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Virtual Screening through Molecular Docking Analysis to Identify Potential Natural Inhibitor(s) of Lyn Tyrosine Kinase- An *In-silico* Approach

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SK and SC did the conception and design. Author SK performed the research and data acquisition. Authors SK, SB and CG did the analysis and interpretation of the data. Authors SK, SB, TS, MS and SC did the manuscript draft, review, and editing. Author SC did the study supervision. All authors read and approved the final manuscript.

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ABSTRACT

Breast cancer on becoming one of the leading cancer types, emerged as an important barrier in increasing life expectancy of the overall population. In the current study, some compounds were screened based on literature survey for the identification of natural bioactive compounds as potential inhibitors of Lyn tyrosine kinase. Therefore, a multi-step molecular docking was carried out using AutoDock embedded in the MGL Tools. After initial screening, molecules having a higher docking score and binding free energy compared to Tamoxifen were considered for further assessment. Some already known synthetic lyn tyrosine kinase inhibitor have been used for better understanding of the comparative study. Based on *in silico* Lipinski filter analysis, toxicity prediction, pharmacokinetic analysis, four compounds were proposed to be promising inhibitors of Lyn tyrosine kinase. Furthermore, the binding interactions of all proposed inhibitors of Lyn showed

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strong ligand efficiency in terms of energy score obtained with the help of molecular modelling analyses. Hence, the proposed compounds out of which three are bioactive compounds might be taken forward as potential next-generation Lyn kinase inhibitors for managing Lyn associated breast cancer after experimental authentication.

Keywords: Bioactive compounds; breast cancer; flavonoids; lyn tyrosine kinase; molecular docking; potential inhibitors.

1. INTRODUCTION

Cancer is a special kind of heterogeneous disease which is mainly triggered by the irreversible impairments of cellular homeostasis and their functions. An uncontrolled cell growth and differentiation is the key reason behind progression of cancer which leads to an exceptional loss of apoptotic functions that causes huge expansions among the neoplastic cell population [1,2]. Breast cancer, being one of the common cancer types, women of every age are being detected with this deadly disease [3]. Currently, in 2020, the WHO confirmed 2.3 million of breast cancer cases worldwide along with 6, 85,000 global death recorded [4].

Lyn is one of the most important members of src family kinases (tyrosine), it has several regulatory impacts upon the signalling intermediates. It is crucial for modulating and also relying several to different input in order to control various outputs, like proliferation, differentiation, apoptosis, migration and metabolism also. Within same and as well as different cellular contexts Lyn can regulate and maintain both the negative and positive processes of signalling [5]. LYN has been identified as a mediator of invasion and a potential therapeutic target, with certain significance in clinically-aggressive basal-like breast cancer [6].

The basal-like breast cancers are the typical molecular classification of breast cancers. They showed the triple (ER/PR/HER2)-negative (TNBC) type of phenotypic features [7]. Among TNBC, the expression level of the LYN is very high. It is expressed in the tumor cell origins too. Activity of LYN is regulated generally by several factors- PIN1, a prolyl-isomerase where in the case of BRCA1 mutant it is getting unregulated with the help of TNBC PIN1 that independently activates LYN in c-KIT. In addition, spliced isoform of LYN initiates invasion along with migration of TNBC aggressive cells, where the ratio of the spliced variant is very crucial for the breast cancer progression. Hence, the uncoupled dual mechanism from the spliced isoform ratio along with upstream signals initiates and drives LYN activity towards aggressive breast cancers [8]. Several research studies claim that in case of breast cancers this LYN is highly overexpressed and a very potential target as drug especially for TNBC [9]. In breast cancers, the point mutations of LYN are very rare (near about 0.6%) [10] but have been linked with resistance towards anti-estrogen in the ER+ tumors subsets [11]. Around 6%-10% of breast cancers show amplification of LYN [10,12].

Prostate cancer being one of the most commonly found malignancies in men, Src related protein Lyn have a crucial impact on activation of B-cells. In support to this several research studies have been inferred that it is attached with the proliferation and inhibition like phenomenon of apoptosis. It has been proved that in normal prostate epithelia, Lyn is highly expressed (examined 95% of prostate cancer cell lines of humans), whereas in case of knockout mice of Lyn shows abnormal morphogenesis of prostate glands. It plays a significant role in the development of prostate epithelium and hence it is also a candidate target for prostate cancer therapy [13]. As an anti-carcinogenic agent, the secondary plant metabolite derivative, especially, flavonoids are found to be very effective. They usually having major contribution in the colour and aroma of flowers, and maintain immunity by regulating antibacterial, antiviral, antioxidant, anti-allergic and anti-inflammatory mechanisms in plants as well [14-16]. Multiple signal transduction pathways of carcinogenesis are highly interfered by the flavonoids. Utilizing this aspect of flavonoids, it is possible to inhibit the proliferation, angiogenesis by increase in apoptosis or, metastasis [17].

Depending upon C-ring saturation and oxidation property, flavonoids can be classified into six major classes- flavanones, flavanols, flavonols, flavones, anthocyanins and isoflavones. According to a study, uptake of flavonols and flavones, but not other flavonoid subclasses or complete flavonoids, appears to be linked to a

lower risk of breast cancer, particularly in postmenopausal women [18]. Flavonol; as defined by its characteristic property, it has an unsaturated C ring at the position of C2-C3 that found oxidized at C4 position whereas C3 position is hydroxylated [14]. It is widely found in lettuce, kale, onion grapes and berries and categorized in six major groups- Quercetin, Kaempherol, Myricetin, etc. Flavones; being composed of an unsaturated C ring at the position of C2-C3 has a ketonic group at C4 position. Though like flavonols they do not have any hydroxylation at C3 position. Generally, they are found in the leaves, flowers and fruits. The highly rich sources of flavones are ginko biloba, red peppers, mint, celery, chamomile and parsley [14]. It mainly categorized in, apigenin, baicalein, tangeretin, etc.

Here, some flavonols and flavones are selected based on literature survey, keeping in mind their availability and activities against breast cancer. Tamoxifen which is not only a commercially available drug for both prevention and treatment of breast cancer but, it is the most elderly and also enormously prescribed SERM (selective estrogen receptor modulator). Here it has been taking as control [19]. Some already established Lyn inhibitors like Bosutinib, Dasatinib and 1-Tert-Butyl-3-(4-Chloro-Phenyl)-1h-Pyrazolo[3,4-D] Pyrimidin-4-Ylamine [20] are considered for the study in order to carry out the comparative analysis.

2. METHODOLOGY

The overall schematic workflow is given in Fig. 1.

2.1 Protein Preparation

In this study, Fig 2(a) represents the crystal structure of the target protein, Lyn tyrosine kinase has been retrieved Protein Databank, PDB ID: 3A4O [21,22] (http://www.rcsb.org). The structure Fig 2(b) shows the different domains of 3A4O. The structure quality was determined by the webservers like VADAR, QMEAN4, ProSa and Verify3D (SAVES6.0), after energy minimization with the help of SPDBV software [23,24]. By deleting the existing ligands and the water molecules these crystal structures are developed in that mean time those missing hydrogen atoms are integrated tat pH 7 to the protonation state of amino acid by employing the Auto Dock version 4.2 program. After that, each protein getting merged with the non-polar hydrogens while the polar hydrogens are added on. Consequently, the proteins are saved in .pdbqt format, that is, in the required format for molecular docking and this step is repeated every time the protein is to be docked.

2.2 Ligand Preparation

The bio-active flavonoids, flavones and flavonols to be precise, were used as ligands, in order to identify the natural inhibitors of Lyn, the target protein for the study. Since, tamoxifen is used in both prevention and cure of breast cancer; it was taken as reference for the study. The PDB of tamoxifen and also other flavones and flavonols which were to be used for docking, were retrieved from the Drugbank (https://go.drugbank.com/) [25]. The structures for which PDB were not available in Drugbank, were downloaded in SDF format from PubChem (www.pubchem.ncbi.nlm.nih.gov) and converted to PDB format through Open Babel [26]. Polar hydrogen charges of Gasteiger were computed and the non-polar hydrogen molecule were incorporated with carbons. The ligands and protein were then further converted to pdbqt format using AutoDock tools. The top docked ligands (Bosutinib, Baicalein, Myricetin and Quercetin) along with the reference (Tamoxifen) is given in Fig. 3.

2.3 Molecular Docking and Screening of Potential Natural Inhibitors

With the help of AutoDock 4.2 online software, the molecular docking of the target protein (3A4O) with all the selected compounds have been carried out. For further investigation of the binding efficiency of respective ligands towards the macromolecule, this procedure of automation turned out to be very effective. Each PDB file has been changed into PDBQT format to run the carry out the docking, where all the non- protein elements are removed. At the surroundings of the binding sites of protein a grid box has been made along with specialized numbers that represents grid points such as; X: 122, Y: 126 and Z: 126 belonging to the three dimensions, where the grid centres are: X centre: -2.209, Y centre: -12.251, and Z centre: -16.948 with space value of 0.375 for 3A4O. The gasteiger partial charges as well as Hydrogen atoms were added using AutoDock tools (Version- 4.2). Following this, the LGA (Lamarckian Genetic Algorithm) have been implemented and through AutoDock, ver-4.2 program by maintaining all default parameters [27]. For the interpretation of results of molecular docking, AutoDock tool comprises

many algorithms and techniques. This procedure involves many assembling tools to assemble the desired result by the similarities of conformations along with their conformational visualization and protein-ligand interactions. To generate affinity potentials the autogrid has been applied. The visualization of the results was carried out by PyMOl and Biovia Discovery Studio [28,29].

2.4 Structural Insights Regarding Drug Surface Hotspots in Lyn Protein

In order to determine the drug surface hotspot of Lyn tyrosine kinase protein (PDB ID: 3A4O), the lyn and its inhibitor complex structure were analysed by, Discovery Studio, and PyMOL [30]. The molecular docking analysis was carried out to study the binding pattern of Bosutinib, Baicalein, Myricetin and Quercetin inhibitor the Lyn protein of Breast cancer, and the results allowed us to do comparative structural analysis of screened the natural Lyn inhibitors [31].

2.5 MD-Simulation

Biological functions of each protein are explained by their dynamic structures. But, this classical process of MD-Simulation is carried out in CABS-flex server, which is a much known and commonly used tool for fast simulation. The experimental investigation of biologically relevant proteins by applying simulation study is in high demand for *in silico* studies of this sort [32,33]. However, based on some literature surveys, we used CABS-flex web server for our study as this server (developed in 2013) is usually used for carrying out quick simulations [34]. In comparison to the classical procedure of MD-simulation, CABS flex is quite efficient for all-atom molecular dynamics [35].

RMSF (Root Mean-Square Fluctuation) plot was derived from the server which allowed us to measure and compare the average deviation of the amino acid residues involved [36]. In molecular dynamics this RMSF is important to evaluate the displacement of any particular atom or, group of atoms that is relative to the reference structure that is averaged over the number of atoms. To determine the structural stability along with time scale of the simulation or, the diverging condition from the initial coordinates RMSF is frequently used [37].

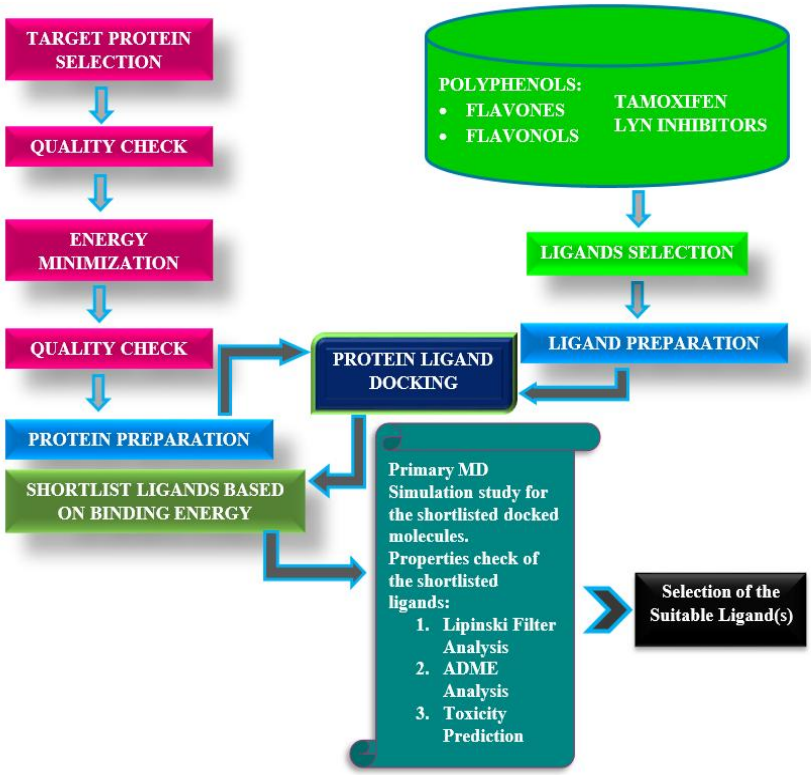


Fig. 1. Schematic workflow

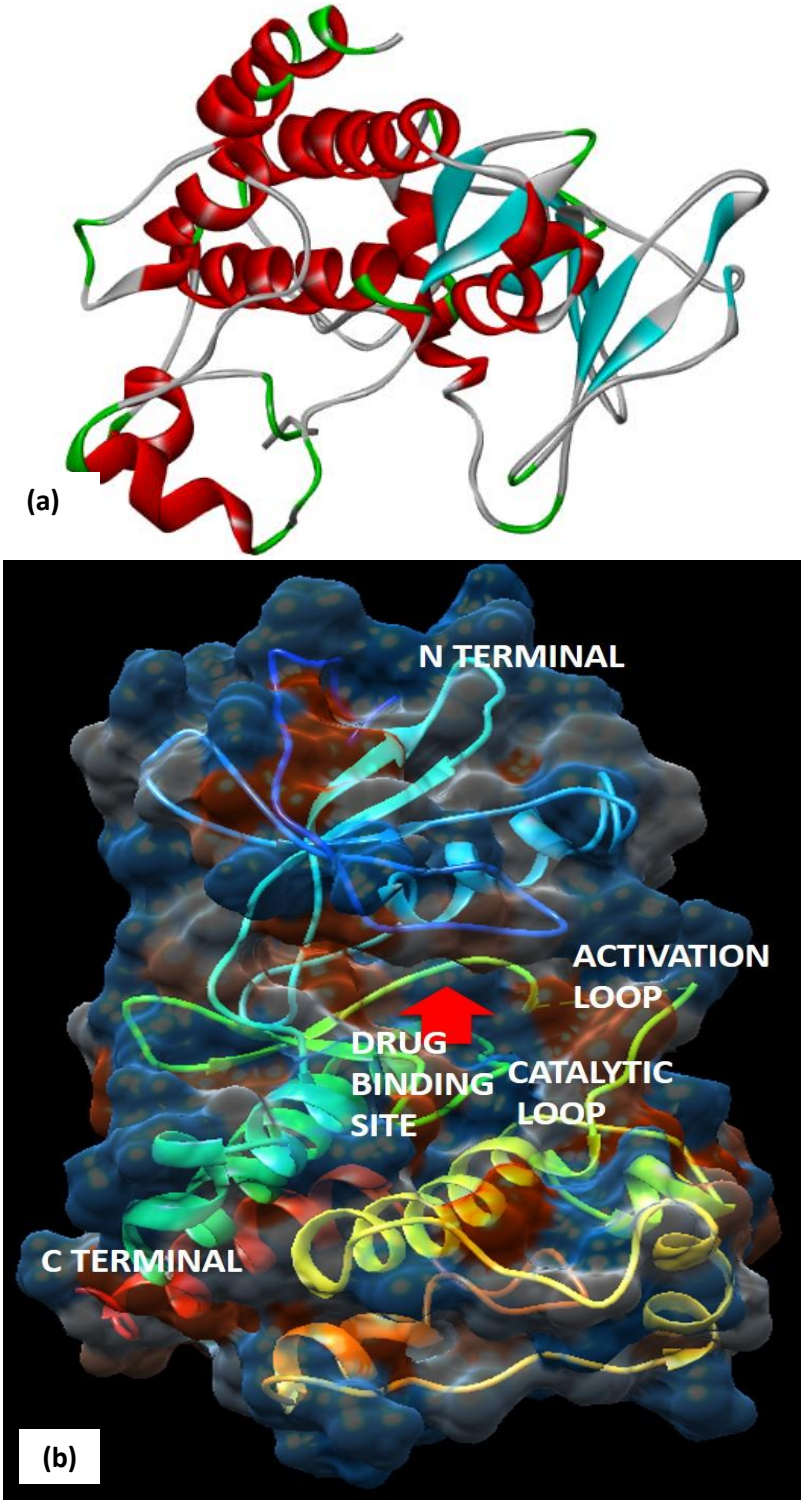


Fig. 2. (a) 3D Structure of 3A4O; (b) Different domains of 3A4O

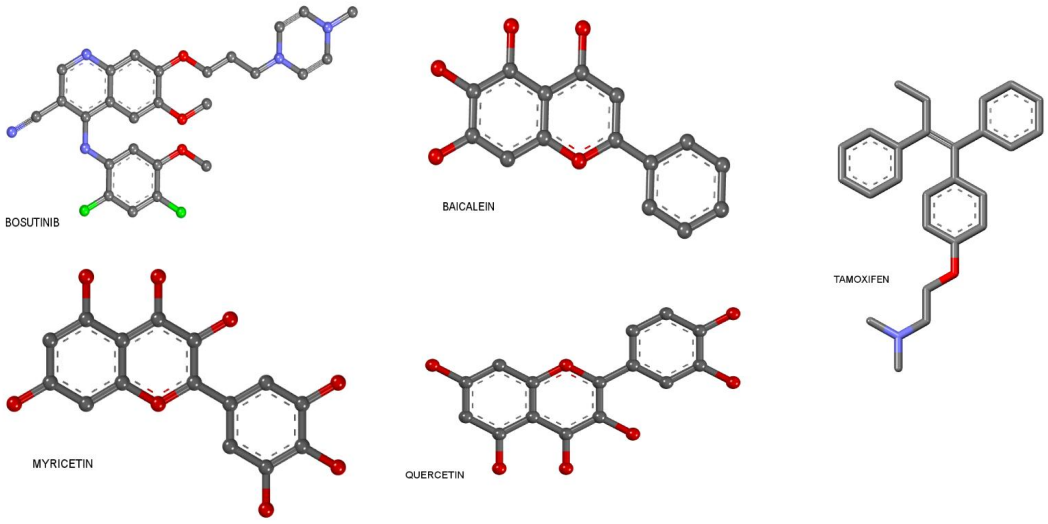


Fig. 3. 3D structure of the top 4 docked Flavonoids and Tamoxifen

2.6 Absorption, Distribution, Metabolism, and Excretion (ADME) Prediction of Top Docked Ligands

Adsorption, Distribution, Metabolism and Excretion all-together abbreviated as ADME is a very valuable server to assess pharmacodynamics properties of the ligands/ small molecules /drugs of concern [38]. The parameters based on which the assessment is done are- solubility of water- Log S (SILICOS-IT, ESOL, Ali), lipophilicity – Log P0/w (XLOGP3, iLOGP, MLOGP, WLOGP, SILICOS-IT), drug likeness rules (Lipinski, Veber, Ghose, Egan and Muegge) and the medicinal chemistry (Synthetic accessibility Brenk, PAINS, Lead-likeness). All the properties are evaluated [39,40] using the SWISS-ADME website (https://www.swissadme.ch) [39].

2.7 Toxicity Prediction of Top Docked Ligands

The prediction of the level of toxicity, in case of drug designing, is a very crucial step. The determination of the tolerance capacity of the animal models, as well as humans, before proper ingestion and application is very important, otherwise can turn fatal. An online server, pkCSM, is utilized for this purpose, where the small molecules can be drawn virtually or, it can be submitted in SMILES format obtained from Drugbank. The pkCSM allows the study of toxicological effects by determining AMES toxicity, hERG-I and hERG-II inhibitor, Oral Rat Chronic Toxicity (LOAEL), Oral Rat Acute Toxicity (LD50), maximum tolerance dose for human, skin toxicity, hepatotoxicity, Minnow toxicity and *T. pyriformis* toxicity. After logging in into the website, the SMILES of compounds under study, which were previously downloaded from DrugBank, were submitted and the mode of toxicity was selected and results were downloaded [41]. Hence, the pharmacokinetics of the small molecules can be determined in this manner.

3. RESULTS

3.1 Protein Quality Determination

The structure of the target protein (PDB ID: 3A4O), as determined by several online servers like VADAR, QMEAN4, ProSa and Verify3D (SAVES6.0), after energy minimization is represented in Fig. 4(a), (b), (c), (d), (e) and (f).

3.2 Screening of Potential Natural Lyn Inhibitors

Based on the docking analysis of bioactive flavonoids with Lyn tyrosine kinase of breast cancer, the results of binding energy are determined. The small molecules with higher binding affinity and more negative binding energy for the target protein 3A4O were ranked higher. A total of ten small molecules including Tamoxifen (reference) were docked with 3A4O

and represented in Table 1(a) along with a graphical representation (Fig 5(a)). According to the observation of the docking analysis, four top docked ligands are shortlisted depending on the binding affinities to 3A4O are represented in Table 1(b) along with graphical depiction (Fig 5(b)). It was observed that Tamoxifen which was used as reference small molecule/ ligand for this study had a binding affinity of -7.83 kcal/mol for 3A4O. Out of the four top docked ligands/ small molecules, Bosutinib (a quinolone derivate, already known lyn inhibitor) with binding affinity of (-9.02 kcal/mol) was top docked, then the flavonols Myricetin (-8.79 kcal/mol) and Quercetin (-8.45 kcal/mol), followed by flavone Baicalein (-8.04 kcal/mol) as given in Table 1(b).

Table 1(a). Bioactive compounds binding affinity towards the target protein Lyn- Tyrosine Kinase (3A4O)

Sl No.	Compounds	Binding energy with LYN-Tyrosine Kinase (kCal/mol) LYN-Tyrosine Kinase
1.	Tamoxifen	-7.83
2.	Bosutinib	-9.02
3.	Dasatinib	-7.71
4.	1-Tert-Butyl-3-(4-Chloro-Phenyl)-1h-Pyrazolo[3,4-D]Pyrimidin-4-Ylamine	-6.96
5.	Apigenin	-7.91
6.	Baicalein	-8.04
7.	Tangeretin	-7.11
8.	Kaempherol	-7.86
9.	Myricetin	-8.79
10.	Quercetin	-8.45

Table 1(b). Top Docked Bioactive compounds with target protein Lyn- Tyrosine Kinase (3A4O)

Sl No.	Compounds	Binding energy with LYN-Tyrosine Kinase (kCal/mol)
1.	Bosutinib	-9.02
2.	Baicalein	-8.04
3.	Myricetin	-8.79
4.	Quercetin	-8.45

Table 2. The amino acids residues involved in the 3A4O interaction with each small molecules/ ligands

Sl no.	Molecules	Amino Acid Residues
1.	Bosutinib	Phe27, Gly28, Glu29, Gly25, Ala24, Lys44, Val30, Leu22, Ala42, Tyr90, Met91, Val72 & Leu143
2.	Baicalein	Lys44, Val30, Gly23, Leu22, Ala42, Gly94, Met91, Thr88, Leu143 & Glu89
3.	Myricetin	Leu22, Met91, Leu143, Glu89, Ala42, Ala153, Val30, Asp154, Lys44 & Ile86
4.	Quercetin	Glu89, Met91, Ala42, Leu143, Glu59, Asp154, Lys44, Val30 & Leu22

Table 3. Average, maximum and minimum RMSF values Lyn protein bound with proposed inhibitors and Bosutinib derived from the RMSF plots. [Source: CABSflex]

RMSF (nm)	Bosutinib	Baicalein	Myricetin	Quercetin
Minimum	0.72×10^{-1}	0.61×10^{-1}	0.79×10^{-1}	0.72×10^{-1}
Maximum	42.19×10^{-1}	37.58×10^{-1}	34.48×10^{-1}	48.59×10^{-1}
Average	21.455×10^{-1}	19.095×10^{-1}	17.635×10^{-1}	24.655×10^{-1}

Table 4(a). ADME/tox filtering and Lipinski analyses

a. Lipinski Filter Analysis				
Lipinski filters	**Bosutinib**	**Baicalein**	**Myricetin**	**Quercetin**
Molecular weight (g/mol)	530.456	270.24	318.237	302.238
No. heavy atoms	36	20	23	22
No. rotatable bonds	9	1	1	1
No. H-bond acceptors	7	5	8	7
Hydrogen bond donor	1	3	6	5
Molar Refractivity	150.65	73.99	80.06	78.03
Lipinski violation	1	0	1	0
XLOGP3	5.38	3.16	1.18	1.54
iLOGP	4.68	2.43	1.08	1.63
MLOGP	2.06	0.52	-1.08	-0.56
Silicos-IT Log P	4.86	2.52	1.06	1.54
WLOGP	4.43	2.58	1.69	1.99
Consensus Log P	4.28	2.24	0.79	1.23

Table 4(b). Admet SAR (lipophilicity)

b. admet SAR				
Absorption (Probability)	**Bosutinib**	**Baicalein**	**Myricetin**	**Quercetin**
Blood-Brain Barrier	-1.541	-0.967	-1.772	-1.355
Human Intestinal Absorption	90.764	91.233	66.197	74.994
Bioavailability Score	0.55	0.55	0.55	0.55
Caco-2 Permeability	1.023	1.067	0.241	0.587
P-glycoprotein Substrate	YES	YES	YES	YES
P-glycoprotein Inhibitor	YES	NO	NO	NO
CYP450 Substrate				
Renal Organic Cation Transporter Inhibitor	YES	NO	NO	NO
GI absorption (%)	High; 90.764	High; 91.233	Low, 66.197	High; 74.994
Log Kp (Skin Permeation)	-5.72 cm/s	-5.70 cm/s	-7.40 cm/s	-7.05 cm/s

Table 4(c). Solubility Analyses

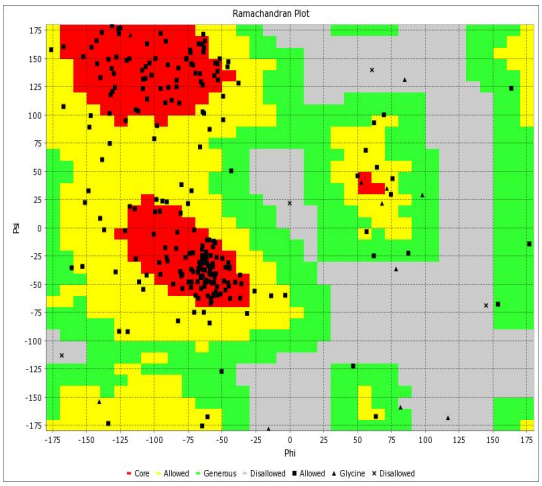
c. Solubility Analysis				
Water Solubility	**Bosutinib**	**Baicalein**	**Myricetin**	**Quercetin**
• Log *S* (ESOL)	-6.25	-4.03	-3.01	-3.16
• Solubility	2.96e-04 mg/ml; 5.58e-07 mol/l	2.51e-02 mg/ml; 9.28e-0 mol/l	3.14e-01 mg/ml; 9.88e-04 mol/l	2.11e-01 mg/ml; 6.98e-04 mol/l
• Class	Poorly Soluble	Moderately Soluble	Soluble	Soluble
• Log *S* (Ali)	-6.87	-4.74	-3.96	-3.91
• Solubility	7.08e-05 mg/ml;	4.93e-03 mg/ml;	3.50e-02 mg/ml;	3.74e-02 mg/ml;
• Class	1.34e-07 mol/l	1.82e-05 mol/l	1.10e-04 mol/l	1.24e-04 mol/l
	Poorly Soluble	Moderately Soluble	Soluble	Soluble
• Log *S* (SILICOS-IT)	-8.75	-4.40	-2.66	-3.24
• Solubility	9.37e-07 mg/ml;	1.07e-02 mg/ml;	6.98e-01 mg/ml;	1.73e-01 mg/ml;
• Class	1.77e-09 mol/l	3.94e-0 mol/l	2.19e-03 mol/l	5.73e-04 mol/l
	Poorly Soluble	Moderately Soluble	Soluble	Soluble
• Aqueous solubility (LogS)	-4.698 log mol/L	-3.059 log mol/L	-2.988 log mol/L	-3.372 log mol/L

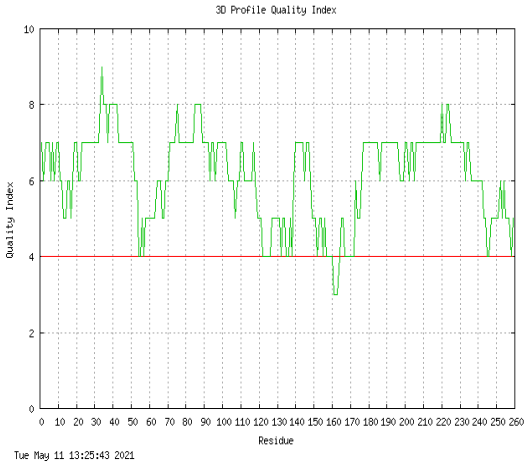
Table 5. Toxicity Prediction based on SWISSADME and pkCSM data

TOXICITY	Bosutinib	Baicalein	Myricetin	Quercetin
AMES Toxicity	**NO**	**NO**	**YES**	**YES**
Hepatotoxicity	YES	NO	NO	NO
Oral Rat Chronic Toxicity (LOAEL)	1.628 log mg/kg_bw/day	1.316 log mg/kg_bw/day	3.728 log mg/kg_bw/day	3.032 log mg/kg_bw/day
Maximum Tolerated Dose (Human)	0.027 log mg/kg/day	0.302 log mg/kg/day	0.997 log mg/kg/day	1.062 log mg/kg/day

Table 6. LD50 Value of top docked compounds

Compounds	Oral Rat Acute Toxicity (LD50 VALUE)
Bosutinib	2.805 mol/kg
Baicalein	2.279 mol/kg
Myricetin	2.358 mol/kg
Quercetin	2.295 mol/kg





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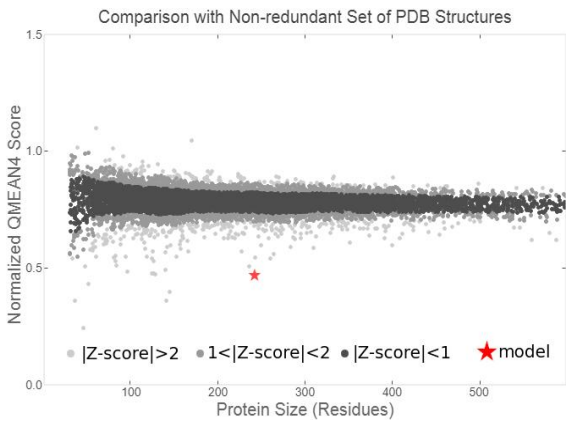

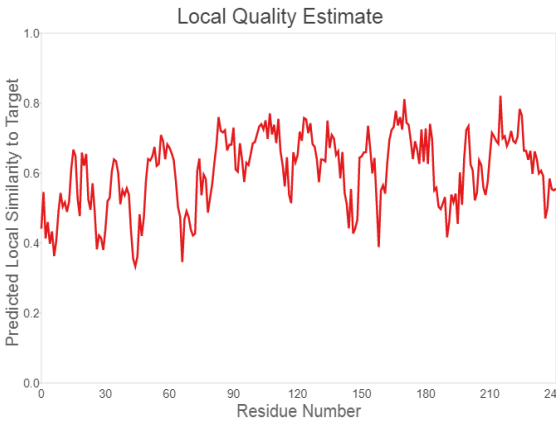

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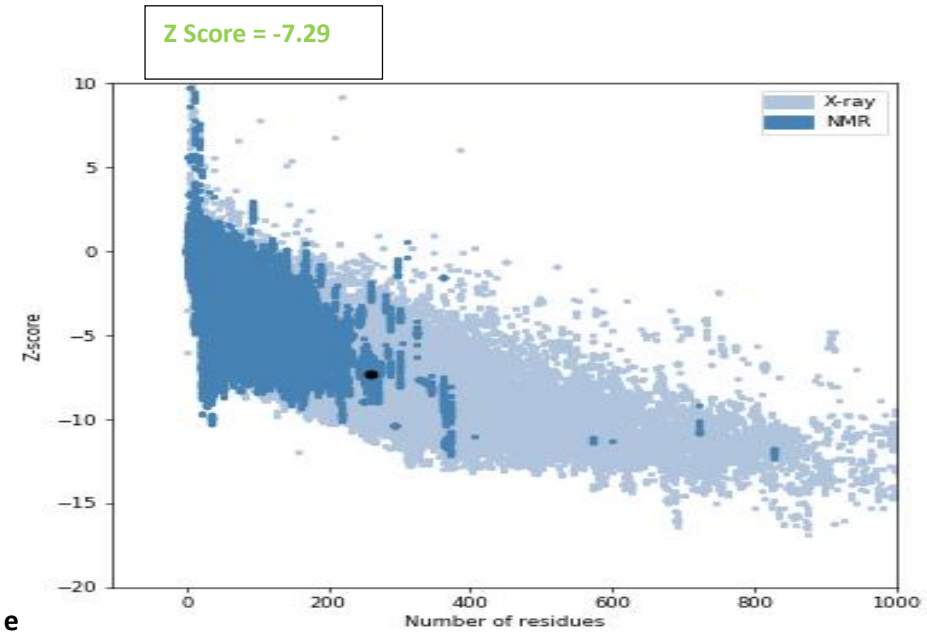

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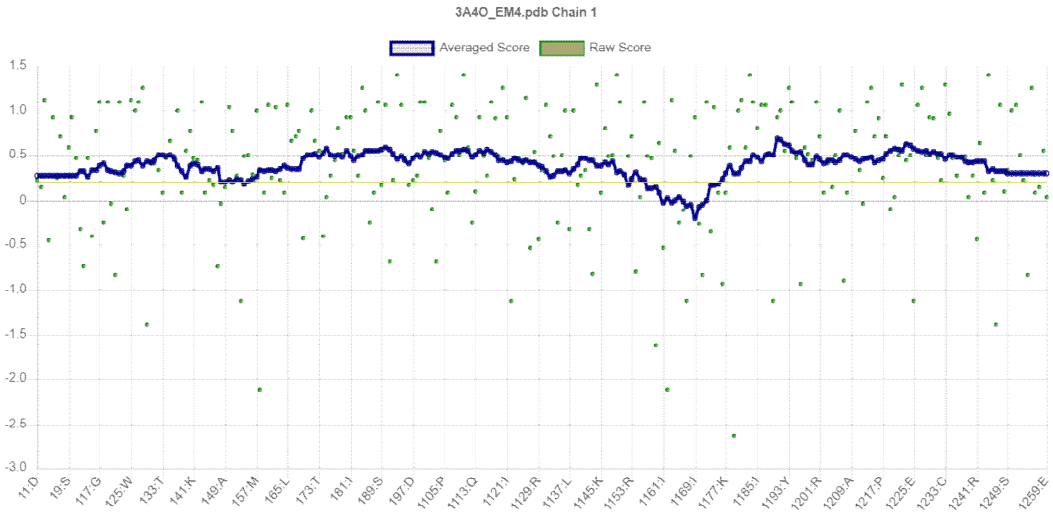

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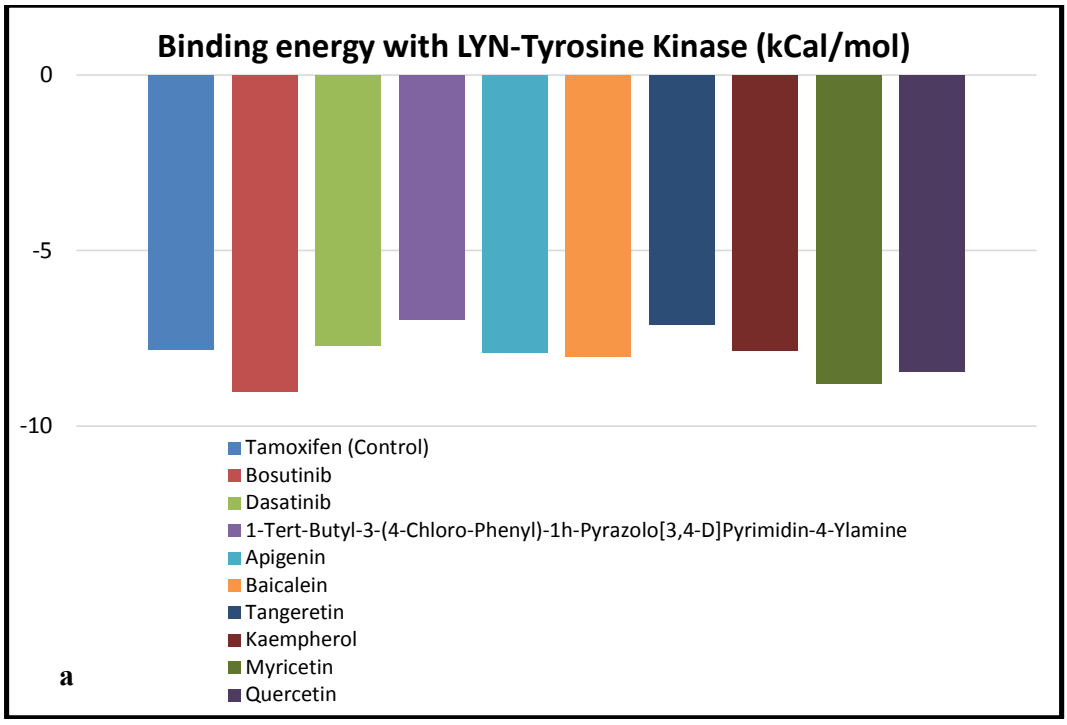
Fig. 4. (a) Ramachandran plot of 3A4O after energy minimization; (b) 3D Quality Index of energy minimized 3A4O; (c) Normalized QMEAN4 Score vs Protein Size (Residues) Plot (QMEAN4 Value: -7.40); (d) Predicted Local Similarity to Target vs Residue Number; (e)The structure validation by ProSa, which shows the Z-score (= -7.29) of the energy minimized model of 3A4O (black dot), when compared to a non-redundant set of crystallographic structures (light blue dots) and NMR structures (dark blue dots). The structure of 3A4O presents good overall quality score compared to that of NMR structures. (f) Structure validation by Verify3D, which shows the 3D-1D score for each atom of the model 3A4O. The graphic shows that 91.89% of the residues of the structure 3A4O presented a compatibility score of >=0.2, which indicates that the structure is a good-quality structure according to Verify3D



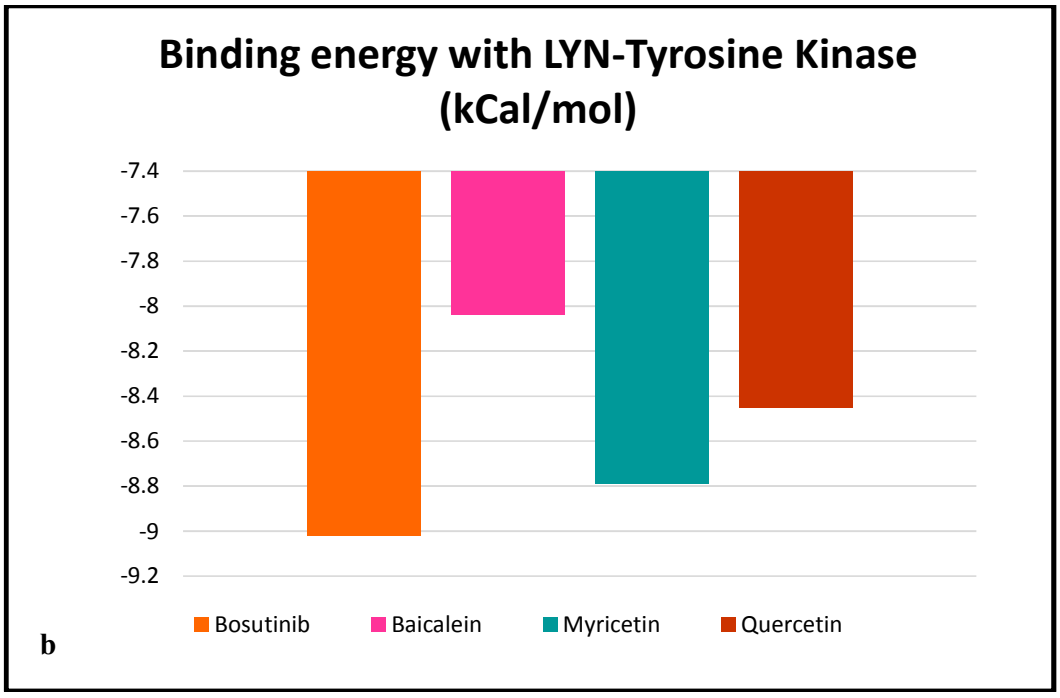


Fig. 5. (a) Graphical representation bioactive compounds binding affinity towards the target protein LYN- Tyrosine Kinase (3A4O); (b) Graphical representation the four top docked small molecules/ligands (bioactive compounds flavones (Baicalein), flavonols (Myricetin and Quercetin) along with Lyn inhibitor (Bosutinib)), binding affinity towards the target protein LYN- Tyrosine Kinase (3A4O)

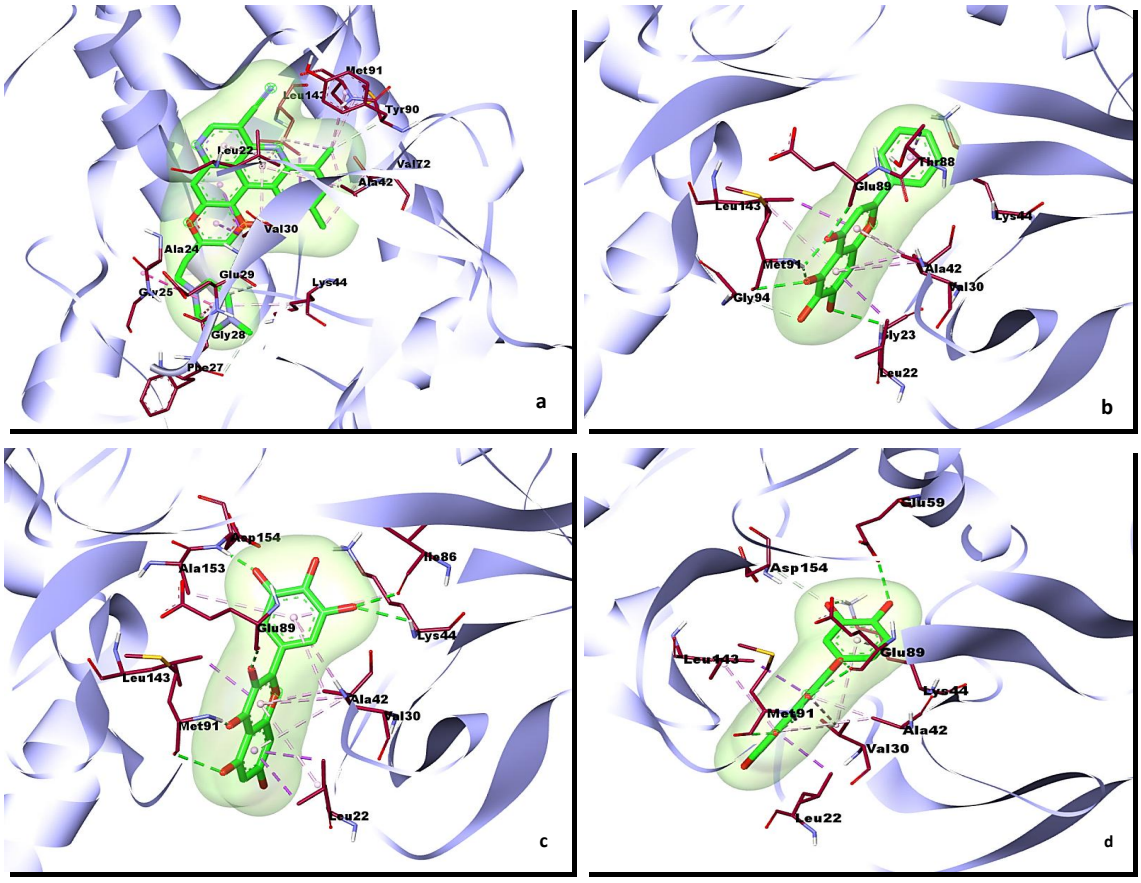
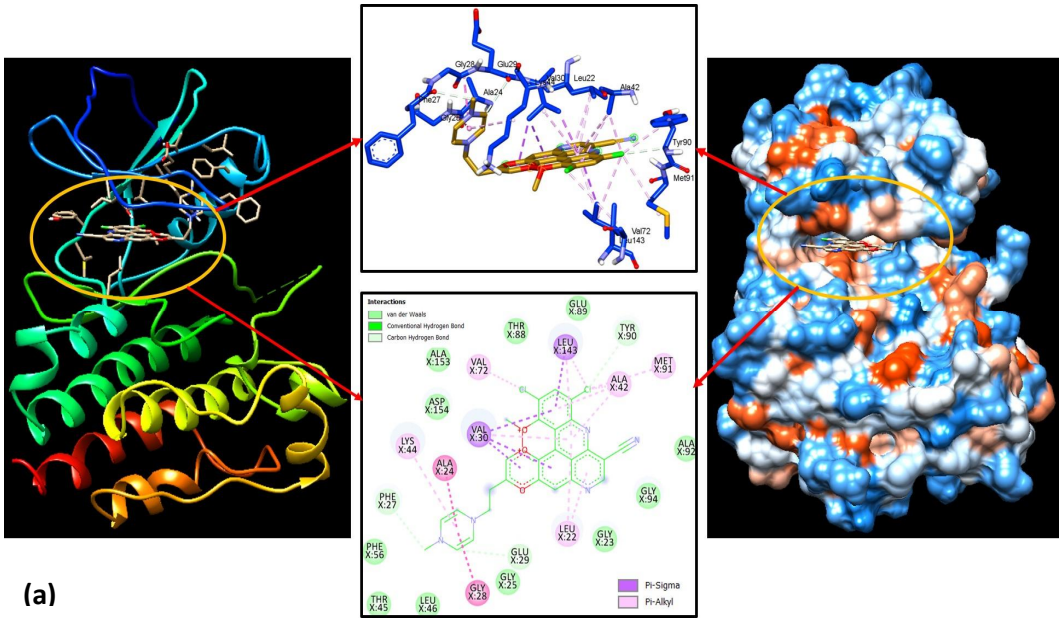
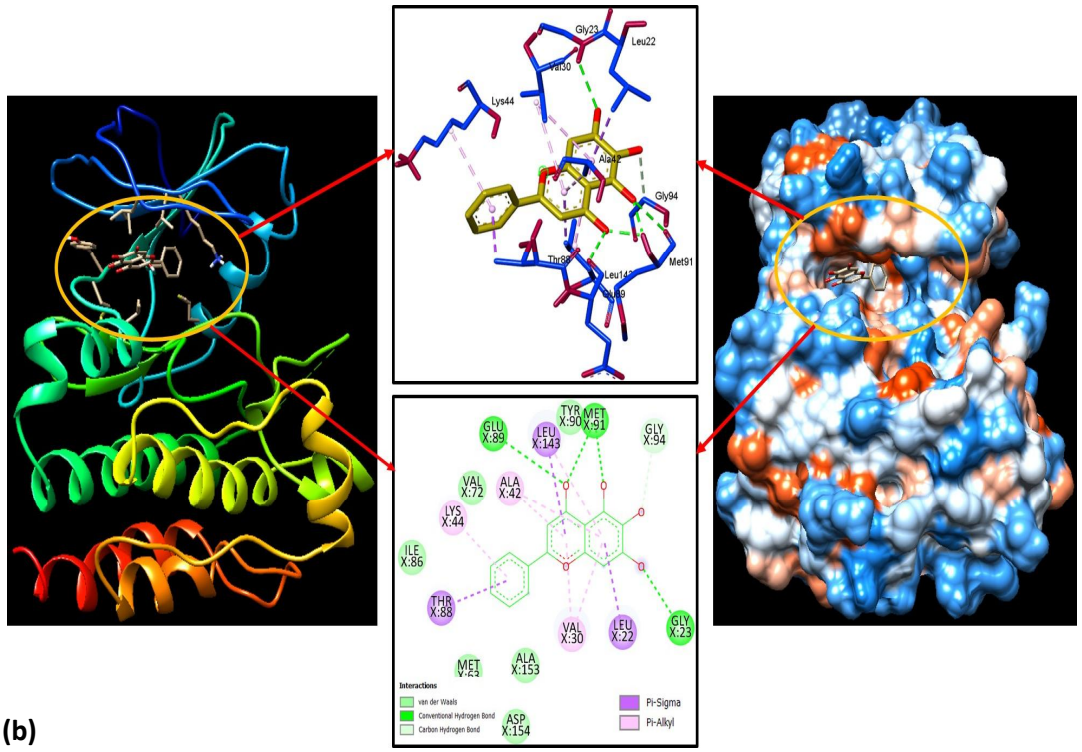


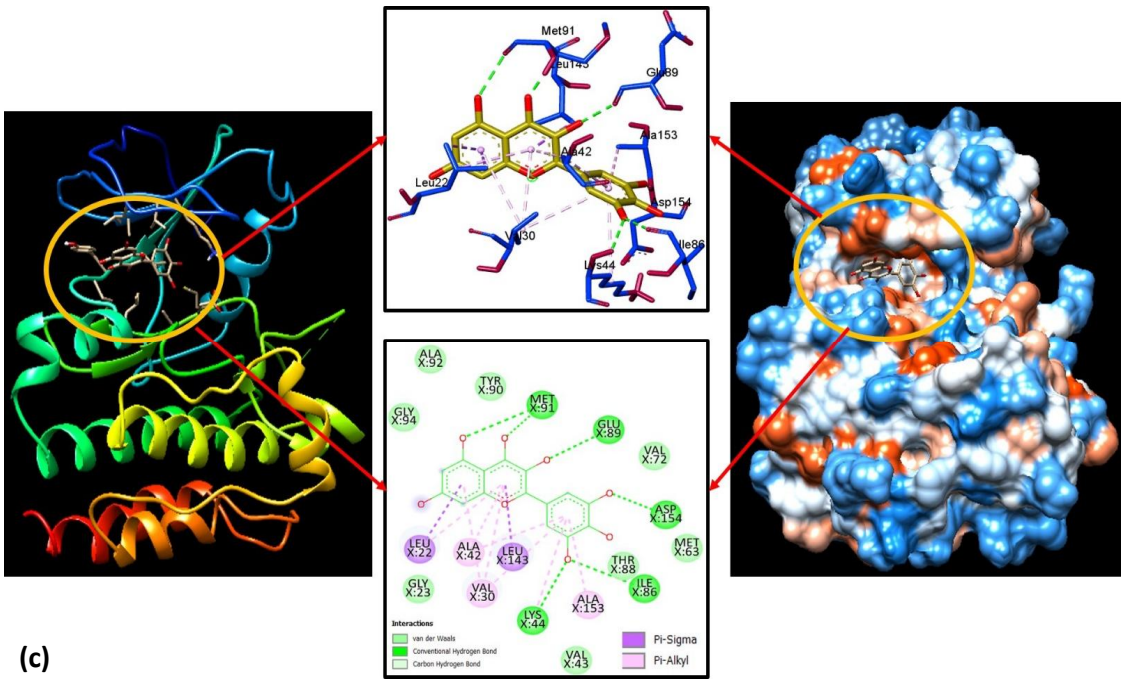
Fig. 6. Visualization of 3A4O 3D interaction with ligands (a) Bosutinib (Lyn inhibitor), (b) Baicalein (Flavones), and (c) Myricetin and (d) Quercetin (Flavonols)



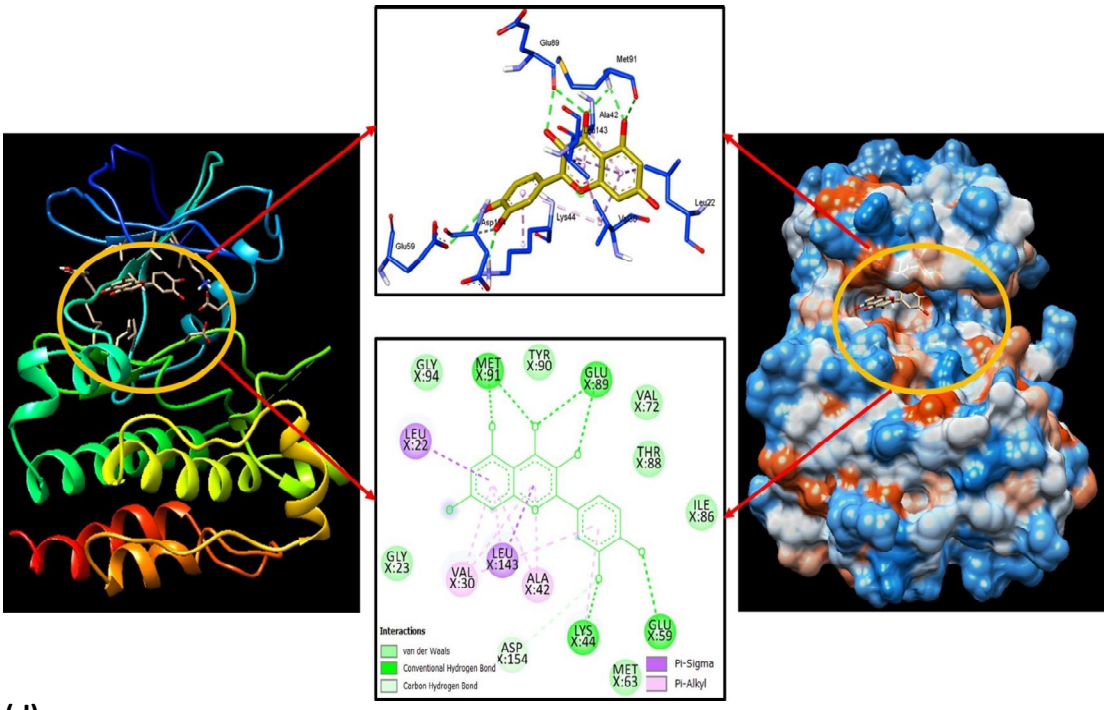
(a)


Gly23
Leu22
Val30
Lys44
Ala42
Gly94
Met91
Leu143
Ile89
TYR X:90
MET X:91
GLU X:89
LEU X:143
GLY X:94
VAL X:72
ALA X:42
LYS X:44
ILE X:86
THR X:88
VAL X:30
LEU X:22
GLY X:23
MET X:63
ALA X:153
ASP X:154
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Pi-Alkyl

(b)


Met91
Leu143
Glu89
Ala153
Ala42
Leu22
Asp154
Val30
Ile86
Lys44
ALA X:92
TYR X:90
MET X:91
GLY X:94
GLU X:89
VAL X:72
ASP X:154
LEU X:22
ALA X:42
LEU X:143
MET X:63
THR X:88
GLY X:23
VAL X:30
ILE X:86
ALA X:153
LYS X:44
VAL X:43
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Pi-Alkyl

(c)



(d)

Fig. 7. Complete representation and Close insight of the 3A4O interaction with the respective four ligands/ small molecules ligands (a) Bosutinib (Lyn inhibitor), (b) Baicalein (Flavones), and (c) Myricetin and (d) Quercetin (Flavonols)

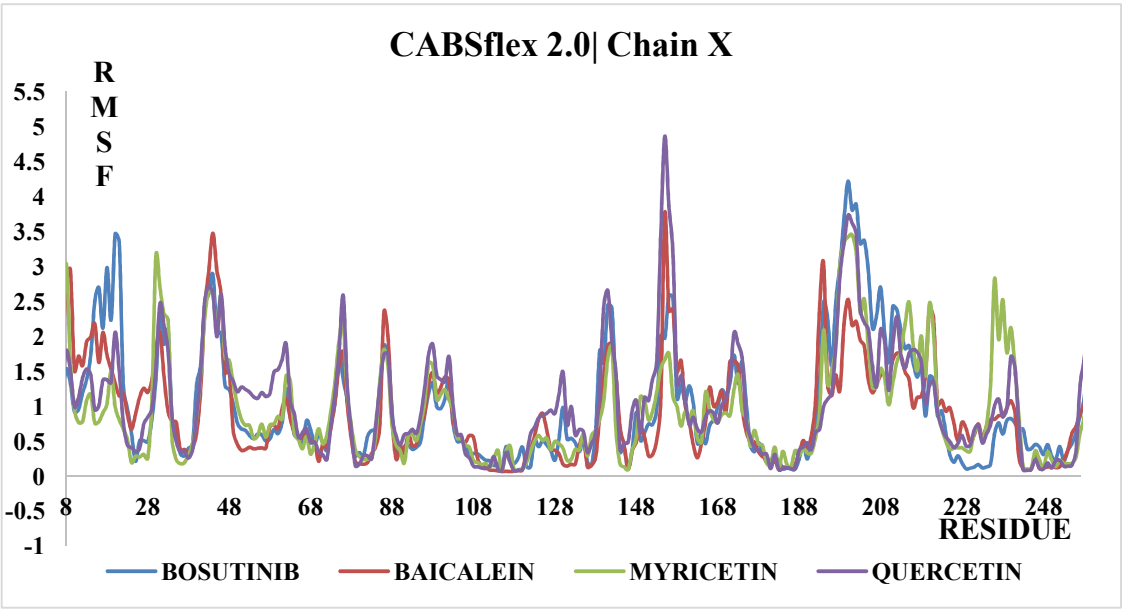


Fig. 8. RMSF of individual amino residue of Lyn bound with proposed natural inhibitors and Bosutinib

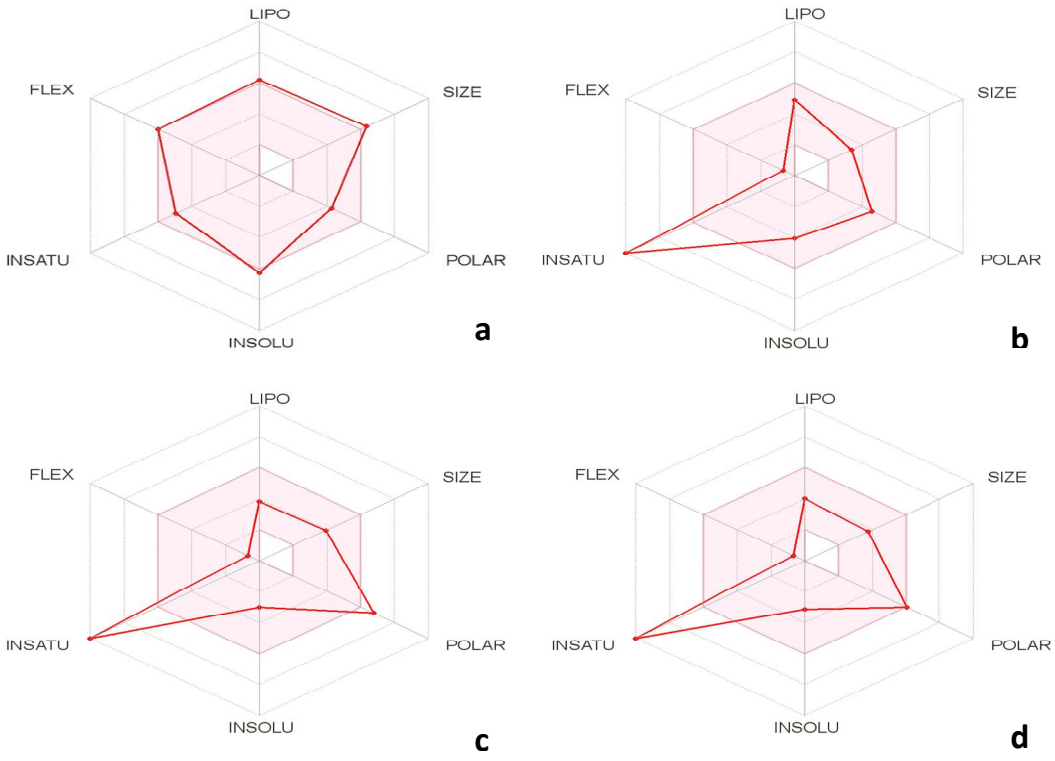


Fig. 9. Bioavailability Ladder for the four ligands/ small molecules ligands (a) Bosutinib (Lyn inhibitor), (b) Baicalein (Flavones), and (c) Myricetin and (d) Quercetin (Flavonols) [LIPO: Lipophilicity; FLEX: Flexibility; INSATU: INSATU: Saturation; INSOLU: Solubility; and POLAR: Polarity]

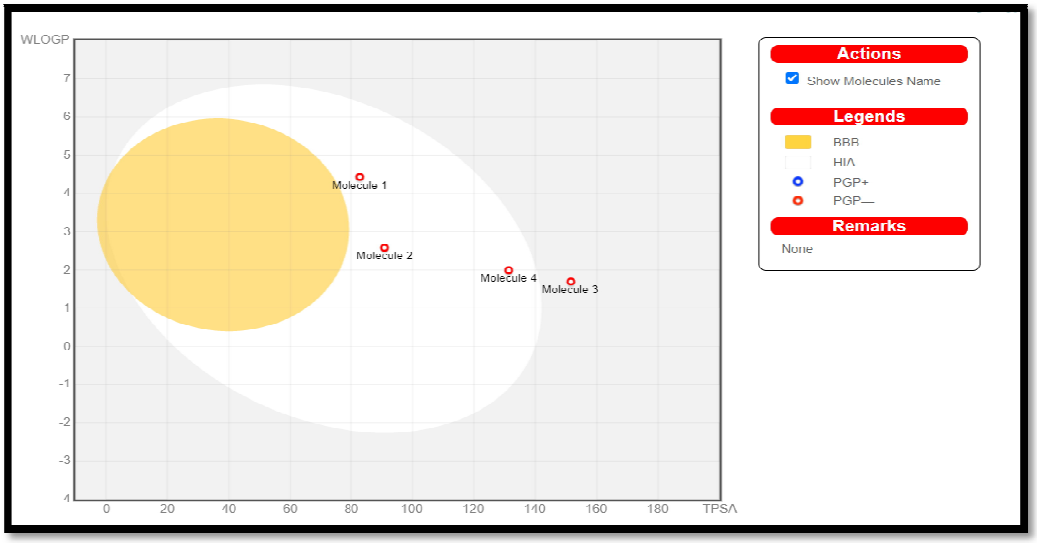


Fig. 10. The BOILED-Egg plot for the small molecules/ ligands. Molecule 1 (Bosutinib); Molecule 2 (Baicalein); Molecule 3 (Myricetin); and Molecule 4 (Quercetin). [BBB: Blood Brain Barrier; HIA: passive gastrointestinal absorption; $PGP^{+/-}$: P-gp substrates/ non-substrates]

3.3 Structural Insights Regarding Drug Surface Hotspots through Visualization

The protein-ligand interactions in the binding sites were visualized in Biovia Discovery Studio. The 3D binding interactions at the binding sites are given for the four top docked ligands; Bosutinib (Lyn inhibitor) (Fig 6(a)) and Baicalein, Myricetin and Quercetin (Fig 6(b), (c), (d)), followed by 2D diagrams and close insight to the interactions of 3A4O active site with the same set of four top docked ligands is given (Fig 7(a), (b), (c), (d)). On visualizing the protein-ligand interaction with the help of Discovery Studio, we found several amino acid residues involved in the site of the interaction. The amino acids involved in case of each small molecules/ ligands are given in the Table 2. Lys44, Val44, Val30, Leu22, Ala42, Met91 and Leu143 are found to be the key amino acid residues of 3A4O involved in the interaction.

3.4 RMSF Plot (MD-Simulation)

The MD-simulation study on CABSflex server, we could derive the RMSF plots of all the top protein-ligand docked complexes. For all the complexes, default parameters were kept. Each amino acid residue has its unique yet crucial role impacting the protein-ligand stability. The fluctuation state of each amino acid residues is determined by plotting the RMSF graph. The analysed RMSF is plotted in the graph (Fig 8). Here in this graph a similar pattern of fluctuations is observed for RMSF variation of each amino residue involved in the interaction between Lyn tyrosine kinase and small molecules/ ligands (flavonoids and Bosutinib). Not a single amino acid was found to have an RMSF value of more than 4.859 nm. Due to binding interaction with the ligand, the RMSF of catalytic amino residues was found significantly low. Maximum, minimum and average RMSF values were calculated and are given in Table 3. The fluctuation range of all complexes was found to be 0.061 to 4.859 nm. Difference between maximum and average, and, average and minimum can give an idea of fluctuation of the amino residues of Lyn. It was of 2.0735 and 2.0735 nm, 1.8485 and 1.8485 nm, 1.6845 and 1.6845 nm, and 2.3935 and 2.3935 nm for the Lyn complexed with Bosutinib, Baicalein, Myricetin and Quercetin respectively. The low values clearly indicat that the individual amino residue remained almost intact during the MD simulation.

3.5 ADME Prediction

The SwissADME database has been implemented to predict the properties for ADME analysis. The results are observed after the submission of the small molecules. Based on the ADME/tox analysis and Lipinski filter analysis (Table 4(a)), the physiochemical properties like number of heavy atoms, hydrogen bond donors, hydrogen bond acceptors, molar refractivity, topological polar surface area of molecule are observed. The lipophilicity of the small molecules, XLOGP3, iLOGP, MLOGP, WLOGP, SILICOS-IT LOGP and Consensus Log P are represented in (Table 4(b)).

The servers allow to analyse the property of water solubility is measured on the basis of the parameters like Log *S* ESOL, Log *S* Ali, Log *S* SILICOS-IT, and overall aqueous solubility as represented in Table 4(c).

The pharmacokinetic properties are gastro-intestinal absorption (GI), blood brain barrier, Human Intestinal Absorption, bioavailability score, Renal Organic Cation Transporter, Skin penetration etc, were determined through this study.

3.6 Toxicity Prediction

The online server pkCSM, efficiently evaluated the results of toxicity prediction. The results revealed that Bosutinib and Baicalein do not have AMES toxicity whereas Myricetin and Quercetin show AMES toxicity. According to the toxicity prediction results, the maximum tolerated dose observed for human are 0.027 log mg/kg/day, 0.302 log mg/kg/day, 0.997 log mg/kg/day and 1.062 log mg/kg/day for Bosutinib, Baicalein, Myricetin and Quercetin respectively as shown in Table 5. The acute rat toxicity (oral); i.e.; the LD50 values for Bosutinib, Baicalein, Myricetin and Quercetin are 2.805 mol/kg, 2.279 mol/kg, 2.358 mol/kg and 2.295 mol/kg respectively (Table 6). The oral rat chronic toxicity (LOAEL) are Bosutinib (1.628 log mg/kg_bw/day), Baicalein (1.316 log mg/kg_bw/day), Myricetin (3.728 log mg/kg_bw/day) and Quercetin (3.032 log mg/kg_bw/day). Except Bosutinib none of the above compound has shown hepatotoxicity. The small molecules are predicted to cause no skin sensitivity. The evaluated GI absorption revealed that except Myricetin, all have shown high absorption. Lastly, the bioavailability scores represented as bioavailability ladder for all the

small molecules/ ligands (Bosutinib, Baicalein, Myricetin, and Quercetin) are shown in Fig 9. To estimate the gastrointestinal absorption and brain penetration (the two major ADME behaviours impacting pharmacokinetics) globally, a graphical output is generated in SwissADME server which gives an enhanced BOILED-Egg plot for each of the small molecules, Molecule 1 (Bosutinib), Molecule 2 (Baicalein), Molecule 3 (Myricetin) and Molecule 4 (Quercetin), represented in Fig 10.

4. DISCUSSION

Breast cancer has already become a huge concern for the population all over. In order to overcome this situation, entire world is running after the therapeutics, and preventive measures that can be utilized against this deadly disease. Usually, doctors tend to follow the conventional line of treatment, i.e., chemotherapy, estrogen modulator medications, hormone therapies, radiations and even surgeries [42]. This study provides a close insight towards identifying natural potent inhibitors for Lyn tyrosine kinase with the help of a comparative study. The study involves a detailed comparative analysis among the already established Lyn inhibitors derived from DrugBank and flavones and flavonols which are easily available, taking Tamoxifen (commercial drug against breast cancer) as control. Phytochemicals are considered to have immense amount of medicinal values. Furthermore, phytochemicals are non-toxic, unlike synthetic molecules that act as inhibitors. After primary screening based on binding energy found from molecular docking, we shortlisted 4 small molecules; Bosutinib (lyn inhibitor), Baicalein (flavone), Myricetin and Quercetin (flavonols), whose binding energies were found to be quite close, especially Bosutinib and Myricetin and higher than our control molecule (Tamoxifen). On analysing the RMSF plots derived for each complex of Lyn with proposed inhibitors and Bosutinib, very interestingly we found that the fluctuation pattern of the flavonoids were almost same to the Bosutinib (Lyn inhibitor). Consequently, we evaluated other properties of the small molecules through the online servers- SwissADME and pkCSM. On analysis of the results evaluated on these servers, it is observed that flavones and flavonols which were considered for the study, have much lesser toxicity almost in all aspects. Baicalein shows no AMES toxicity whereas Myricetin and Quercetin do. None of the flavone and flavonol molecules show hepatotoxicity unlike Bosutinib. The maximum tolerated dose for all the flavonoids is much higher than Bosutinib as well. Like Bosutinib, Baicalein, Myricetin and Quercetin show no skin sensitivity at all. According to the SwissADME server, the bioavailability score for all the compounds under study are equal (0.55). The intestinal and oral adsorption of the drugs were analysed through the Lipophilicity study. As per results, the GI absorption and human intestinal absorption seem to be higher for Baicalein and Quercetin whereas a little low for Myricetin. The small compounds possess all the characteristics of good small biomolecules. The bioactive flavonoids have good molar refractivity values, indicating that they are permeable through the membrane and can strongly maintain constant molar refractivity even when there is a weak or strong solute-solvent interaction. The water solubility results confirmed that they are freely water soluble. The release of phosphate ion from ATP and at the same time binding of the ADP to the glycoprotein was predicted through pharmacokinetic properties. The bioactive flavonoids do not block or hinder the metabolism of other therapeutics drugs such as anti- malarial, anaesthetic, anti-ulcer, anti-sedative drugs etc. From the overall toxicity prediction, it is observed that the small molecules which are analysed in this study are safe and can be used as drug considering the prescribed amount of dosage for human consumption. Hence, the complete prediction and analysis suggest that Baicalein, Myricetin and Quercetin – all the three are better than the synthetic Lyn inhibitor Bosutinib. This study is another confirmation that flavonoids can be utilized as candidate drugs against breast cancer [43]. Further *in-vitro* and clinical studies should be done considering the advantages of using natural molecules over any kind of synthetic molecules as inhibitors of disease-causing proteins.

5. CONCLUSION

Since the Lyn (SRC-family kinase) is overexpressed in triple-negative/basal-like breast cancer (TNBC), inhibition of the same is very important. Most of the established lyn inhibitors are chemical inhibitors like Bosutinib, Dasatinib, 1-Tert-Butyl-3-(4-Chloro-Phenyl)-1h-Pyrazolo[3, 4-D]Pyrimidin-4-Ylamine etc. These kinds of inhibitors have a lot of limitations like some blocks invasion (but not proliferation) and chances of other side effects are relatively high. Hence, a detailed molecular docking based virtual screening was done to identify the

potential natural inhibitors for Lyn tyrosine kinase (PDB ID: 3A4O). This is to pave a path for dysregulated Lyn associated breast cancer therapeutic applications. Phytochemicals, in general, are completely natural and proved to nurture our health in the best possible way. Flavones and flavonols already proved to act against breast cancer. This study further emphasizes on a more precise mode of action by targeting Lyn tyrosine kinase protein for the treatment of breast cancer caused by Lyn associated dysregulation. In addition, the protein-ligand interaction was also studied for Bosutinib (established Lyn inhibitor) to establish the differences between using a synthetic inhibitor and natural molecules as inhibitors. Furthermore, many studies focussing on the delivery of these natural molecules when used as drug have been done [44]. Nano mediated delivery of flavonoids like Quercetin have been done and found to be really successful [45]. Baicalin, also known as baicalein-7-glucuronide, is a conjugate of baicalein, being a papain like protein (PLpro), not only act as an efficient Lyn inhibitor but also have anti-tumor, anti-inflammatory and anti-virus effects [46]. Hence the drug delivery of these compounds at the site of action would not be an issue for taking these molecules to the next step towards breast cancer therapeutics. The scope for precision treatment against cancer is also increasing with everyday research [47] as this study focuses on screening natural inhibitors for Lyn protein to be precise. A Lyn targeted therapy can be discovered doing the follow up *in vitro* research focusing the same and using this study as the base.

6. RESEARCH SIGNIFICANCE

This study highlights the effectiveness of "traditional medicine" utilizing the plant phytochemical compounds especially, flavonoids. It is an ancient tradition and is still used in some parts of India. This ancient concept needs to be carefully evaluated in the light of modern medicine and can be partially used if found appropriate.

DATA AVAILABILITY

All data generated and analyzed during this study is included in the main manuscript or supplementary materials/files.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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