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Research Article

MOLECULAR DOCKING STUDIES, INSILICO ADMET SCREENING, MM-GBSA BINDING FREE ENERGY OF SOME NOVEL PHENYL PYRAZOLE SUBSTITUTED 9-AMNOACRIDINES AS HER2 INHIBITORS TARGETING BREAST CANCER

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ABSTRACT

9-aminoacridines play an important role in the field of antitumor DNA-intercalating agents, due to their antiproliferative properties. Series of some phenyl pyrazole substituted 9-aminoacridines1a-x were designed for anti-breast cancer activity. Molecular docking targeted againstHER2by Glide module, *insilco* ADMET screening by qikprop module and binding free energy by Prime-MMGBSA module of Schrodinger suite-2016. The binding affinity of the designed molecules towards HER2 was selected on the basis of GLIDE score and interaction patterns. Most of the compounds 1a-x have good Glide scores when compared with standard drugsledacrine and tamoxifen. The Phenyl pyrazole substituted 9-aminoacridine derivatives 1a-x have good binding affinity with Glide score in the range of -3.43 to -8.086 compared with the standard ledacrine and tamoxifen. The results reveals that, Phenyl pyrazole substituted 9-amino acridines as HER2 inhibitor and the compounds, 1v,u,w,t,n,k with good Glide score may produce significant anti-breast cancer activity for further refinement.

INTRODUCTION

Many types of cancer can be treated by chemotherapy and the search for new chemotherapeutic agents still plays a major role in the fight against cancer. For about 1 in 5 women affected with breast cancer, the cancer cells have too much of a growth-promoting protein known as HER2 on their surface. Human epidermal growth factor receptor HER2 over expressionis present in approximately 20–30% of breast cancertumors. HER2 over expression is associated with a more aggressive disease, higher recurrence rate, and shortened survival[1].

These cancers, known as breast cancers, tend to grow and spread more aggressively. The benefit of anti-HER2 therapies demonstrated to one of the most promising molecules for targeted therapy[2].

Human epidermal growth factor receptor-2 (HER2) is a membrane tyrosine kinase and that is over expressed and gene amplified in human breast cancers. HER2 amplification and over expression have been linked to important tumor cell proliferation and survival pathways[3].Breast cancers can have up to 25–50 copies of the HER2 gene, and up to 40–100 fold increase in HER2 protein resulting in 2 million receptors expressed at the tumor cell surface (ERBB2 amplification in breast cancer analysed by fluorescence in situ hybridization[4].

In general, 9-aminoacridine derivatives are inhibiting DNA due to the ability of acridine nucleus to intercalate into DNA base pair and forming the 'ternary complex' which involve DNA, intercalated compound. The currently available 9-aminoacridine derivatives such as amsacrine and CI-921 a well-known antiproliferative agent used in the treatment of acute leukaemia. They are biologically unstable because ofthe Amsacrine (m-AMSA) and CI-921 possess a methane sulfonyl and a methoxy function at C-1' and C-3' of the 9-anilino ring and readily undergo reversible oxidation either chemically or microsomally converted in to quinonediimine. More than 50%of the dose is excreted as the glutathione conjugate. The half-life of m-AMSA in the presence of fresh mouse blood at 37°C is 30 min. To improve the current drawbacks of 9-aminoacridines, the effective strategy is to design modified drugs and synthesized to overcome the above problems.

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In the same context, acridines have gained importance for their various biological activitieslikeantimicrobial [5], antimicrobial [6], anticancer [7-11], antimalarial[12,13], anti-inflammatory[14], analgesic[15], antileishmanial[16], antinociceptive[17], acetyl cholinesterase inhibitors[17] and antiherpes[19] etc. The chemical modification ofacridines such as the introduction of different substitutions were allowed expansion of research on the structure activity relationship to afford new insight into molecular interactions at the receptor level[20]. The structural modification on 9-aminoacridines may bring various improvement in pharmacological effects. Similarly, pyrazolesare also be an important class of compounds with a wide range of biological activities[21-23]like antimicrobial, anticanceretc.

As part of our ongoing research on searching new potent antitumor agents [24-27], we have designed some novel 9-aminoacridine analogues bearing the phenyl pyrazole residue on 9-aminoacridine rings by molecular docking studies by using by using Schrodinger suit-2016. The results revealed that the newly designed 9-aminoacridine derivatives exhibited significant inhibition with HER2 exhibit anti breast cancer activity.

MATERIALS AND METHODS

Protein Preparation

The Human epidermal growth factor receptor 2 (HER2) with co crystallized ligand (PDB ID: 3PP0, resolution 2.25 A^o) was retrieved from protein data bank. Protein preparation wizard module of Schrödinger suite 2016-2was used for the protein preparation. Water molecules without hydrogen bonds are deleted. Missing chain atoms are added by using prime module of Schrödinger suite 2016-2. The possible ionization states were generated for the heteroatom present in the protein structure and the most stable state was chosen. Finally, minimization of the protein structure was carried out using OPLS3 force field to reorient side-chain hydroxyl groups and alleviate potential steric clashes. A grid box was generated to defined the centroid of the active site for docking studies [28]

Ligand Preparation

The structures of the ligands (1a-x) were generated and subjected to LigPrep module of Schrodinger suite 2016-2. 2Dstructures were converted to 3D structures by including stereo chemical, ionization, tautomeric variations, as well as energy minimization and optimized for their geometry, desalted and corrected for their chirality and missing hydrogen atoms. The ionization and tautomeric states were generated between pH of 6.8 to 7.2 using Epik module. In the final stage of LigPrep, compounds were minimized using Optimized Potentials for Liquid Simulations-3(OPLS-3) force field in Impact package of Schrodinger suit until a root mean square deviation of 1.8A^o was achieved. A single low energy ring confirmation per ligand was generated and the optimized ligands were used for docking analysis.

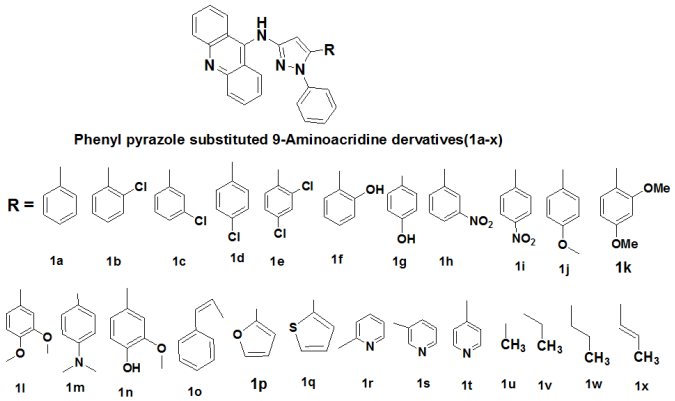

Figure 1 Structure of designed compounds(1a-x)

Glide Ligand docking

The designed phenyl pyrazole substituted 9-aminoacridines (1a-x) are docked in to catalytic pocket of HER2 protein (PDB ID: 3PP0) by using Glide module of Schrödinger suite 2016-2. The best docked compounds are selected by using Glide score function. The favourable interactions between ligand molecules and the receptor were scored using Glide ligand docking program. All the docking calculations were performed using extra precision (XP) mode and OPLS-3 force field. The flexible docking mode was used for docking process in which automatically generates conformations for each input ligand. This algorithm recognizes favourable hydrophobic, hydrogen-bonding and metal-ligation interactions, and penalizes steric clashes. Finally, the minimized poses were re-scored using Glide Score scoring function[29].The XP-Glide score of active compounds were summarized and compared with the Glide score of standard compound containing acridine derivative ledacrine and the anti-breast cancer drug tamoxifen.

The *in-silico* ADME properties of the proposed compounds were determined by qikprop of Schrodinger suit-2016.

Validation of the docking programme

The accuracy of the docking procedure was determined by finding how closely the lowest energy pose (binding conformation) of the co-crystallized ligand predicted by the object scoring function, Glide score (G Score), resembles an experimental binding mode as determined by X-ray crystallography. Extra precision Glide docking procedure was validated by removing the co-crystallized ligand from the binding site of the protein and redocking the ligand with its binding site. The hydrogen bonding interactions and the root mean square deviation (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation were used for analyzing the results.

Binding free energy calculation by using Prime/MM-GBSA approach

The binding free energies of complex are computed by Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) using the Prime module of Schrödinger suite 2016-2 which incorporates the OPLS3 force field and VSGB solvent model to search algorithms.

RESULTS AND DISCUSSION

Docking studies

The molecular docking studies of the designed ligands(**1a-x**) to protein active sites were performed by molecular docking program Schrodinger suit-2016-2 for determining the binding affinities of the ligands. The designed analogues are docked towards the HER2 (3PP0) in order to ascertain their HER2 inhibition activity against breast cancer. The compounds **1a-x** showed good affinity to the receptor when compared with standard acridine derivative with anticancer activity ledacrine and anti-breast cancer agent tamoxifen. The Glide scores of docking studies against HER2 inhibitor (PDB id 3PP0) are shown in the Table1. The docking results reveals that the interactions are mainly with lipophilic factors due to the presence of aromatic rings of acridine and heterocyclic rings. The ligand interactions ofthe compound **1v** with high Glide score (-8.086) are mainly dominated in the region of Ser728 to ASP 863 residues which are the active site region (Figure 2). The hydrophobic/philic interaction map of compound **1v**is given in the Figure 3. The phenyl pyrazole substituted 9-aminoacridine rings are located in the hydrophobic pocket and the amino group is located in the hydrophilic pocket. The best docked poses of the compounds **1v, u, w, t, n, k** with high Glide score were shown in the Figure 4.The compound**1n** is exhibited hydrogen bonding with ARG849 (H-bond length 4.65), ASP863(H-bond length 3.31) residues (Figure 5).

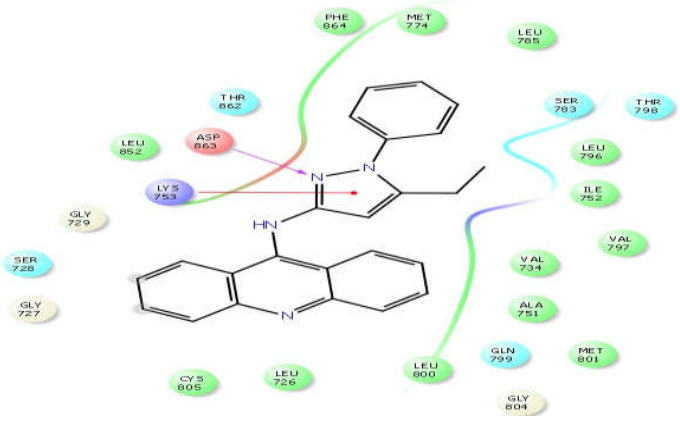

Figure 2 Ligand interaction of compound 1v with HER2 (3PP0)

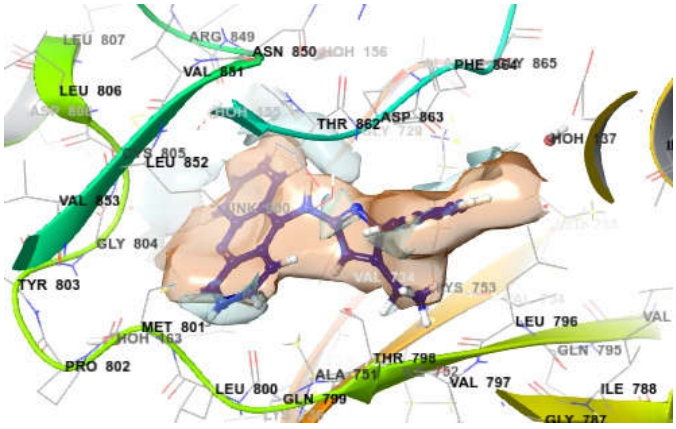

Figure 3 Hydrophobic/Philic map of compound 1v with HER2 (3PP0)

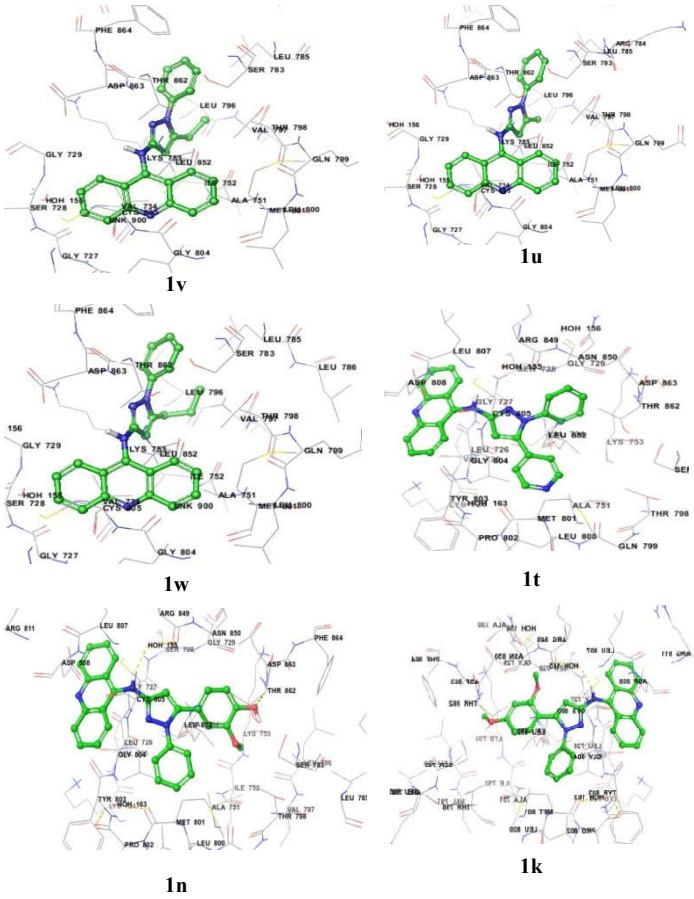

Figure 4 Best affinity mode of docked compounds 1v, u, w, t, n, k with HER2 (3PP0)

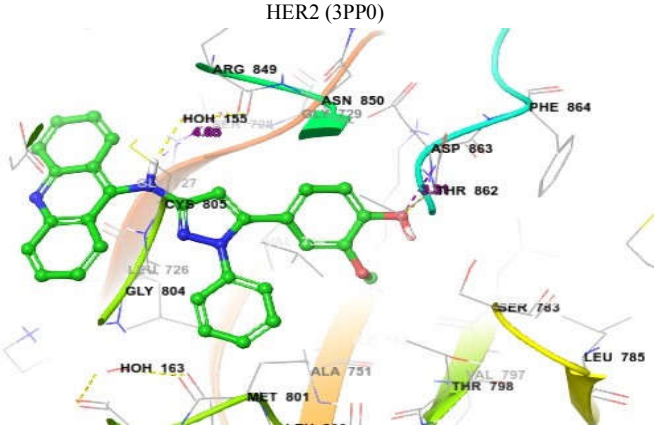

Figure 5 Hydrogen bonding affinity of docked compounds 1n with HER2 (3PP0)

Insilico ADMET Screening

The ADMET properties for the designed compounds (**1a-x**) can be determined *in-silico* by using qikprop module of Schrödinger suite 2016-2. Molecular weight of the designed compounds are between 350 1nd 473. Computed dipole moment of the compounds between 2.13 and 6.99. Estimated no. of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution of the compounds are in the range of 1-2. Estimated no. of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution of the compounds are in the range of 2.5- 4. Number of likely metabolic reactions of the compounds are in the range of 1-2.

Table-1 Docking studies for compounds 1a-x with HER2 (3PP0)

Cpd	Glide score	Glide energy	Lipo philic EvdW	H Bond	Phob En	Low MW	Rot Penal	XP Electro	XP Penalties
1v	-8.086	-51.932	-8.685	0	-0.334	-0.285	0.186	0.032	1
1u	-7.966	-53.905	-8.591	0	-0.238	-0.332	0.2	-0.004	1
1w	-7.406	-38.887	-8.183	-0.179	0	-0.238	0.232	-0.037	1
1t	-7.177	-37.521	-4.984	-0.7	0	-0.122	0.198	-0.145	0
1n	-6.574	-33.659	-5.502	-1.18	-0.2	0	0.164	0.144	0
1k	-5.829	-37.067	-5.375	-0.843	0	0	0.155	0.165	0
1f	-5.566	-30.783	-5.05	-0.661	-0.225	-0.072	0.185	0.178	0
1c	-5.559	-40.159	-5.69	0	0	-0.01	0.172	-0.127	0
1i	-5.418	-42.997	-5.482	0	0	0	0.165	-0.114	0
1m	-5.326	-37.802	-5.383	0	0	0	0.166	-0.118	0
1s	-5.252	-39.869	-5.471	0	0	-0.122	0.198	0.036	0
1g	-5.216	-27.046	-4.843	-0.7	0	-0.072	0.185	0.136	0
1a	-5.191	-33.053	-5.167	0	0	-0.125	0.199	-0.098	0
1r	-4.907	-35.401	-5.553	0	0	-0.122	0.198	0.009	0
1j	-4.89	-29.025	-5.259	-0.7	-0.127	-0.025	0.175	0.02	0
1l	-4.815	-42.158	-5.277	0	0	0	0.155	-0.124	0
1b	-4.617	-23.937	-4.33	-0.915	0	-0.01	0.172	-0.194	0
1h	-4.541	-44.931	-5.478	-0.124	-0.125	0	0.165	-0.175	0
1p	-4.493	-35.448	-5.051	0	0	-0.159	0.208	-0.004	0
1d	-4.428	-34.339	-5.336	0	0	-0.01	0.172	-0.036	0
1e	-4.377	-48.615	-5.344	-0.35	0	0	0.15	-0.236	0
1q	-4.038	-45.595	-4.996	-0.314	0	-0.105	0.194	0.001	0
1x	-3.581	-43.976	-3.884	-0.692	0	-0.245	0.234	-0.554	0
1o	-3.43	-40.044	-3.973	-0.35	0	-0.038	0.222	-0.371	0
ledacrine (std)	-6.318	-32.208	-5.284	-0.593	-0.125	-0.419	0.305	-0.202	0
Tamoxifen(std)	-3.787	-22.744	-4.179	0	0	-0.262	0.42	-0.023	0

Prediction of binding to human serum albumin for the compounds are in the range of -7.1 to -8.2. Number of violations of Lipinski's rule of five is 1.

All the compounds have 100 % Human Oral Absorption. So almost all the properties of the compounds are within the r

Table 2 Insilico ADMET screening for proposed compounds (1a-x)

Compounds	mol MW	dipole	Donor HB	Accpt HB	QPlog HERG	# metab	QPlog Khsa	Rule Of Five	%Human Oral Absorption
1a	412.49	5.206	1	2.5	-8.184	1	1.621	1	100
1b	446.93	6.273	1	2.5	-8.055	1	1.723	1	100
1c	446.93	4.086	1	2.5	-8.046	1	1.742	1	100
1d	446.93	4.215	1	2.5	-8.089	1	1.744	1	100
1e	481.38	5.139	1	2.5	-7.96	1	1.847	1	100
1f	428.49	6.188	2	3.25	-8.063	2	1.369	1	100
1g	428.49	4.698	2	3.25	-8.051	2	1.379	1	100
1h	457.49	4.948	1	3.5	-8.038	2	1.569	1	100
1i	457.49	5.991	1	3.5	-8.08	2	1.572	1	100
1j	442.51	4.579	1	3.25	-7.926	2	1.623	1	100
1k	472.54	5.787	1	4	-7.35	3	1.529	1	100
1l	472.54	3.553	1	4	-7.716	3	1.651	1	100
1m	455.56	6.63	1	3.5	-8.015	2	1.778	1	100
1n	458.51	6.99	2	4	-7.876	3	1.386	1	100
1o	438.53	2.13	1	2.5	-7.014	1	1.586	1	100
1p	402.45	5.357	1	3	-7.98	2	1.345	1	100
1q	418.51	5.482	1	2.5	-7.926	2	1.545	1	100
1r	413.48	5.069	1	3.5	-8.219	2	1.394	1	100
1s	413.48	5.527	1	4	-8.024	3	1.276	1	100
1t	413.48	4.124	1	4	-8.017	3	1.276	1	100
1u	350.42	5.167	1	2.5	-7.185	2	1.15	1	100
1v	364.44	5.171	1	2.5	-7.19	2	1.261	1	100
1w	378.47	5.296	1	2.5	-7.415	2	1.389	1	100
1x	376.46	5.229	1	2.5	-7.556	2	1.395	1	100
Recommended values	130-725	1- 12.5	0– 6	2-20	-2-8.5	1 – 8	–1.5 – 1.5	max 4	>80% is high <25% is poor

MW- Molecular weight of the molecule,
Dipole – Computed Dipole moment of the molecule
donorHB - Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution.
accptHB- Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution
#metab- Number of likely metabolic reactions.
QPlogKhsa- Prediction of binding to human serum albumin.
RuleOfFive Number of violations of Lipinski's rule of five.
%Human- Oral absorption- Predicted human oral absorption on 0 to 100% scale.

Dr.S.P.Dhanabal, JSS College of pharmacy, Ooty for the technical support.

Table 3 Binding free energy calculation using Prime/MM-GBSA approach (1a-x)

Compound code	ΔGbind (Kcal/mol)	ΔGbind Coulomb	ΔGbind Vander	ΔGbind HBond	ΔGbind covalent	ΔGbind Lipophilic
1v	-44.2899	-26.0875	1.048949	0.246785	4.928729	-23.4258
1u	-58.1881	-6.31151	-1.30722	-0.26307	8.711433	-29.071
1w	-51.6117	-15.9219	1.368953	0.993958	5.366778	-27.0684
1t	-49.8116	-18.2499	3.018464	-1.26064	0.537611	-22.2536
1n	-49.8233	-14.3084	2.400292	-1.91122	3.836515	-24.847
1k	-46.6152	-25.1295	1.229076	-2.64785	10.65352	-23.7267
1f	-36.8186	3.161912	2.659625	1.916831	-5.27681	-22.1385
1c	-29.7095	-9.30606	2.144612	2.542939	10.61557	-19.6965
1i	-45.2658	6.617119	4.269057	0.774799	1.278663	-23.3002
1m	-35.4657	46.63129	3.846093	2.816668	-8.1205	-22.8826
1s	-49.5063	-39.2171	2.861231	-1.64852	15.30527	-24.0959
1g	-45.2356	-30.1209	2.364717	-3.5977	16.84194	-24.9449
1a	-52.57	-30.4867	1.250214	-1.45764	14.96417	-26.6798
1r	-34.5396	21.94787	2.16225	2.870107	3.095313	-21.38
1j	-54.8638	-5.59448	2.127224	-0.97921	-3.5634	-24.2049
1l	-52.0923	-35.0743	2.531662	-3.65594	-1.43618	-18.8706
1b	-45.8137	-34.6972	2.115495	-2.48436	5.602946	-18.0172
1h	-48.5946	-3.41409	1.321958	-0.37195	9.776811	-21.4937
1p	-40.4358	-34.8556	1.553369	0.072809	12.13234	-18.9228
1d	-50.0709	-18.4602	3.820505	-0.31639	-5.21596	-20.4024
1e	-49.5041	-26.5996	3.047551	-2.59757	12.93218	-26.3152
1q	-51.1409	-38.4616	-0.51145	-0.4076	5.200194	-20.9033
1x	-35.5724	-24.8988	2.393937	-0.0357	15.32881	-20.3309
1o	-54.3834	-46.9223	1.699911	-3.92628	7.372585	-22.9238

Binding free energy calculation using Prime/MM-GBSA

Molecular docking was also evaluated with MM-GBSA free binding energy[30] which is related to post scoring approach for HER2 (PDB ID: 3PP0) target. The accuracy of docking is confirmed by examining the lowest energy poses predict by scoring function. The Glide scores are almost resembles to the experimental binding mode as determined by the X-ray crystallography. The Glide score and MM-GBSA free energy values are obtained by the docking of ligands in to the binding pocket. The details of the MM-GBSA free binding energy for the compounds **1a-x** are shown in the Table-3.

CONCLUSION

Generally, 9-Aminoacridine derivatives exhibited various biological activities. The molecular docking study revealed that phenyl pyrazole substituted 9-aminoacridine derivatives showed better alignment at active site by interacting with many amino acid residues. Thus, the *in silico* method adopted in the present study helped in identifying the lead molecules and also may partly explain their beneficial effect in *in vitro* and *in vivo* study. On this basis, authors recently demonstrated that diverse compounds of the phenyl pyrazole substituted 9-aminoacridine series exerted HER2 inhibition as anti-breast cancer activity. Results observed in the present study clearly demonstrated that some derivatives of the phenyl pyrazole substituted 9-aminoacridine family may exert interesting antitumour activity. The compounds 1v, u, w, t, n, k have significant anti-breast cancer activity with therapeutic potentials and are likely to be useful as drugs after further refinement.

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