257, and ILE289 of Tyrosine Kinase are important for strong hydrogen bonding interaction with these inhibitors. To the best of our knowledge GSN235, AsN257, and ILE289 are conserved in the domain and may be important for structural integrity or maintaining the hydrophobicity of the inhibitor-binding pocket. After synthetic method development trials was found that not possible of making the thiozolidine moiety on 3-{[(3*E*)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]amino}-2-phenylquinazolin-4(3*H*)-one derivatives with thioglycolic acid.

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