*IN-SILICO*DESIGN,SYNTHESISANDANTI-MICROBIALEVALUATIONOF2,4,6-TRISUBSTITUTED1,3,5–TRIAZINE-BASEDALDEHYDEDERIVATIVES

ABSTRACT

Background & Objective: Cancer is a serious public health concern, particularly in developing countries. Thecurrent investigation was to discover novel EGFR and aromatase inhibitors utilizing ligand- and structure-baseddrugdevelopmenttechniques.Thisstudyalsofocusesontheantimicrobialactionofthedesignedcompounds.*In-vitro*Anti-microbial evaluation of thedesigned compoundswas assessed.

MaterialsandMethods: Aseriesoftriazinebasedaldehydesubstitutedderivativesweredesignedandmoleculardocking studies were carried out against Aromatase inhibitors, HER-2, Dihydrofolate reductase targets by usingAutodock 1.5.6. The designed compounds were synthesized by condensing triazine with various aldehydes andcharacterisedusingMassspectrometry.*Invitro*Antimicrobialactivitywasstudiedforthesynthesizedcompoundsagainstthe standard drugAmpicillin.

Results and Discussion: *In silico* study revealed that the designed compound had a 3D structure that iscomparable to other well-studied anti breast cancer proteins. According to molecular docking, proteins exhibithigh binding energy to enzymes. Minimum inhibitory concentration validated the efficiency of the designedcompoundsutilisedforantibacterialactivitytesting.Antimicrobialsusceptibilitytestsrevealedthatthecompoundshaveanantimicrobial action.

Conclusion: The present study suggests that the synthesized compounds possessing drug-like properties as aplausibletherapeuticcandidateagainstmicrobialactivity.However,thedesignedcompoundscouldbeemployedasinhibitors ofEGFR andaromatase after passingthrough *invivo* and *in vitro* evaluation.

Keywords:*Breast cancer,Triazine,Moleculardocking, Aromatase,Pharmacologicalevaluation.*

1. INTRODUCTION

Breast cancer is one of the most frequent cancer in women and, after lung cancer, it is the second most seriousdisease in terms of mortality [1].Breast cancer patient cases are growing in both developed and developingnations [2].Itisanticipatedthatby2030,therewouldbe26millionnewcasesofcancerand17milliondeathsdueto cancer worldwide [3].Though the majority of breast cancers remain benign and treatable with surgery, one-quarter of these cancers have a dormant and insidious nature, developing slowly but metastasizing quickly.Current medicines considerably slow tumour development, but recurrence is unavoidable, resulting in highfatality rates. Breast cancer cell function is seeded in its inception. Embryonic mammary cells have motile andinvasivecapabilities,andmammarydevelopmentischaracterizedbycellmobilityandalterationsin cellcontact.Several measures, such as preventative behaviours in general and screening programs, are critical in terms ofreducing the incidence rate of breast cancer and implementing early treatment. Currently, that Breast HealthGlobal Initiative (BHGI) is in charge of developing appropriate recommendations and ways to give the mostadequate breast cancer control worldwide. We focused on female breast cancer especially in the present studysince,as previously said,it is now themostcommon diseaseamong females [4,5].Breast cancer patients have benefitted tremendously from surgery, radiation, chemotherapy, hormone treatment,and gene-targeted therapy. Current therapy and detection technologies have improved, contributing to a higherrate ofsurvival [6].Breast cancer develops when the normal cells in the tissue of the breast expand and alter, becoming a mass ofcellsknownasatumor.Thetumorthatformscanbeeitherbenignormalignant.Breastcancerhasseveralphasesthatreflect how the diseasehasprogressed. StagescompriseI,II,andIII [7].Addressed treatment targeting the oestrogen receptor is important in systemic therapy; endocrine resistancepathwayshavealsobeenaddressed.BiologicaltreatmenttargetingtheHER2receptorhasrecentlybeendeveloped,as acombinationofantibodydrugconjugateslinkingcytotoxictherapytoHER2antibodies.Thenewefficientsystemicmedicinesresultedinsignificantincreasesinsurvival,andpatientswithmetastasesareexpectedto livelonger [8].There are several pharmacologically active nitrogen-containing heterocyclic compounds, the most important ofwhich being s-Triazine. It has anti-bacterial, anti-tubercular, anti-fungal, anti-malarial, antiplasmodial, antiviral,anticancer,respiratory stimulant, CNS depressant,and herbicidal properties,among others [9].Different triazine moiety substitutions at different positions have been shown to have varied activities. Manyresearchers (1,3,5) advocate for the successive incorporation of different piperazine and piperidine substituentsintothe triazine ring [9].Medical microbiology is the field of research that focuses on understanding how to prevent and treat diseasesbroughtonbymicrobes.Inorder totackle microbialresistance,therehasbeenanincreasinginterestininvestigatingandcreatingnovelantimicrobialagentsfromdifferentsources.Asaresult,approachesforscreeningandmeasuring antimicrobial activity havereceived moreattention

[10].A logical and promising area of research in contemporary medicinal chemistry is the design of novel smallmolecules that resemble drugs and are based on pharmacologically active scaffolds [11]. In order to createinnovative treatments compounds with a wide range of pharmacological actions, medicinal chemists havecombinedanumberof biologically activepharmacophores to createanumber ofdifferent molecules [12, 13].One of the most fundamental and significant methods for drug discovery has been molecular docking studies. Itenablesthe prediction ofthe molecularinteractions that bind aproteinto aligand and keep themtogether [14].Drug development is a difficult process, and choosing the proper lead molecule is critical to the project's overallsuccess. According to a study conducted by the Tufts Center for the Study of medicine Development, the cost ofdeveloping and bringing a new medicine to market has climbed by over 145% in the previous decade [15].Moreover, while the average amount of time to get a medicine to clinical trials has been lowered, the successpercentage of pharmaceuticals receiving FDA clearance has declined to 12% [15].By guiding experimentalresearch toward ideal molecules more rapidly, computer-aided drug design (CADD) has made it possible to cutthecosts and time associated with drug development.

2. MATERIALSANDMETHODS

The reagents and solvents utilized in the synthesis were acquired from Supra Scientific Lab, and they wereemployed without additional purification. The melting points of synthesized compounds were determined usinga precision melting point apparatus LH-109, which is a research-grade instrument equipped with a 128×64graphical LCD display and an icon-based navigation menu. Thin-layer chromatography was carried out on pre-coatedaluminumsheets.Various solventsystemswere employed,andthespots wereidentifiedusing UVlight.

2.1 Synthesisof4,6-dichloro-N-(puridin-4-yl)-1,3,5-triazine-2-amine

A mixture of 2, 4, 6-trichloro 1,3,5-triazine (0.01mol) in acetone with water ratio (1:1) and 4-aminopyridine(0.01mol)was taken in a conical flask .To this mixture,10% sodium carbonate was addeddrop-wise at 0- 10°C temperature. The solution was stirred for 2h. The reaction mixture was thenpouredintocrushedicewithconstantstirring.Thesolidformedwasfiltered,washedwithwater,driedand recrystallized using acetone. Molecular formula $C_8H_5Cl_2N_5$,Molecular weight 242.0648g/mol,Percentageyield 78%, Rf value 0.7, Melting point 150°C-155°C.

2.2 Synthesisof4-cloro-N-(pyridin-4yl)-6-(pyridin-4-yloxy)1,3,5-triazine-2-amine

A mixture of 4,6-dichloro-*N*-(pyridin-4-yl)-1,3,5-triazin-2-amine (0.01mol) in acetone with waterratio(1:1)and4-hydroxypyridine(0.01mol)wastakeninconicalflask.Tothismixture,10%sodiumcarbonate was added drop-wise at room temperature and was stirredfor 3hr.The reaction mixturewasthenpouredontocrushedicewithconstantstirring.Thesolidobtainedwasfiltered,washedwithwaterandrecrystallizedfromacetone.MolecularformulaC13H9ClN6O,Molecularweight300.031g/mol,Percentageyield64%, R f value0.8, Melting point148°C-153°C.

2.3 Synthesisof1-(4-{[4-phenoxy-6-(pyridin-4-ylamino)-1,3,5-triazine-2yl]amino}phenyl)ethanone

Amixtureof4-chloro-*N*-(pyridin-4-yl)-6-(pyridin-4-yloxy)-1,3,5-triazin-2-amine(0.01mole)and4-aminoacetophenone(0.01mole)weredissolveinacetonewithwaterratio(1:1) andthereactionmixturewasrefluxedfor12hr.sodiumcarbonate10%solutionwasaddedtoneutralizethereactionmixtureanditpouredintocrushedice.Thesolidseparatedoutwasfiltered,washedwithwaterandpurifiedbyrecrystallization.MolecularformulaC21H17N7O2, Molecular weight 399.4058g/mol, Percentage yield 60, R f value 1.04, Melting point 171°C -176°C.

2.4 Synthesis of (2Z)-3-phenyl-1-[4-({4-[(pyridine-4-yl)amino]-6-(pyridine-4-yloxy)-1,3,5-triazine-2yl}amino)phenyl]prop-2-en-1-one

Amixtureof1-[4-({4-[(pyridine-4-yl)-6-(pyridine-4-4yloxy)-1,3,5-triazine)phenyl]ethan-1-one(0.01mole) and substituted aromatic aldehyde (Benz aldehyde) (0.01mole) was taken in 30ml DMF40% KOH was added into the reaction mixture. The reaction mixture was stirred for 2hr and laterovernight. Above reaction mixture was poured into ice water, neutralized with HCL, filter, washedwithwater,andpurifiedbyrecrystallizationfromethanoltoyieldproduct(1)compoundsthehomogeneityofcompoundswascheckedbyTLC.MolecularformulaC28H21N7O2,Molecularweight487.5120g/mol,Percentageyield56%, R f value0.9.

2.5 Synthesis of 2-N-[4-(2-aminopyrimidin-4-yl)phenyl]-4-N-(pyridin-4-yl)-6-(pyridin-4-yloxy)-1,3,5-triazine-2,4-diamine

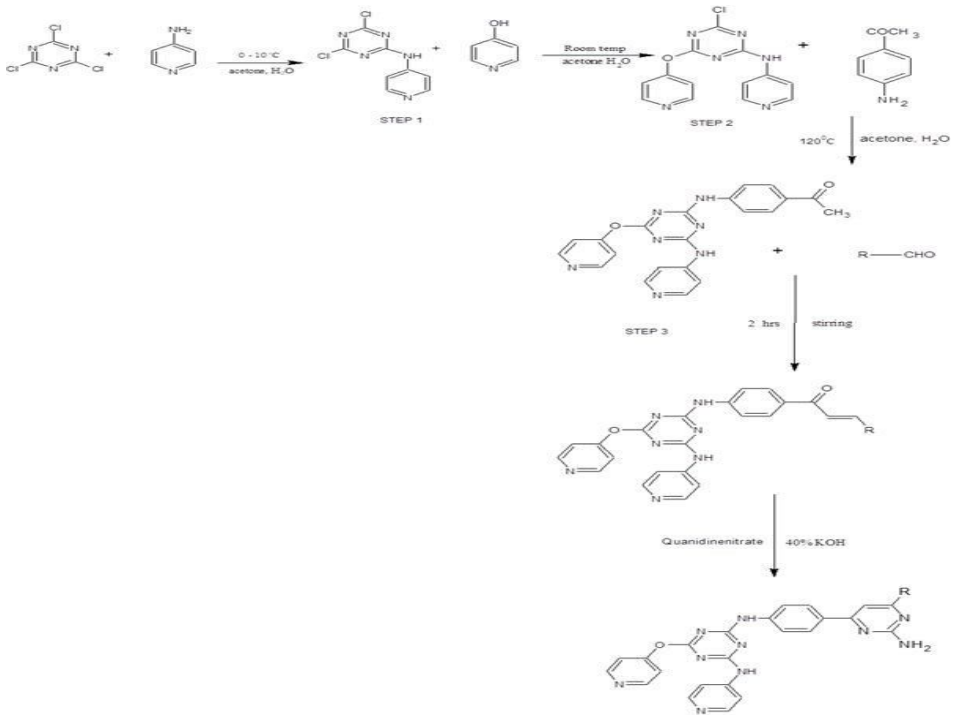


Fig. 1. SCHEMEFORTHESYNTHESISOFDESIGNEDDERIVATIVES

A mixtureof(2Z)-3-phenyl-1-[4-({4-[(pyridine-4-yl)amino]-6-(pyridine-4-yloxy)-1,3,5-triazine-

2yl}amino)phenyl]prop-2-en-1-onewastakenin25mldioxinguanidinenitrate(0.01mole)and40%KOH(5ml)was added and the mixture refluxed for 15hr. then the reaction mixture was cooled and poured into ice coldwater,filtered,driedandthen purifiedby recrystallizationfrom alcoholtogive thefinal product.

2.6 PROPOSEDSCHEMEDETAILS

2.6.1 ANTIBACTERIALAVTIVITY

Thecompoundswesynthesized(TZ1-TZ3)weresubjectedtotestingagainstbothgram-positivebacterialstrains,which include *B. subtilis* and *S. aureus* (MTCC-96), as well as three gram-negative strains, specifically *E. coli*(MTCC-443) and *P. aeruginosa* (MTCC-424). We followed the standard protocol for the agar well diffusionmethodonMueller-Hintonagar(MHA).Toinitiatethetesting,weselectedisolatedcoloniesfromculturesgrownon agar plates for 18 to 24 hours, typically using a non-selective medium like blood agar. In aseptic conditions,we punched holes with diameters ranging from 6 to 8 mm, positioning them at a distance of 25 mm apart fromeach other. Subsequently, we introduced 10 µL volumes of the tested compounds dissolved in DMSO into thesewells. Afterward,theplateswereincubatedat 37°Cforaduration of24 hours.To ensurethat thesolvent didnothinder bacterial growth, we also screened a DMSO control (at a concentration of 5% v/v). We measured theinhibition zones with precision to the nearest whole millimeter using a meter ruler, and we conducted theseexperimentsmultipletimesforconsistency.Furthermore,wedeterminedtheMinimumInhibitoryConcentration(MIC) values using the agar dilution method. The MIC represents the lowest concentration at which bacterialgrowthisinhibited.Forthispurpose,wedilutedthestocksolutiontoviarousconcentrations,including200,400, 600, 800,and 1000 µg/mL.

2.7 MOLECULARDOCKING

Docking is a method which predicts the preferred orientation of one molecule to a second molecule when boundto each other to form a stable complex in three dimensional spaces. Auto dock is a software used for modelingflexible small molecule such as drug molecule binding to receptor proteins. ADT is used to prepare, run andanalysethe docking stimulation, inaddition to itsseveralfeatures necessary formodelingstudies.

Building the receptor: In this step the 3D structure of the receptor should be downloaded from PDB andmodified. This should include removal of water molecules from the cavity, stabilizing charges, filling in themissing residue, generation of side chain etc., according to the parameters available. After modification thereceptorshould bebiologically activeand stable.

Identification of active site: After the receptor is build, the active site within the receptor should be identified.Thereceptor may havemany activesites but the oneof theinterests shouldbeselected.

Ligandpreparation: LigandcanbeobtainfromvariousdatabaseslikeZINC,PubChemorcanbesketchedusingtools like chemsketch. While selecting the ligand, the Lipinski rule of 5 should be applied.The rule is veryimportant for drug development where the pharmacologically active lead structure is optimized stepwise

forincreasedactivity and selectivity as well as druglikeproperties.

Docking: Thisisalaststep,wheretheligandisdockedintoreceptorandtheinteractionarechecked.Thescoringfunctiong enerates depending on which ligand with best fit wasselected.

3. RESULTANDDISCUSSION:

Inthiscurrentstudy,wedigitallydesignedacollectionofsmallchemicallibrariesconsistingofaminopyrimidinederivati ves with triazine-based aldehyde substitutions. Three compounds were synthesized by the condensationof amino pyrimidine with different aldehydes, specifically chlorobenzaldehyde, orthonitrobenzaldehyde, anddimethylaminobenzaldehyde.Thecompletionofthereactionswasverifiedthrough Thin-LayerChromatography(TLC) using a mobile phase consisting of chloroform and methanol in an 8:2 ratio. The spots were visualizedusingaUVchamber.Thestructureofthesynthesizedcompoundswascharacterizedby MASSspectroscopyan d results werefound tobeconsistentwith the predicted structure.

To sum up, the correlation shown between docking scores and inhibition zones for dimethylaminobenzaldehyde, orthonitrobenzaldehyde, and chlorobenzaldehyde shows the significance of chemical structure in determining antimicrobial action. The interaction between electron-donating and electron-withdrawing groups greatly affects binding affinity as well as chemical reactivity. Subsequent investigations ought to concentrate on clarifying particular modes of action for these substances by means of experimental verification in conjunction with computational modeling.

3.1*IN-VITRO*ANTIBACTERIALACTIVITY

A synthesized series of triazine derivatives was evaluated for their antibacterial activity through an in vitroscreening method, specifically the agar well diffusion technique on Mueller Hinton agar. The assessmentencompassed the inhibition of the growth of selected pathogens, including eight Gram-positive bacterial strains(suchas*B. subtilis*and*S.aureus*)andthreeGram-negativebacterialstrains(namely*E. coli,P.aeruginosa,andK. pneumonia*).Thepercentageofinhibitionagainstboth Gram-positive and Gram-negative bacteria is presented in a table. The size of the inhibition zones was measuredin millimeters using the established agar well diffusion method. These values were compared against a control(Ampicillin), which was prepared in the same manner with DMSO as the solvent. Additionally, the minimuminhibitoryconcentration (MIC) values weredeterminedusing the agar dilution method.Thevariousfeaturesinsynthesizedcompoundsliketriazineunitsatthecentralandinclusionof variousbenzaldehydesontheterminalaminopyrimidineunitand4,6-branchingatthecentraltriazinesubstitutionsetc.,showedhighestproximityformoleculardocking.*In-silico*studyshowedthatthecompounds2and3werefound topossessvery goodantimicrobial.From theAntibioticsusceptibility,MICforcompound**2B**wasfoundtobe 1000 µg/mL against*K. pneumonia*

Table 1.Anti-bacterialSusceptibilitytestforsynthesizedcompounds

Code	ZoneofInhibition(inmm)at10mg/10ml				
	Grampositive		Gramnegative		
	B. Subtilis	*S. auereus*	*E. Coli*	*K. pnuemoniae*	*P. aerugonosia*
Compound TZ1	14	16	13	10	14
Compound TZ2	17	18	21	15	23
Compound TZ3	13	15	19	21	16
Ampicillin	25	26	27	25	19

MINIMUMINHIBITORYCONCENTRATIONOFSYNTHESIZEDCOMPOUNDS

3.2 EstimationofMICby agardilutionmethod

Minimum inhibitory concentrations (MICs) were determined by agar dilution technique. The procedure fordetermining the Minimum Inhibitory Concentration (MIC) is identical to the method used for measuring theZone of Inhibition. Based on the results obtained from the Zone of Inhibition assay, it was observed thatcompound 2A exhibited superior activity when compared to the other synthesized compounds. As a result,compound 2A was selected for further investigation in Minimum Inhibitory Concentration (MIC) studiesagainst Klebsiella pneumonia. Various concentrations, including 200 µg/mL, 400 µg/mL, 600 µg/mL, 800µg/mL, and 1000 µg/mL, were employed in these MIC studies. The result of MIC was tabulated in tableandthefigurerepresented thezoneof inhibition ofcompound at variousconcentrations.

Table2.MinimumInhibitoryconcentrationofsynthesizedcompounds

Testedcompound	Zone ofInhibition(inmm)				
	200µg/ml	**400µg/ml**	**600µg/ml**	**800µg/ml**	**1000µg/ml**
Klebsilapneumonia					
Compound	15	16	19	20	**22**

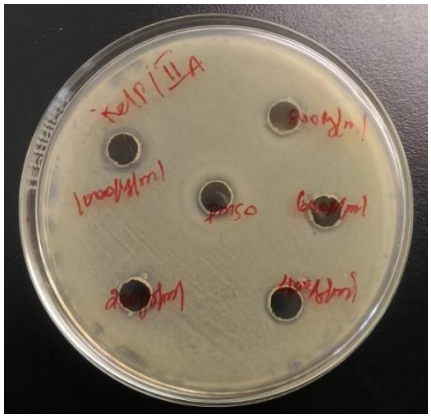


Fig. 2. MinimumInhibitoryconcentrationofsynthesizedcompounds

3.3 MOLECULARDOCKING

The synthesized compounds underwent preliminary Quantitative Structure-Activity Relationship (QSAR) studiesusing MOLINSPIRATION software, and the results are provided in the table. Some of the synthesized compoundsadheredtoLipinski'sRule.Furthermore,dockingstudieswereconductedforallthedesignedcompoundsemploying AUTODOCK version 1.5.6 software. These studies aimed to predict the interactions between thedesignedligandsandthe anti-cancer enzymeDihydrofolatereductase.InregardtoAromatasecytochromeP450,thebindingenergiesofthecompoundsfellwithintherangeof -11.4to-11.7.Notably,compoundTZ2exhibitedthehighestbindingaffinitywithascoreof-11.74,surpassingthestandardLETROZOLE,whichhadabindingenergyof-8.34.For the HER2 (erbb2) receptor, the binding energies of the compounds varied from -8.0 to -10.0. Notably,compoundTZ3displayedthehighestbindingaffinitywithascoreof-10.31,outperformingthestandardNERATINIB,whichhada bindingenergyof-6.38.In the case of Dihydrofolate reductase, the binding energies of the compounds ranged from -13.2 to -13.4.Remarkably, compound TZ2 exhibited the highest binding affinity with a score of -13.48, surpassing the standardMETHOTREXATE,whichhadabindingenergyof -10.81.Allofthesynthesizedcompoundsdemonstratedfavorablebindingenergyagainstvariousenzymes.RegardingtheADMEproperties,thedesignedcompoundswereevaluatedusingSWISSADMEsoftware.Thesecompoundswerefound to be polar with good to moderate water solubility, suggesting that they are likely to possess good oralabsorption and bioavailability. Furthermore, the toxicity profiles of all the designed compounds were assessedusing the pKCSM web server. Encouragingly, none of the synthesized compounds were found to exhibit Amestoxicity.However,it'sworthnotingthatallthreecompoundsdisplayedhepatotoxicityinthestudy.Thisimpl

iesthatfurtherinvestigationandpotentialmodificationsmaybenecessarytomitigatethisadverseeffectwhilepres ervingthedesired therapeutic properties.

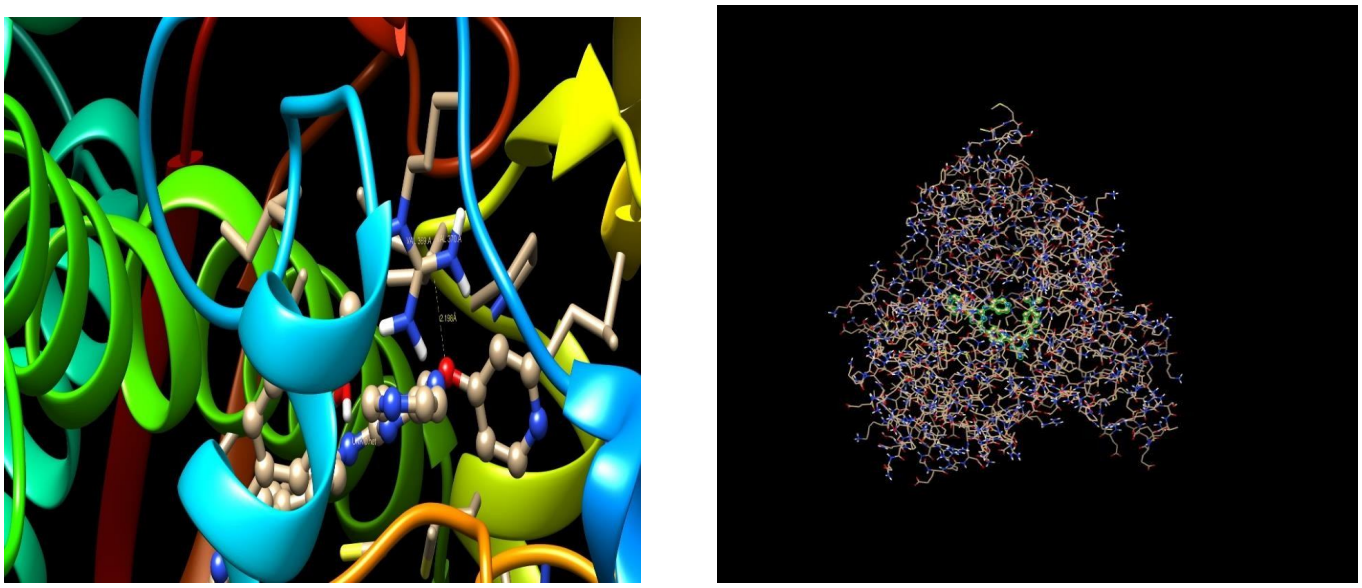


Fig. 3.

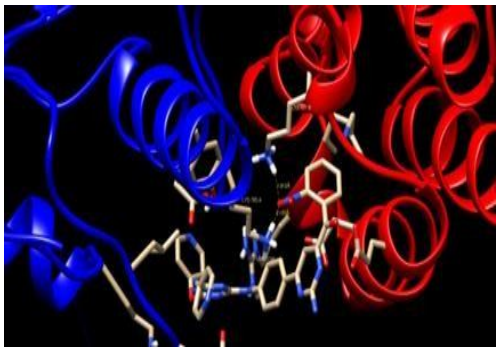
Dockedposesofthesynthesizedtriazine(TZ3)intheactivesiteofTargepro tein(3EQM)

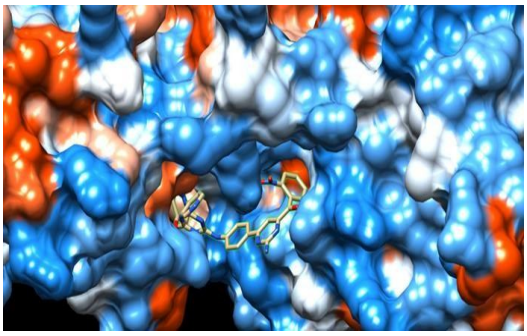
Table – 3: EnergyMinimalization

COMPOUNDCODE	LIGANDEFFICIENCY	INHIBITORYCONSTANT	INTERMOLENERGY	Vdw-hb-DESOLVATIONENERGY	BINDINGENERGY
TZ13EQM	-0.27	4.19	-14.11	-14.49	-11.43
TZ2	0.26	2.47	-14.73	-15.36	-11.74
TZ3	-0.26	3.79	-14.47	-15.03	-11.49

STANDARD	- 0. 38	776. 48	-9.23	-9.27	-8.34
TZ13 PP0	- 0. 2	667. 68	-11.11	-10.51	-8.42
TZ2	- 0. 2	306. 38	-11.87	-10.45	-8.89
TZ3	- 0. 23	27.6 3	-13.29	-9.71	-10.31
STANDARD	- 0. 16	20.9 4	-9.37	-8.91	-6.38
TZ14 KAK	- 0. 32	188. 7	-15.95	-14.98	-13.27
TZ2	- 0. 3	132. 36	-16.46	-16.21	-13.48
TZ3	- 0. 3	150. 99	-16.38	-14.5	-13.4
STANDARD	-	12.0	-13.49	-11.82	-10.81

	0. 33	1			





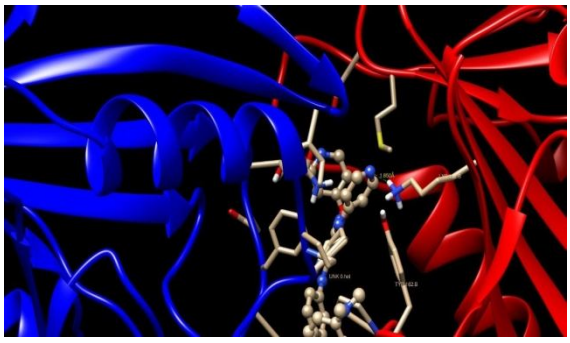


Figure- 4: dockedposesofthesynthesizedtriazine(TZ2)intheactivesiteofTarget protein(3PP0)

3.4 PRELIMINARYQSARSTUDIES

Table 4.DRUGLIKENESSPROPERTYUSINGMOLINSPIRATIONWEBTOOL

Compound code	GPCRLIGAND	IONCHANNELMODULATOR	KINASEINHIBITOR	NUCLEAR RECEPTOR LIGAND	ENZYMEINHIBITOR	PROTEASE INHIBITOR
TZ1	0.15	-0.54	0.32	-0.41	0.07	-0.07
TZ2	0.03	-0.78	0.15	-0.56	-0.11	-0.10

TZ3	-0.06	-0.79	0.05	-0.62	-0.15	-0.15

Table 5.TOXICITYPROFILEUSINGpKCSMWEBTOOL

COMPOUNDCODE	**AMESTOXICITY**	**HEPATOTOXICITY**	***T.PYROFORMIS* TOXICITY** (logµg/l)	**MINNOWTOXICITY** (logmM)
TZ1	NO	YES	0.285	0.948
TZ2	NO	YES	0.285	0.948
TZ3	NO	YES	0.285	-0.752

Table 6.<u>Molecular property of Designed Compound- SWISSADME</u>

Compound	Molecular Weight g/mol	Tpsa A^2	Log p	No.of Hydrogen Bond Acceptor	No.of Hydrogen Bond Donor	Water Solubility	GI Absorption	BBB Penetration
T21	491.54	112.03	3.39	5	4	Moderately Soluble	High	No
T22	487.51	109.03	3.31	4	4	Moderately Soluble	High	No
T23	516.51	162.68	3.80	9	3	Poorly Soluble	Low	No

4. CONCLUSION

Insummary,thefusionofbenzaldehydeswithacompacttriazinepharmacophorehasyieldedafreshcategoryoftriazine derivatives. These compounds showcase notable antibacterial capabilities and demonstrate encouragingpotential in molecular docking assessments against a crystalline structure. These outcomes further

inspire us toembarkonanexpandedsyntheticexploration,withtheobjectiveofuncoveringtheStructure-ActivityRelationship (SAR) within this fascinating group of compounds. Designed compounds possessing drug-likeproperties as a plausible therapeutic candidate against microbial activity. However, the designed compounds canbeemployed as inhibitorsof EGFR andaromataseafter passingthrough *in vivo* and*in vitro* evaluation.

Competing interests

The authors declare that they have no competing interest.

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