https://doi.org/ 10.33472/AFJBS.6.5.2024. 336-349

African Journal of Biological Sciences

Exploring Plant Metabolites as Potential Inhibitors of HIV-1 Nef Protein: A Computational Approach

Manisha Kotadiya*[1a], Bhakti Shah[2a], Ravi R. Patel[2a], Kalubha S. Zala[2b], Mansi Soni[3a], Manali Patel[3b], Honey Joshi[3c], Ravi Ajudia[a]

[1a, a]Department of Pharmaceutical Chemistry, School of Pharmacy RK University, Rajkot, Gujarat, India, 360370.
[2a, 3a]Department of Pharmaceutics, Shree Swaminarayan College of Pharmacy, Swaminarayan University, Kalol, Gandhinagar, Gujarat, India, 382725.
[2b]Department of Pharmacology, Shree Swaminarayan College of Pharmacy, Swaminarayan University, Kalol, Gandhinagar, Gujarat, India, 382725.
[3b]Department of Pharmaceutical Chemistry, Shree Swaminarayan College of Pharmacy, Swaminarayan University, Kalol, Gandhinagar, Gujarat, India, 382725.
[3c]Department of Chemistry, Shree Swaminarayan College of Pharmacy, Swaminarayan University, Kalol, Gandhinagar, Gujarat, India, 382725.
***Email: manishakotadiya3@gmail.com**

Article History
Volume 6, Issue 5, Apr 2024
Received: 22 Apr 2024
Accepted: 29 Apr 2024
doi: ***10.33472/AFJBS.6.5.2024. 336-349***

ABSTRACT

The HIV-negative factor (Nef) protein is an accessory pathological component that plays a crucial role in the condition known as acquired immune deficiency syndrome (AIDS). The HIV virus which lacks Nef took time to transformed into AIDS. As a consequence, attacking the Nef molecule is viewed as a significant strategy for HIV/AIDS treatment. Only a few drugs have been discovered as Nef inhibitors until date. The present effort seeks to find putative HIV-1 NEF antagonists among a collection of plant compounds. Following ADME assessment, among the one hundred, twenty phytocompounds that were docked in conjunction with HIV-1 NEF to determine their inhibitory effectiveness with respect to the NEF Protein. Ten of the 20 compounds have strong binding affinity. Then, only six of the 10 substances succeeded the tests for toxicity (mutagenicity). These antagonists' reactivity was studied. According to the outcomes of the Molecular dynamic simulations, the affinity of these inhibitors was high in relation to other compounds. These results imply that the selected botanical compounds offer promising treatments to combat HIV in future.

Keywords: HIV-1, NEF, Plant compounds, molecular docking; Molecular simulation, ADME, Toxicity.

INTRODUCTION

In 1983, the first instance of acquired immune deficiency syndrome (AIDS) was described. AIDS is propagated by a virus identified to be the human immune deficiency virus (HIV), which persists one of the oldest and most problematic infectious illnesses[1]. According to estimates, roughly 34 million individuals worldwide are infected with HIV/AIDS[2]. Despite tremendous investments in HIV/AIDS research, there is no cure for this worldwide pandemic exists. At the moment, the only effective treatment available is a combination of numerous medications that target distinct the virus enzyme at various phases of its entire life cycle[3]. The Nef (Negative Factor) is an accessory gene product of HIV that plays an essential role in viral transmission and the progression of AIDS[4]. In the absence of targeted gene in HIV, the impact of AIDS was substantially reduced[5].

In nature, the nef is a tiny protein (24-35kDa) which has been myrisotylated. Its major mode of transport is via the cytosol towards the host membrane6. Nef plays a vital function in the development of immunological synapses by limiting the cell distribution of CD4, MHC-I, MHC-II, and CD28 [7].

Numerous computational tools/approaches for assessing enormous biochemical datasets for hits/leads are now available. Among numerous computing tools/approaches, virtual assessment has been regarded as a must-have in current drug development situations[8]. Virtual screening may be broken into two methods: ligand-based and structure-based[9]. Ligand-based virtual testing approaches examine binding affinity of possible hits for a certain target employing knowledge of present ligands and an unknown receptor location[10]. Structure-based virtual screening approaches, on the other hand, leverage knowledge of the desired protein's 3D structure to predict the binding affinity of substances against the target protein of choice [11]. As a result, the present work applied linked computational techniques of ligands and structure based online screening to give fresh viewpoints on fundamental aspects associated with drug development.

These techniques incorporate a combination of ligand (pharmacophore and shape similarity) and structure-based virtual screening methodologies, molecular dynamics simulation, result analysis, and binding energy estimates [12]. As a result, the current inquiry incorporates a 20 ns MD simulation in addition to analyses of docking complexes' sturdiness and binding capacity throughout an appropriate time scale. The combined in-silico approaches suggested in this study might be a beneficial tool for identifying new compounds as HIV-Nef inhibitors.

MATERIAL AND METHODS

Retrieval of Phytochemicals and Preparation of Library

To pick the best of Indian plants, Indian ancient literatures were examined thoroughly to develop a complete list of herbal remedies that are renowned for having power and containing renewing characteristics, which may reveal antiviral properties. Subsequently, around 100 phytocompounds from diverse plants were obtained through IMPPAT the database13, KNApSAcK[14], the PubChem[15] and the ChEMBL[16] databases.

Retrieving Target Proteins

For this investigation of HIV 1 Nef protein, two distinct protein domains were obtained; initial motif is 1EFN (in combination along with the SH_3 motif) another motif is 2NEF (Nef core domain). The target proteins were downloaded via RCSB PDB along with the PDB identification numbers comprising 1EFN (SH3 Region) and 2NEF (The core framework).

Absorption, Distribution, Metabolism, Excretion, and Toxicity Analysis

In order to choose the most acceptable, drug-like, lead-like, and free of infringe compound, the drug bank was subjected to ensembles for ADME evaluation utilising Swiss ADME17. Compounds that were verified applying ADME evaluation were next subjected to toxicity evaluation using TEST software which concluded by docking assessment.

Protein Preparation

3DligandSite18 and COACH-D19 were utilised to identify the active pocket regions in the identified target proteins where the medicines are most probably interacting. The target is not suitable directly for docking since they may be linked with enormous atomic particles, co-crystallized ligands and water molecules, requiring preparation through the addition of the atoms of hydrogen, eliminating water, cobound hetero-atoms and positioning polar charges. Once the setup procedure had been finished, they were transformed into pdbqt format.

Preparing Phytocompounds for Docking

Before the phytocompounds could be installed for molecular docking, they were energy minimized using various parameters—forcefield mmff94 optimization Then Phytocompounds were converted from. mol2/. mol/.sdf to. pdbqt and were then subjected to docking in Autodock Vina.[20] For multidrug docking, we used PyRx software tool [21], which works for virtual screening.

Docking was carried out twice, 1 for each target, with the same initial steps of preprocessing. After loading the prepared target with set parameters, the prepared ligand file was also uploaded. The grid size was set to 50 × 50 × 50 points with a grid spacing of 0.375 Å with exhaustiveness equal to 8.

Interaction Analysis

The resulting complexes were examined for interactions using Biovia discovery studio visualizer application.[21]

Molecular Dynamics Simulations

The best-docked protein target and ligand complexes were subjected to refinement and molecular dynamics simulation was done using Imods server.

Deformability, B-Factor, and Covariance Computation

The complexes were subjected to deformability, B-factor, covariance, and root-mean-square fluctuation analysis to check for any residues that may still be unstable or deformed after the

coarse-grained simulation. The deformability, B-factor, and covariance analysis were executed using Imods software.[22]

RESULTS AND DISCUSSION

Molecular Docking

The major purpose of this study was to look at natural chemicals that may conceivably and successfully mix efficiently with the HIV-1 NEF proteins. Just twenty of the one hundred ADME-screened substances qualified and were subsequently sent to the docking procedure. A few of the were removed as there was no .sdf/. mol form available, they were only reported in the academic literature, or they generated a parsing error while being exposed to molecular docking studies. Following the docking research, only 10 of the 100 phytochemicals exhibited greater binding affinity with proteins being studied. Table 1 displays phytochemicals such as Amentoflavone, Glabrocoumarin, Glabroisoflavanone, and others. Figures 2 and 3 show botanical compounds connected to the target proteins 1EFN and 2NEF, respectively. Toxicity will be tested following docking. Amentoflavone, Glabrocoumarin, Glabroisoflavanone, Nicotiflorin, Naringetol, and Chrysin with targeting Protein are the best-docked compounds selected for MDS.

Analysis of ADME Properties

The resultant drug library (n=100) was examined for pharmacokinetic descriptors. Botanical compounds with better intestinal absorption, dissolution, flexibility, and outstanding logP values (partition coefficient) were assessed, as were those that failed to meet any of the criteria, including Lipinski's Rule, Veber's, Ghosh's, Egan's, and Mugge's. Finally, phytocompounds with an good leadlikeness, and synthetic accessibility were selected. Then toxicity screening was done to select those with less toxicity or sensitivity to any hazardous receptor, notably Mutagenicity. Chrysin, Nicotiflorin, amentoflavone, Glabrocoumarin, Glabroisoflavanone, and Naringetol were among 10 phytochemicals that passed the mutagenicity test. Table 3 displays the predicted value. Following toxicity testing, phytochemicals were put to a Molecular dynamic modelling investigation in order to develop a stable complex.

Analysis of Interactions

Before submitting the selected complex for MDS around 20 nanoseconds, compounds were assessed further utilising an interaction evaluation to identify a range of linkages produced among docked compounds. Table 2 presents the interaction results. A complex is deemed powerful when the total number of bonds composed of hydrogen is higher than a specific threshold, with only a few hydrophobic connections, bridges of salts, and pi-pi interactions. We evaluated the number of interactions formed by each of the 10 chemicals docked with Protein separately. According to the interaction analysis, the complexes with the greatest proportion of bonds carrying hydrogen having significant binding ability. Amentoflavone generated 5 hydrogen bonds with 1EFN, with ARG D:71, TRP D:113, THR D:117, LEU D:112 and TRY D:115, and 2 with 2NEF. The second phytochemical with a variable number of hydrogen bonds was aromadendrin. It created three bonds with one of the EFN amino acid sequences. Figures 2 and 3 exhibit the interaction of the aforementioned chemicals with the chosen proteins.

Simulations of Molecular Dynamics

Based on the binding data, we did molecular dynamic analysis studies on the docking complex with the lowest energy value and best-posed. The iMods website executed the MD simulation at 300 K and 1 atm constant pressure at the molecular mechanics' level. This augmented normal mode analysis (NMA) technique in inner coordinates is available via the iMods service. Users may utilise NMA or molecular dynamics to generate potential paths between two conformations and explore, directions, visuals, and even huge macromolecules interactively in 3D. Finally, the complexes were exposed to a twenty-nanosecond molecular dynamic simulation.

Establishing an RMSD (root mean square deviation) or root-mean-square deviation serves as a standard measure of structural difference among two proteins for docking and simulation. The initial framework is continuously distorted along the lowest modes to represent probable transitions, and RMSD for the desired structure is reduced. There are two initial superimposition procedures available: global [23] and local [24]. The former evaluates all atoms for the RMSD, whereas the latter chooses the overlap between the **most similar regions.**

Thecalculations involved of deformation, B-factor, and the covariance

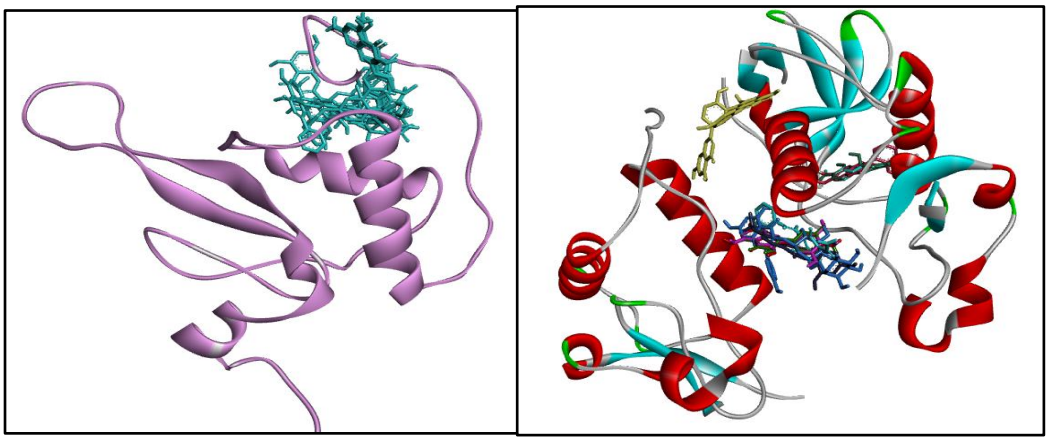
Deformability is an assessment of the capacity of a molecule to change shape of its sites. The Amentoflavone-2NEF complex displayed the highest deformability, with several peaks of about 1.0 deformability indices in contrast to other complexes. The B-factor is determined by taking the PDB framework and analysing it via normal mode analysis (NMA), and then multiplying the findings of the NMA mobility by 8pi 2. The B-factor evaluation also produces an estimated average RMS value. The covariance matrix indicates how the complex's residues are related; the greater the relationship, the better the complex. The red hue reflects a strong relationship between residues, the white shade shows a lack of association, and the blue shade represents anti-correlations. Amentoflavone NEF- suggests a strong relationship with only a few anticorrelations in a majority of situations. The eigenvalues are directly connected to the amount of energy needed to deform the structure. The simpler the deformation, the smaller the eigen value. The amentoflavone-NEF complex displays outstanding deformation with an eigenvalue of 1.54, as illustrated in Figure 3.

REFERENCES

1. Wlodawer A,Vondrasek J. (1998). Inhibitors of HIV-1 pro tease: A major success of structure-assisted drug design. Annu. Rev. Biophys. Biomol. Struct. 27, 249–284.
2. Johnson, B et al (2012). A comparison of the ability of rilpivirine (TMC278) and selected analogues to inhibit clinically relevant HIV-1 reverse transcriptase mutants. Retrovirology,doi:10.1186/1742-4690-9-99.
3. Morah E. (2017). Are people aware of their HIV-positive status responsible for driving the epidemic in Sub-Saharan Africa? The case of Malawi. Development Policy Rev.25, 215–242.
4. Fischl Met al. (1987). The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex—a double-blind, placebo-controlled trial.N. Engl. J. Med.317, 185–191.
5. Bouchet J et al. (2011). Inhibition of the Nef regulatory protein of HIV-1 by a single-domain antibody. Blood, 31, 3559–3568.

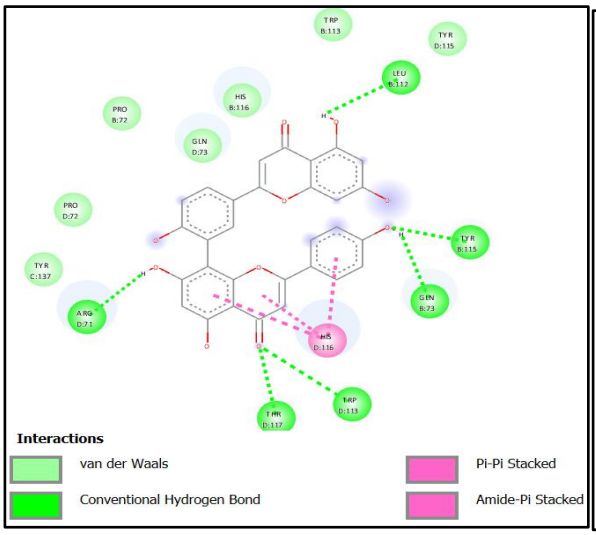
6. Lulf S et al. (2014).Structural basis for the inhibition of HIV-1 Nef by a high-affinity binding single-domain antibody. Retrovirology, 2014;13,11–24.
7. Landi A, Iannucci V, Van Nuffel A, Meuwissen P and Ver hasselt B. (2011). One protein to rule them all: Modulation of cell surface receptors and molecules by HIV Nef. Curr. HIV Res. 9, 496–504.
8. Kotadiya M and Ajudia R. (2024). In-silico Docking and ADME Studies of Natural Phytoconstituents from Different Medicinal Plants as Potential HIV Reverse Transcriptase Inhibitors. International Journal of Pharmaceutical Quality Assurance. 15, 1-4.
9. Feng J, Guo H, Wang J and Lu T. (2014). Screening of drug target proteins by 2D ligand matching approach. Chem. Biol. Drug Des. 83, 174–182.
10. Zavodszky M, Rohatgi A, Van Voorst J, Yan H and Kuhn L. (2009). Scoring ligand similarity in structure-based virtual screening. J. Mol. Recognit. 22, 280–292.
11. Lavecchia A and Di Giovanni C. (2013). Virtual screening strategies in drug discovery: A critical review. Curr. Med. Chem.20, 2839–2860.
12. Shoichet B, Mcgovern S, Wei B and Irwin J. (2008)Hits, leads and artifacts from virtual and high-throughput screening. Mol. Inf.123–135
13. Sheriff M. (2018).IMPPAT: a curated database of Indian medicinal. Sci. rep.8, 4329. http://ayush.gov.in/sites/default/ files/IV.pdf
14. Nakamura Yet al. (2014). KNApSAcK metabolite activity database for retrieving the relationships between metabolites and biological activities. Plant Cell Physiol.55, 1-9. doi:10.1093/pcp/pct176
15. Kim Set al. (2019).PubChem substance and compound databases. Nucleic Acids Res. 47, 1202-13.
16. Gaulton A et al. (2012). ChEMBL: a large-scale bioactivity database for drug discovery. Nucleic Acids Res.40, 1100–1107. doi:10.1093/nar/gkr777
17. Daina A, Michielin O and Zoete V. (2017) Swiss-ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep.7, 1–13. doi:10.1038/ srep42717
18. Wass M, Kelley L and Sternberg M. (2018). 3DLigandSite: predicting ligand-binding sites using similar structures. Nucleic Acids Res.38, 469-473. doi:10.1093/nar/gkq406
19. Wu Q, PengZ, Zhang Y and Yang J. (2018). COACH-D: improved protein ligand binding sites prediction with refined ligand-binding poses through molecular docking. Nucleic Acids Res. 46, 438-442. doi:10.1093/nar/gky439
20. Trott O and Olson A. (2019). Autodock vina: improving the speed and accuracy of docking. J Comput Chem.31, 455-461.
21. Salentin S, Schreiber S, Haupt V, Adasme M and Schroeder M. (2015)PLIP: fully automated protein-ligand interaction profiler. Nucleic Acids Res.43, 443-447. doi:10.1093/nar/gkv315
22. Lopez-Blanco J, Aliaga J, Quintana-Orti E and ChaconP. (2014).IMODS: internal coordinates normal mode analysis server. Nucleic Acids Res.42, 271–276. doi:10.1093/nar/ gku339
23. Damm K and Carlson H. (2006). Gaussian-weighted RMSD superposition of proteins: a structural comparison for flexible proteins and predicted protein structures. Biophys J.90, 4558–73. https://doi.org/10.1529/biophysj.105.066654.

FIGURES AND THEIR LEGENDS

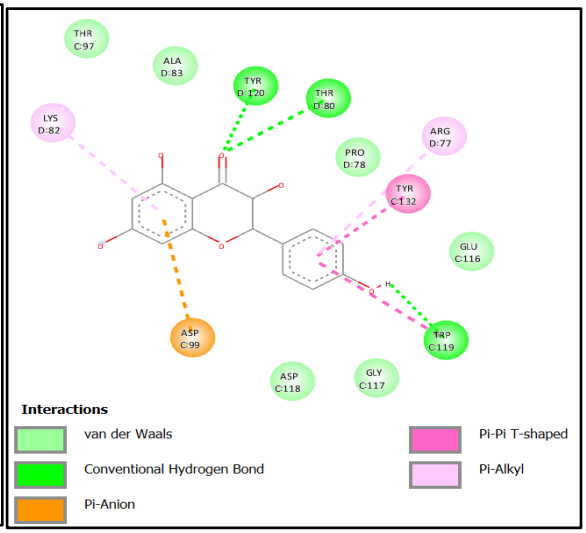


A.Nef core domain (2NEF) **B. Nef complex with SH3 domain(1EFN)**

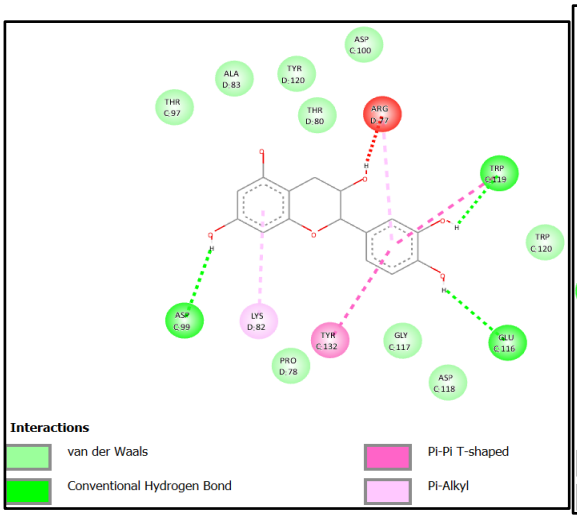
Fig.- 1. Binding orientation of HIV-1 Nef protein with selected Phytocompounds.



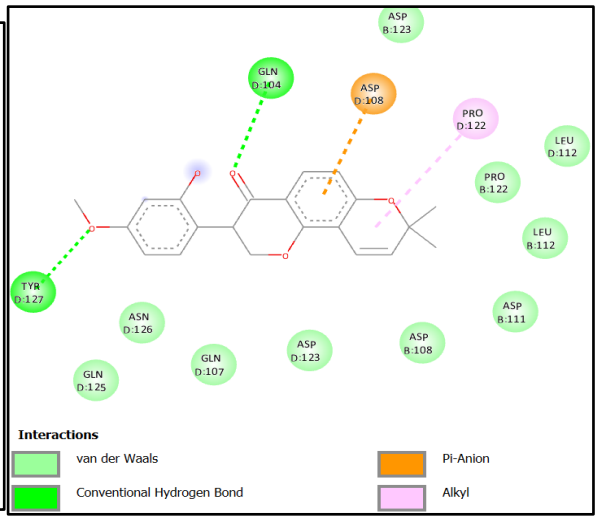
A. Amentoflavone



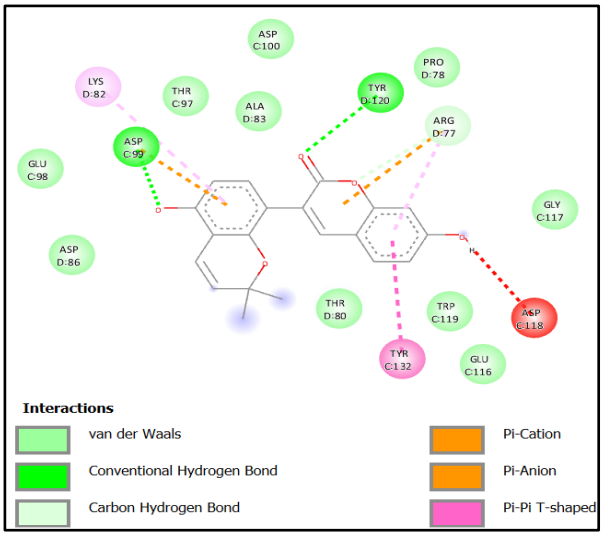
B. Aromadendrin



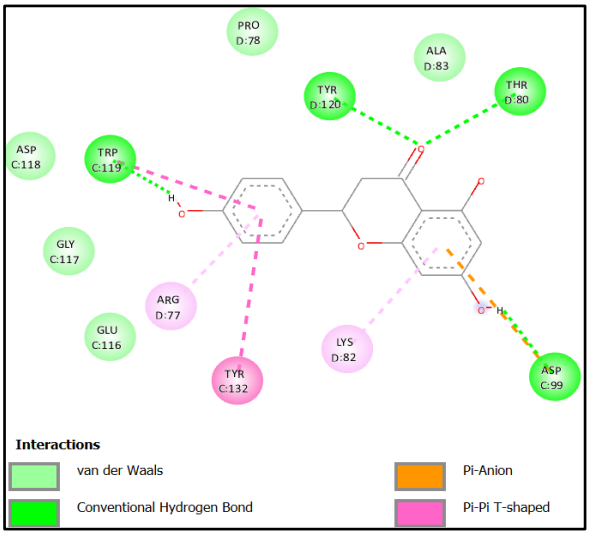
C. Epicatechin



D. Glabroisoflavanone B

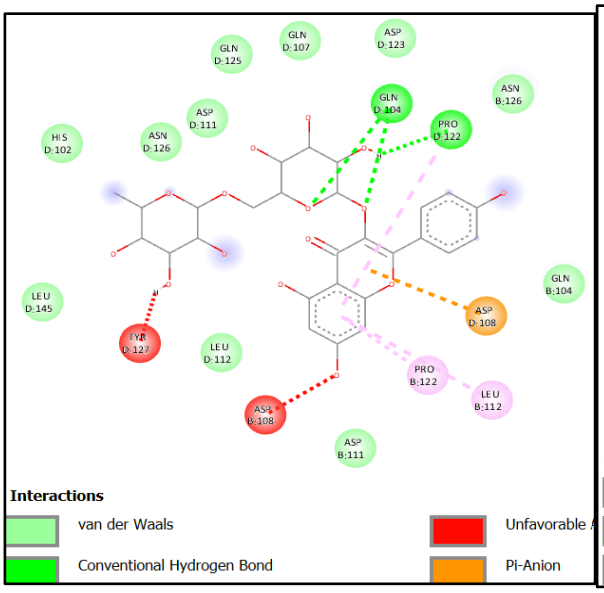


Glabrocoumarin

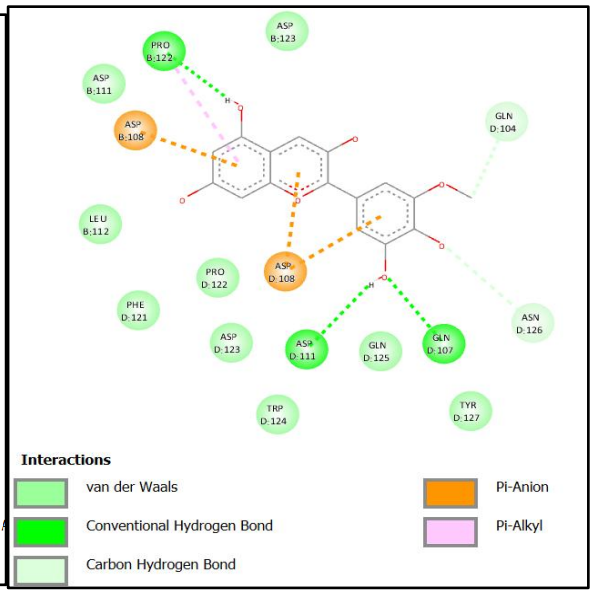


F. Naringetol

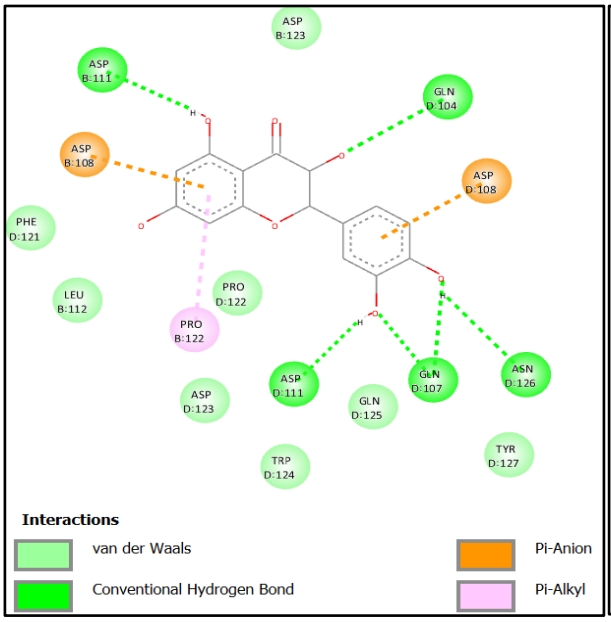
E.



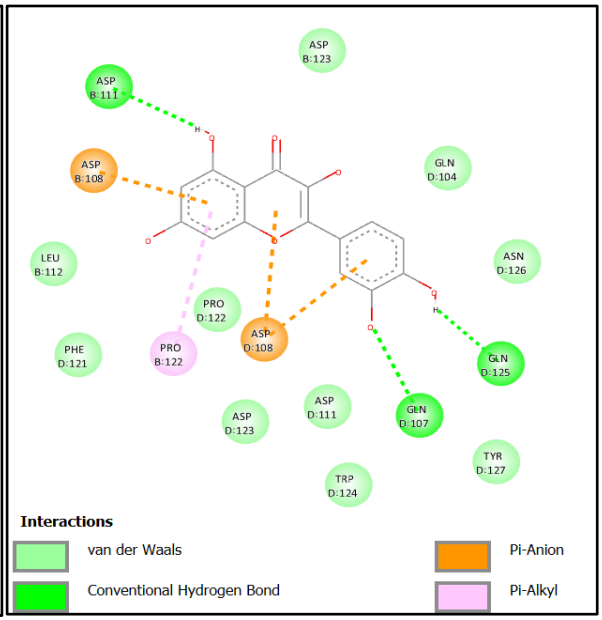
G. Nicotiflorin



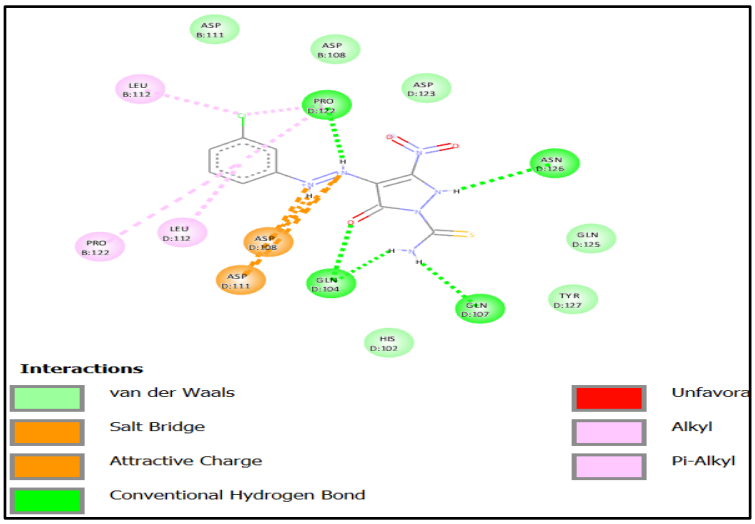
H. Petunidin



I. Taxifolin



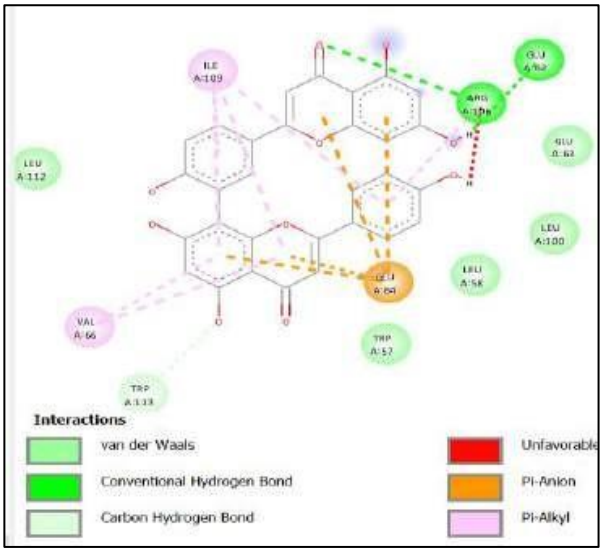
J. Quercetin



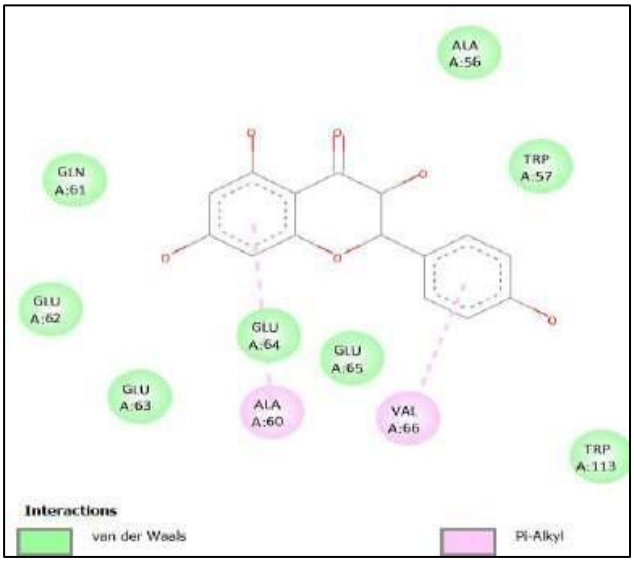
K. 4-(3-Chlorophenyl) diazinyl)-5-hydroxy-3-nitro-1h-pyrazole-1-carbothioamide (Reference Standard)

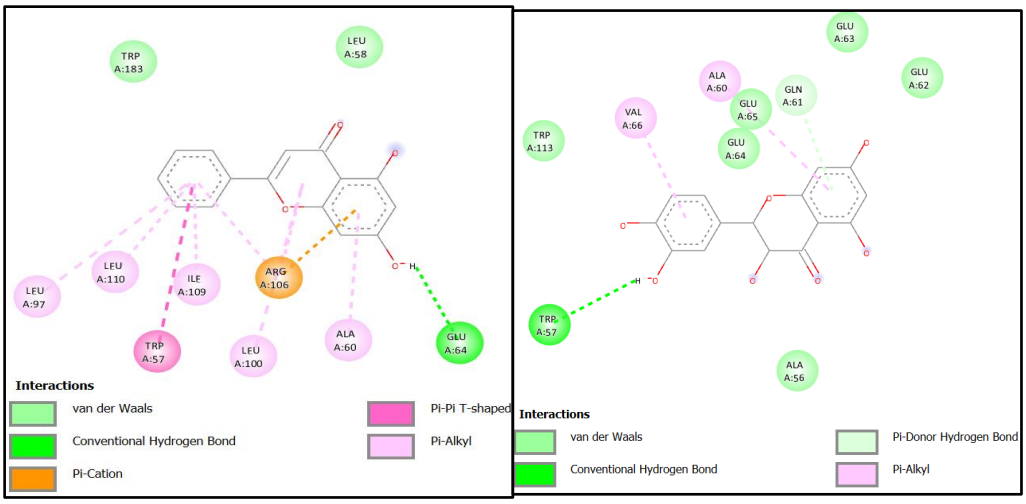
Fig. -2. 2D Interaction schematic presentation of Phytochemicals with Nef Protein with PDB ID: 1EFN

A. Amentoflavone

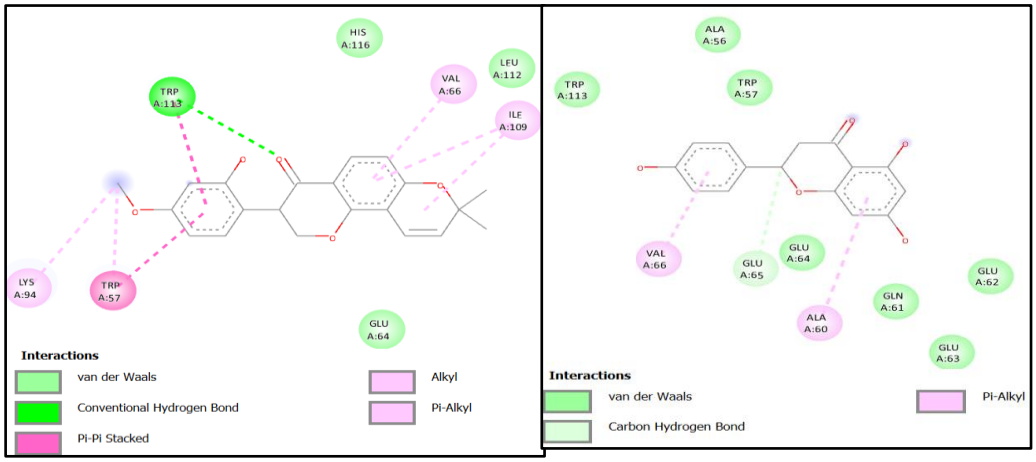


B. Aromadendrin

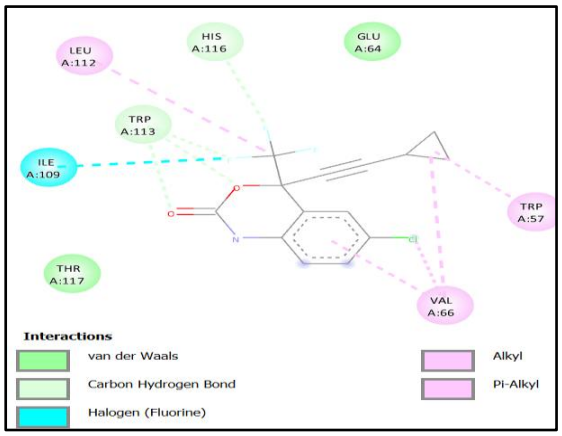




C. Chrysin **D. Glabrocoumarin**



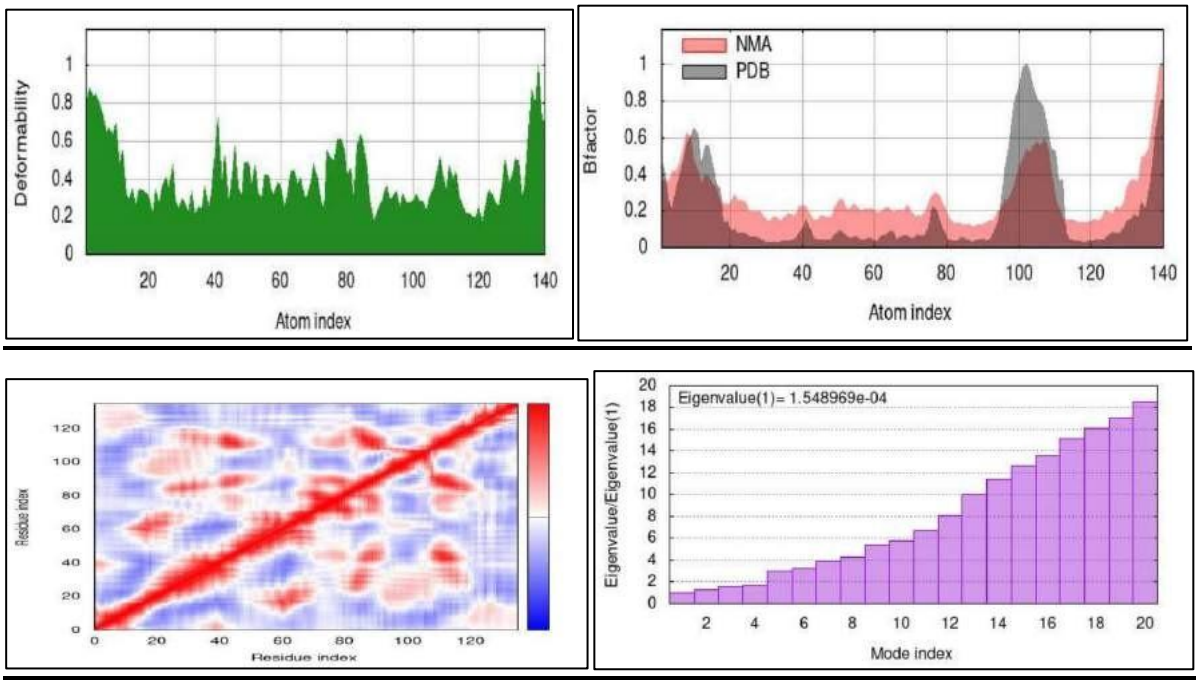
C. Glabroisoflavanone BD. Naringetol



E. Efavirenz

Fig.- 3. 2D Interaction schematic representation of Phytochemicals with Nef Protein with PDB ID: 2NEF

A. Deformability **B. B-factor**



C. Eigenvalues **D. Co-Variance**

Fig.- 4. Molecular dynamic simulation of NEF protein complex with amentoflavone. 3D Structure of interaction (a) Deformability, (b) B-factor (c) Eigenvalues and (d) Co-variance map

TABLES

Table-1: Docking Score of Phytoconstituents.

Sr. No.	Title	Binding energy [2NEF]	Binding energy [1EFN]
1	(-)-Epicatechin	-6	-8.3
2	Amentoflavone	-8.2	-10.9
3	Aromadendrin	-6.4	-8.2
4	Auranetin	-5.3	-7.1
5	Chrysin	-6.6	-7.8
6	Coriandrin	-5.3	-6.8
7	Diosmetin	-9.1	-8.3
8	Glabrocoumarin	-6.4	-9.2
9	Glabroisoflavanone A	-6.2	-8
10	Glabroisoflavanone B	-6.5	-8.2
11	Hirsutidin	-6	-6.9
12	Isorhamnetin	-8.0	-7.5
13	Linderoflavone B	-6	-7.7
14	Naringetol	-6.5	-8.4
15	Pelargonidin	-6.3	-7.7
16	Petunidin	-6.5	-8.4
17	Quercetin	-6.6	-8.7
18	Rosinidin	-6	-7.5
19	Tangeretin	-5.5	-7.2
20	Taxifolin	-6.5	-8.7
21	4-(3-Chlorophenyl) diazenyl)-5-hydroxy-3-nitro-1h-pyrazole-1-carbothioamide	-6.4	-8.2
22	Nicotiflorin	-6.5	-8.8

Table 2. Physicochemical Properties of Phytocompounds

Sr. No.	Title	Mol. Wt	log P	AlogP	HBA	HBD	TPSA	AMR
1	(-)-Epicatechin	290.27	1.55	-2.49	6	5	110.38	26.1
2	Amentoflavone	538.09	2.302	-3.769	2	6	173.98	48.46
3	Aromadendrin	288.26	1.48	-2.47	6	4	107.22	23.94
4	Auranetin	372.37	3.5	-1.1	7	0	76.36	52.52
5	Chrysin	254.24	2.87	-1.42	4	2	70.67	21.67
6	Coriandrin	230.22	2.86	0.16	4	0	52.58	30.61
7	Diosmetin	300.27	2.59	-1.94	6	3	100.13	31.55
8	Glabrocoumarin	336.34	4.06	0.17	5	2	79.9	46.23
9	Glabroisoflavanone A	338.36	3.64	-0.56	5	2	75.99	46.13
10	Glabroisoflavanone B	352.39	3.94	-0.16	5	1	64.99	50.89
11	Hirsutidin	345.33	3.52	-1.54	6	3	99.68	29.64
12	Isorhamnetin	316.27	2.29	-2.39	7	4	120.36	33.23
13	Linderoflavone B	386.36	3.22	-1.01	8	0	85.59	53.27
14	Naringetol	272.07	0.897	-1.814	1	3	86.99	22.61
15	Pelargonidin	271.25	3.2	-1.84	4	4	92.22	38.41
16	Petunidin	317.27	2.92	-2.35	6	5	121.68	20.14
17	Quercetin	302.24	1.99	-2.8	7	5	131.36	28.48
18	Rosinidin	315.3	3.51	-1.48	5	3	90.45	22.33
19	Tangeretin	372.37	3.5	-0.76	7	0	76.36	53.12
20	Taxifolin	304.25	1.19	-2.93	7	5	127.45	26.51

Note* HBD-Hydrogen bond donor, HBA-Hydrogen bond acceptor, TPSA-Total polar surface area, AMR- Molecular reflective index

Table 3.Toxicity analysis

Sr.No.	Phytochemicals	Predicted value	Mutagenicity

1	Aromadendrin	0.63	Positive
2	Amentoflavone	0.34	Negative
3	Glabrocoumarin	0.49	Negative
4	GlabroisoflavanoneB	0.49	Negative
5	Chrysin	-0.03	Negative
6	Nicotiflorin	0.02	Negative
7	Naringetol	0.34	Negative
8	Quercetin	0.67	Positive
9	Epicatechin	0.53	Positive
10	Taxifolin	0.72	Positive