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AN INSILICO APPROACH OF RESVERATROL AND ITS DERIVATIVE ANALOG 3E BINDING PATTERNS WITH SIRT1, COX, ER, AND NF-κB PROTEINS.

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Abstract: Resveratrol (RV), a non-flavonoid polyphenol found in grapes, berries, peanuts and grape products such as red wine, has antioxidant, anti-cancerous, anti-inflammatory, and anti-neurodegenerative properties. These effects are due to its ability to remove reactive oxygen species, inhibition of cyclooxygenases, and activation of anti-inflammatory pathways through interactions with proteins such as SIRT1, COX, ER, and NF-κB. In the present report an insilico approach is adopted to study the interactions between RV and its analog 3E with the above proteins through a flexible molecular docking tool using molecular virtual docker to hypothesize an efficient possible pathway. The results have revealed analog 3E as an efficient molecule with better docking scores to interact with proteins than RV.

Index Terms **– Resveratrol, 3E analog, molecular docking, SIRT1, COX, ER, and NF-KB.**

I. INTRODUCTION

Resveratrol (RV), a non-flavonoid polyphenol found in grapes, berries, peanuts and grape products such as red wine, has antioxidant anti-cancer properties (Kris-Etherton et al, 2002) anti-inflammatory, and anti-neurodegenerative properties (Piotrowska et al, 2012). These effects are due to its ability to remove reactive oxygen species (ROS), inhibit Cyclooxygenases (COX), and activate anti-inflammatory pathways through SIRT1 activation (Miceli et al., 2014; Malaguarnera, 2019).

The various biological effects of RV are due to the diversity of its molecular targets such as cyclooxygenases/lipoxygenase, kinases, sirtuins, cytokines, transcription factors, adenyl cyclase, DNA polymerases, aromatases etc., it is hypothesised that RV provides a complex physiological action because of its capability to mediate different pathways. Specifically, RV reduces oxidative stress and inflammation, improves Ca^{2+} handling, inhibits pathological signalling, decreases apoptosis and modifies autophagy either by intracellular and epigenetic influences (Riba et al, 2017) by direct or indirect molecular interactions.

Sirtuins (SIRT1): Sirtuins, control various metabolic and stress response pathway by SIRT1 activation which intern interrupt the TLR4/NF-κB/STAT axis, reduces cytokine production by inactivated immune cells, and inhibits proinflammatory factors derived from macrophages/mast cells, such as platelet-activating factor and TNF-a (Capiralla et al, 2012). The RV-SIRT1 interaction changes the structure of SIRT1 and promotes binding activity with its substrates, including p65/RelA (RELA proto-oncogene) (Yeung et al, 2004), a component of the NF-κB complex that regulates leukocyte and inflammatory cytokine activation. SIRT1 activation by RV reduces the expression of inflammatory factors such as TNF-α, IL-1β, IL-6, metalloprotease (MMP)-1, MMP-3, and NF-κB mediated Cox-2, which inhibits acetylation of

RelA. (Malaguarnera, 2019). AMP activated protein kinase (AMPK) is another RV target because it regulates SIRT1 activity by regulating cellular NAD+ levels, acting as an energy sensor (Price et al., 2012). Protein kinase A is activated by cyclic adenosine monophosphate (cAMP), resulting in the phosphorylation and activation of SIRT1 (Wan et al., 2016). SIRT1 activation catalyses the deacetylation and activation of PGC-1a, promoting beneficial effects in the metabolism (Ren et al, 2019).

Cyclooxygenases (COX): COX an catalases involved in the synthesis of prostaglandins, The inhibition of cyclooxygenases (COXs) was initially attributed to RV cancer chemo preventive activity (Jang et al, 1997). As an outcome, it is not surprising that RV has anti-inflammatory properties and has also been proposed to have an analgesic effect (Bertelli et al, 2008). Despite strong evidence that RV can directly inhibit COX activity, there is also a large number of documented evidence that RV reduces COX activity *in -vivo* via transcriptional means. RV has significantly reduced COX-2 transcription by downregulating the Akt, MAPK, and NF-κB pathways simultaneously (Das et al, 2007; Kundu et al, 2006). Regardless of the mechanism, COX activity appears to be a clear mode of action through which RV reduces inflammation and tumorigenesis. Furthermore, RV has been shown to inhibit lipoxygenase (LOX) activity (Pinto et al, 1999, MacCarrone et al, 1999) which is involved in leukotriene synthesis, resulting in the production of other inflammatory and carcinogenic signals (Aggarwal et al, 2006).

Estrogen receptors (ER): ER appears to exert powerful transcriptional effects via RV by a biphasic effect on cell proliferation, stimulating growth at low concentrations while inhibiting growth at high concentrations (Bowers et al, 2000 & Mense et al, 2008). Interestingly, RV inhibited cell proliferation in ER-negative cancer cell lines, this demonstrates how RV exerts its biological effects through a variety of mechanisms. While RV's Phyto-estrogenic action may support a chemoprotective role in breast cancer, there is also evidence of potential adverse effects (Mense et al, 2008) which are likely to depend on the distinct patterns of ER and its expressions in different cell types. RV binding acted as an ER agonist, increasing transcription of the estrogen-responsive reporter gene (Gehm et al, 1997). On the other hand, the magnitude of the effect was cell type dependent. Thus, in the MCF-7, human breast cancer cell line, RV produced a more excellent maximal transcriptional response than estradiol, whereas it was weaker in the BG-1 (human ovarian carcinoma cell line) (Gehm et al, 1997).

Nuclear factor-κB (NF-κB): Evidence suggests that RV may interfere with transcription factors nuclear factor-kappaB (NF-kB) family. RelA (p65), NF-κB1 (p50 and p105), NF-κB2 (p52 and p100), c-Rel, and RelB (Tergaonkar et al, 2005) are members of this family. These factors are generally available in the cytoplasm by interacting with IB, which prevents NF-κB and nuclear translocation (Tergaonkar et al, 2005). By promoting IB degradation, NF-κB can migrate into the nuclear compartment, where these transcription factors act as heterodimers, binding the DNA promoters of many inflammatory and immune response genes (Tergaonkar et al, 2005). RV has been widely reported to inhibit NF-κB activity. The initial hypothesis proposed that RV could either reduce NF-κB nuclear presence (Tsai et al, 1999) or inhibit transcriptional activity (Pendurthi et al, 1999). However, the mechanism underlying such actions is largely unknown.

There is no evidence that RV has a direct effect on NF-κB. Most hypotheses to date have converged on the possibility that RV reduces the ability of several pro-inflammatory stimuli, such as tumour necrosis factor (TNF-α) lipopolysaccharide (LPS) or H_2O_2 to induce IB phosphorylation and degradation (Manna et al, 2000), thereby impeding NF-κB translocation, Through this it has been proposed that RV may suppress IB kinase (IKK) activity (Ashikawa et al, 2002, Ren et al, 2013), thereby preventing IB degradation and resulting in NF-κB nuclear translocation. SIRT1 physically interacts with the RelA/p65 subunit of NF-κB, deacetylates it at K310, and thus inhibits its transactivation potential (Yeung et al, 2004). RV is likely to have an indirect negative impact on NF-κB via this pathway.

Therefore, the present study has focused on the insilco analysis of RV and its analog 3E direct interactions with proteins such as SIRT1, COX, ER, and NF-κB which can regulate the oxidative stress, inflammation, inhibits pathological signalling, decreases apoptosis.

II. METHODOLOGY:

The Insilco analysis of RV and its analog 3E interactions with SIRT1, COX, ER, and NFK-B proteins to find out the possible binding patterns and effectivity. Based on our previous preliminary results analog 3E was found to be efficient bioactive compound with respect to anti-inflammatory, anti-oxidant activity (Naini et al, 2017 & Ganesh et al, 2020).

2.1 Ligand and protein docking:

Molegro Virtual Docker (MVD, 2010.4.0.0), a molecular docking programme that includes highly efficient PLP (Chanda Sinha et al., 2015) and the MolDock scoring function (Yang et al., 2004), provided a flexible platform for molecular docking (Bandaru et al., 2014). The proteins' optimised crystal structures were obtained from NCBI RCB SIRT1(4i5i), COX (1cqe), ER(1a52), and NF-κB (1svc), as well as the 3D structures of the compounds RV (CID:445154) and 3E compound (Ganesh et al., 2020).

Each protein molecule was first examined for its active cavity using MVD software. Docking parameters were set to 0.20 as grid resolution, 1500 spinoffs, and 50 population size. After docking, the energy minimization and hydrogen bonds were optimized. RV and its analog 3E were docked in the active cavities of each protein using molegro and standard mol grid algorithms. Based on the scores, the binding patterns were further analysed and classified. Biova discovery studios were used to visualize the interactions of RV and 3E molecules with proteins.

The internal ES (Electrostatic Interaction), internal hydrogen bond interactions, and sp2-sp2 torsions were used to evaluate the inhibitor's binding affinity and interactions with protein (Srinivas et al, 2015).

III. RESULTS AND DISCUSSIONS:

According to the binding scores of all the proteins docked with RV and its analog 3E, analog 3E exhibited a greater binding affinity towards the proteins than RV. Table 1 shows the rerank and docking score, as well as binding inspections and hydrogen-bond interactions. The binding scores obtained from docking studies are an indicator of the formation of ligand-receptor complexes. The higher negative MolDock score indicates a more favourable binding mode with a better fit between the ligand and receptor. The binding patterns were compared to the among the molecule (Manubotula Durga Sahithya et al, 2021).

3.1 SIRT1 docking with 3E and RV

Based on the docking score, rerank score and MolDock score it is evident that the RV analog 3E is having a better fit with active cavity of SIRT1. The h-bond interactions values for -7.05 with that of RV showing -7.35 with a slight difference of -0.3 indicating similar form of interactions with RV. SIRT1was found to be activated by RV and reduces the expression of inflammatory factors and NF-κB mediated COX, from the above binding patterns it could be further assumed that analog 3E can be a better fitter with SIRT1 irrespective of the steric forms.

Table 1: MolDock Algorithm Aided Docking of Compounds in SIRT1, COX, ER, and NFK-B proteins with RV and its analog 3E.

Protein	Compound	STERIC POSE	MolDock Score	Rerank Score	Docking Score	HBOND
SIRT1	3E	[03] 3E	-104.216	-79.5997	-102.475	-7.0528
	RV	[01] 445154	-96.3881	-76.7427	-99.8284	-7.3539
COX1	3E	[00] 3E	-129.329	-107.513	-130.03	-5.1594
	RV	[01] 445154	-114.488	-98.1161	-115.384	-5.0000
ER	3E	[02] 3E	-95.7999	-80.5189	-100.13	-3.9767
	RV	[00] 445154	-88.7887	-74.6063	-95.3507	-7.3116
NFKB	3E	[00] 3E	-109.187	-77.7201	9880.98	-1.6450
	RV	[00] 445154	-109.091	-90.3524	9883.34	-9.6978

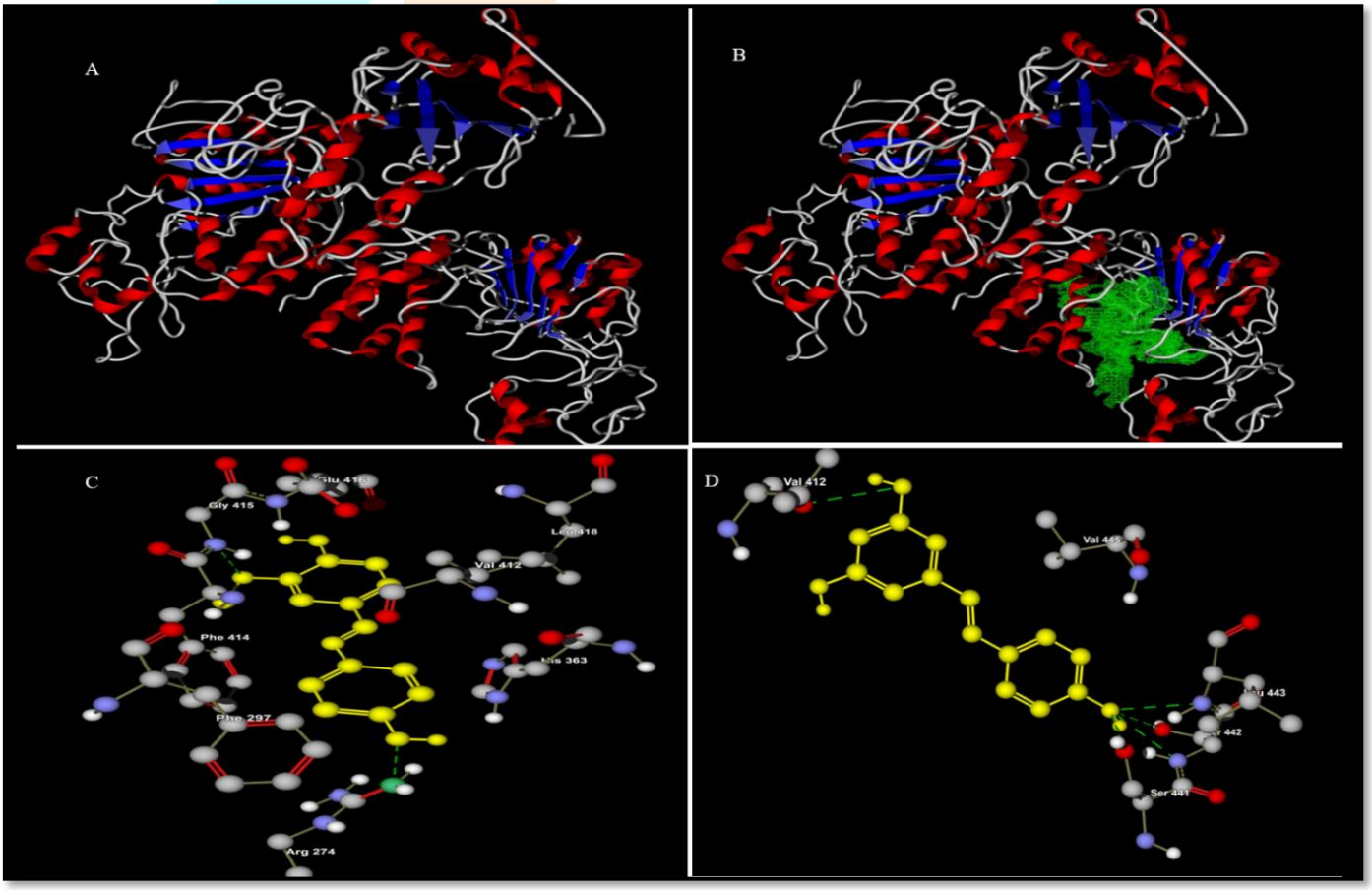

Fig 1: A) 3D back bone view of the SIRT1 protein, B) active cavity of the SIRT1 protein (Green mesh), C) interaction of 3E with SIRT1 with two hydrogen bonds at Arg274 and Gly415 residues, D) interaction of RV with SIRT1 with five hydrogen bonds at Val412, Leu443, Ser442, and two bonds with ser441residues indicated with green doted lines.

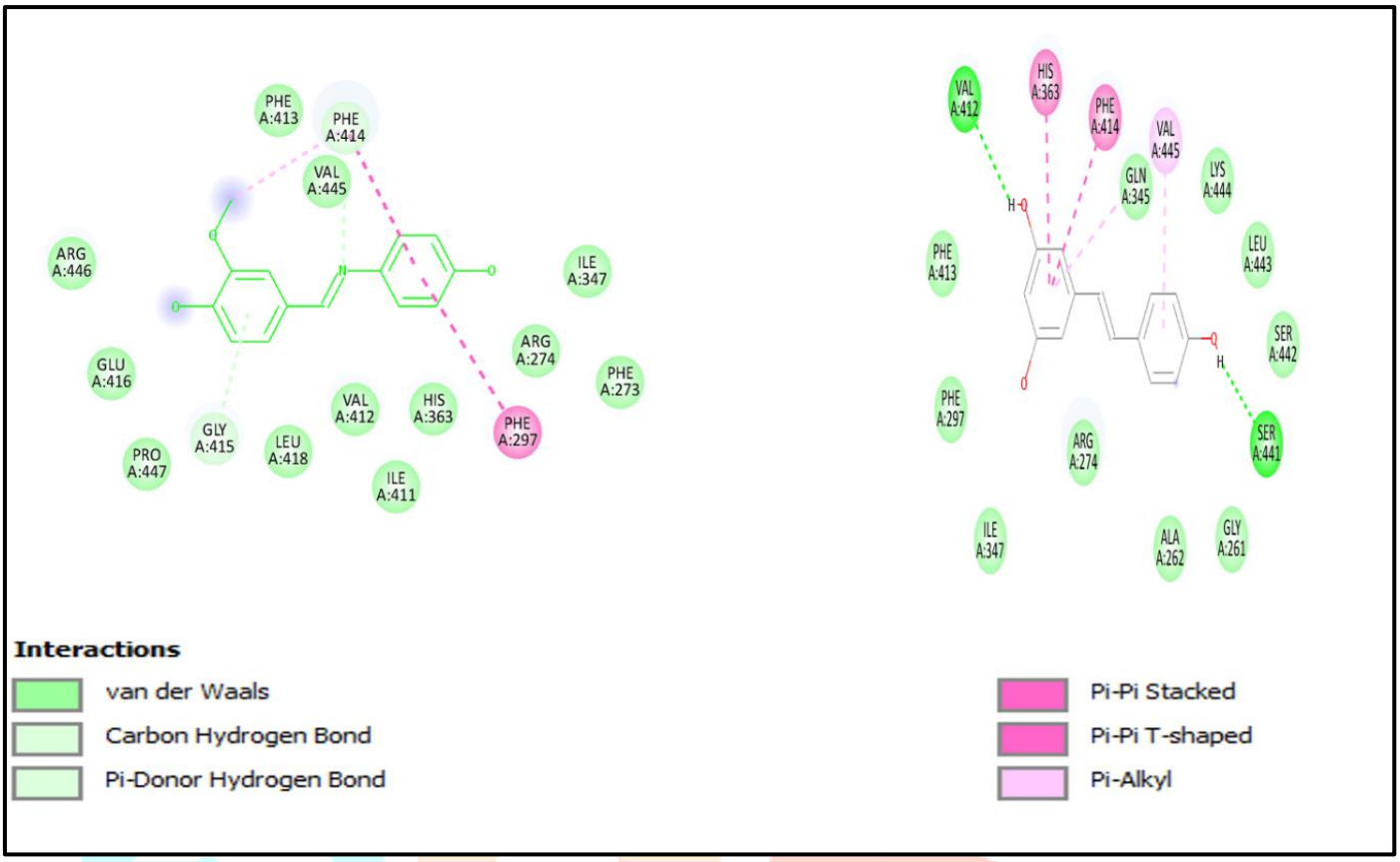

Fig 2: A 2D view of the interactions of 3E and RV with SIRT1, residues circled in green participate in van der wall force interaction, residues circled in light green participate in H-bond interactions with green dotted lines and pink residues indicate participate in PI-PI interactions.

3.2 COX 1 docking with 3E and RV

A similar form of higher docking score, rerank score and MolDock score was observed with the COX1 protein when docked with RV and 3E analog. 3E molecule showed a higher h-bond interaction valued for -5.15 when compared with RV having -5.00. COX despite having evidences that it can regulate the expression of Akt, MAPK, and NF-B pathways transcriptionally there is no appropriate mechanism disposed, from the docking patterns it was assumed that analog 3E can perform the better activity than RV.

3.3 ER docking with 3E and RV

RV being the agonist with ER which transcriptionally increases the ER mediated genes based on the cell line. The relative binding patterns when docked with ER the analog 3E showed a better interactive score when compared with RV, evident that 3E could be a better agonist to ER but an experimental support is needed for the assumption in terms of dosage.

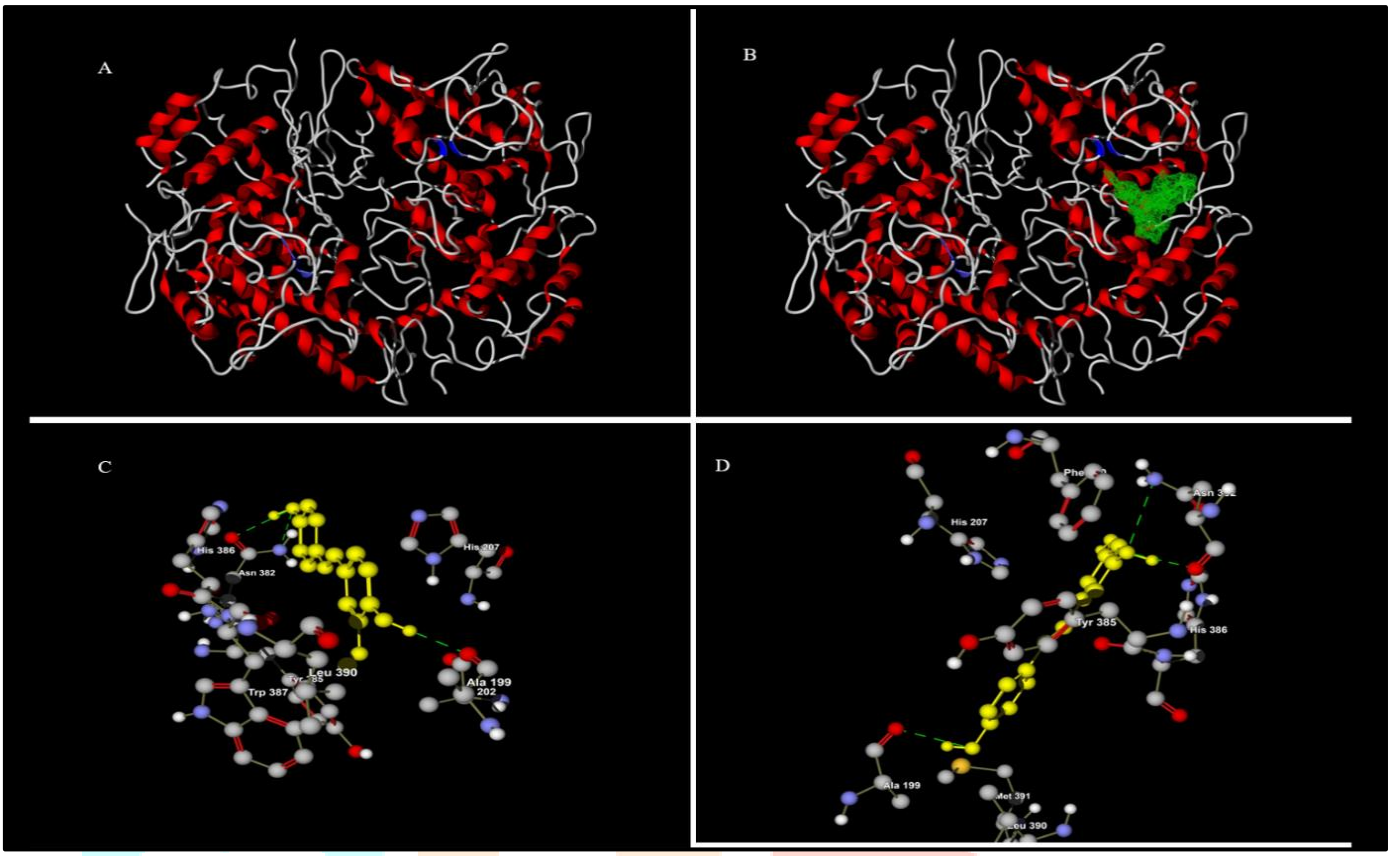

Fig 3: A) 3D back bone view of the COX1 protein, B) active cavity of the COX1 protein (Green mesh), C) interaction of 3E with COX1 with three hydrogen bonds at Ala199 and two bonds with Asn382 residues, D) interaction of RV with COX1 with three hydrogen bonds, two at Asn382, and Ala199 residues indicated with green doted lines.

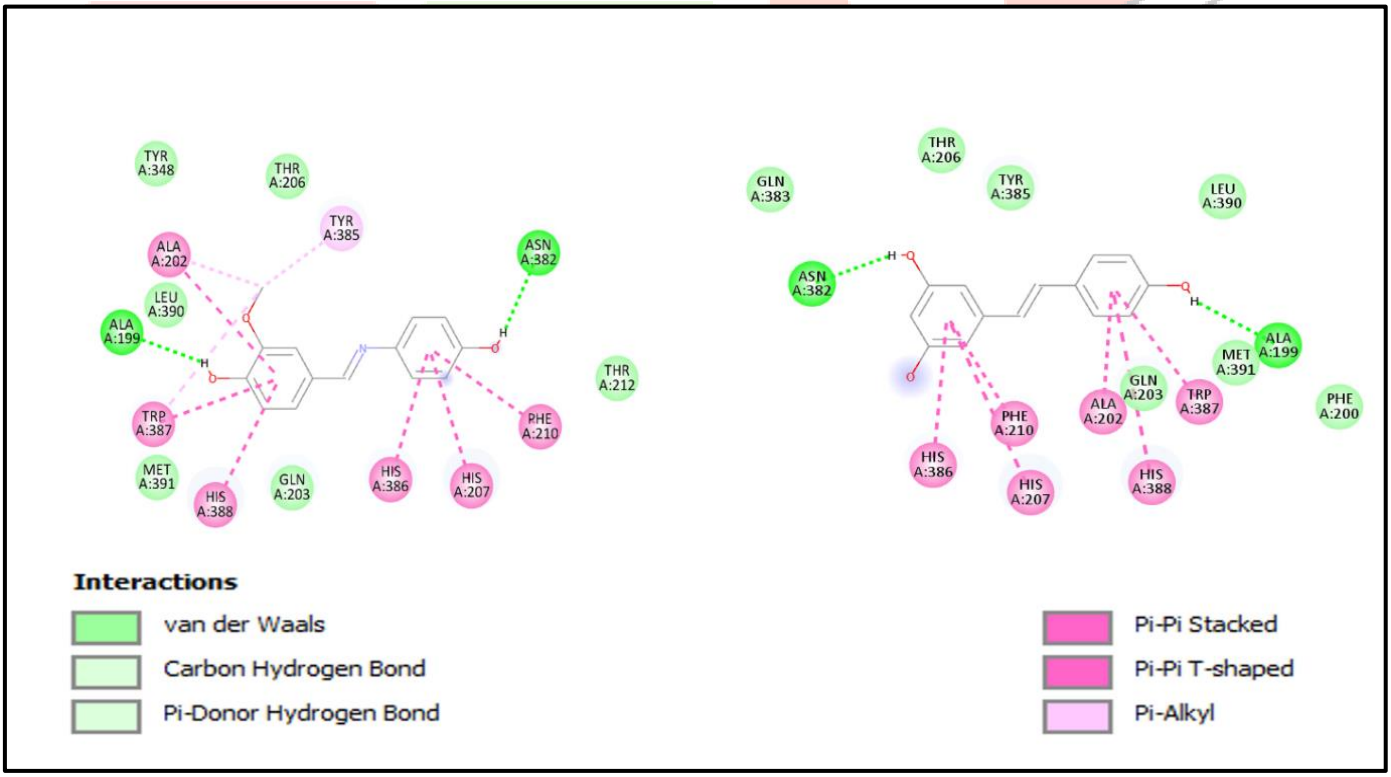

Fig 4: A 2D view of the interactions of 3E and RV with COX, residues circled in green participate in van der wall force interaction, residues circled in light green participate in H bond interactions with green dotted lines and pink residues indicate participate in PI-PI interactions.

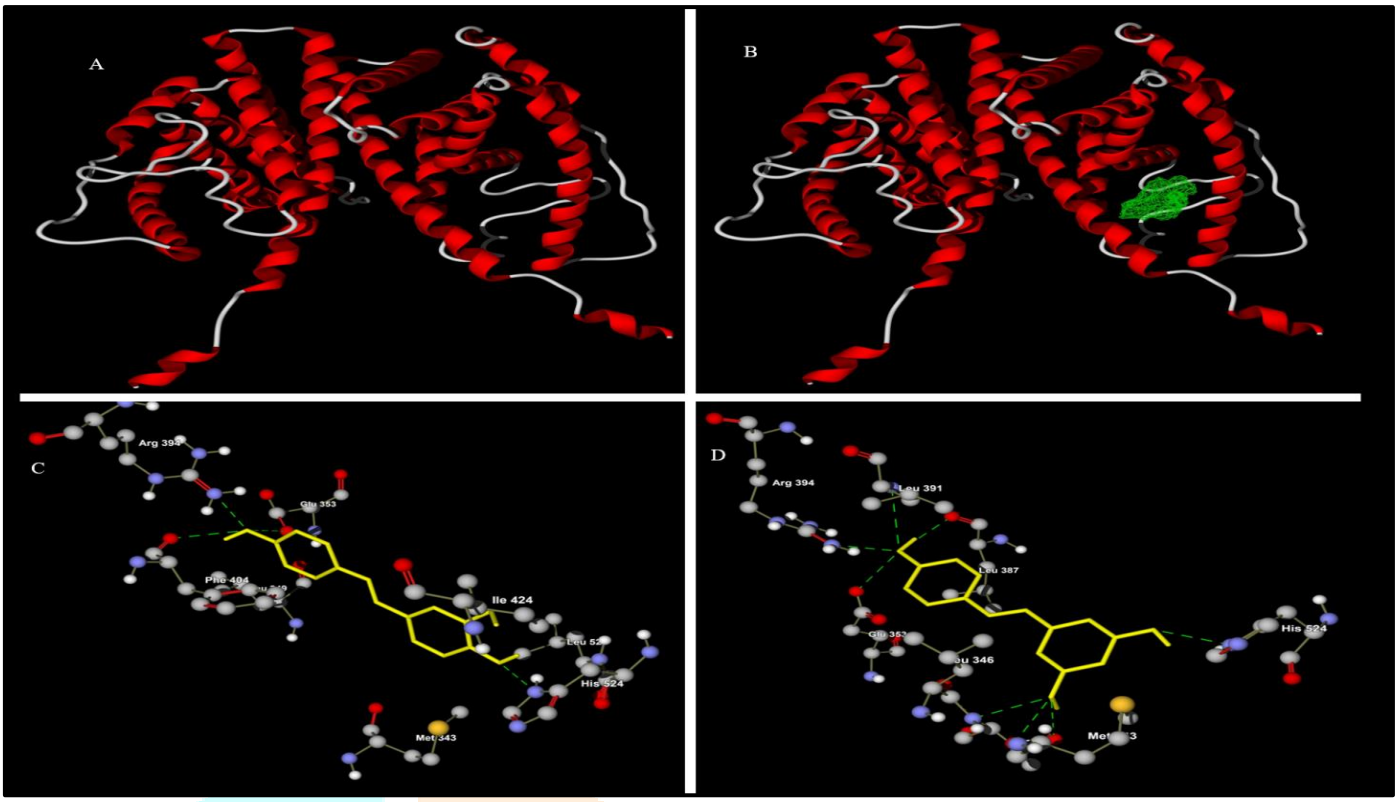

Fig 5: A) 3D back bone view of the ER ALPHA protein, B) active cavity of the SIRT1 protein (Green mesh), C) interaction of 3E with ER ALPHA with three hydrogen bonds at His524, Phe404, Arg394 and Glu353 residues, D) interaction of RV with ER ALPHA with eight hydrogen bonds at His524, Met343, two bons with Thr347, Glu353, Leu391, Arg394, and Leu391 indicated with green doted lines.

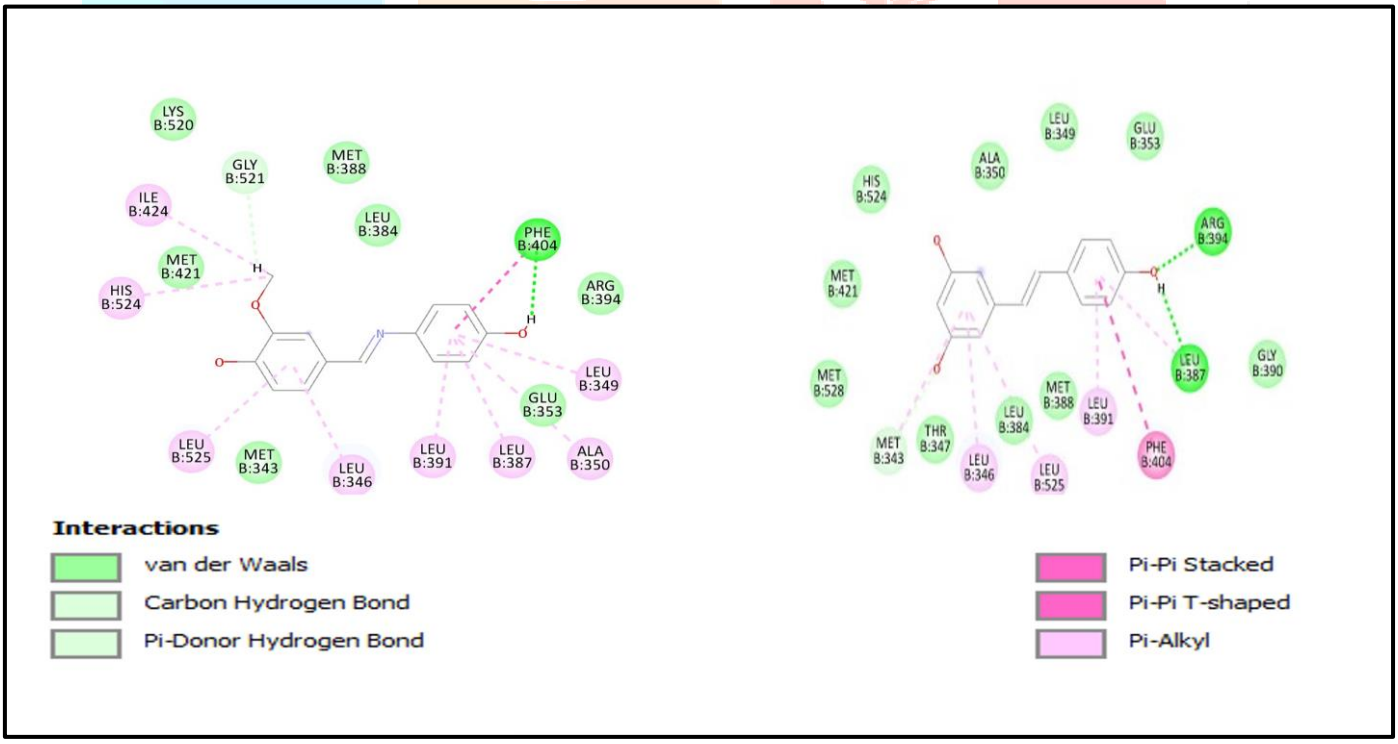

Fig 6: A 2D view of the interactions of 3E and RV with ER ALPHA, residues circled in green participate in van der wall force interaction, residues circled in light green participate in Hbond interactions with green dotted lines and pink residues indicate participate in PI-PI interactions.

3.4 NF-κB docking with 3E and RV

Based on the evidences RV could interactive with transcription factor NF-κB family promoting the transcription of inflammatory cytokines in the cells. RV and 3E can inhibit the activity of NF-κB

by binding, and further reduces the steric interaction of NF-κB with DNA, the 3E molecule could be assumed to be having the better interactive face with NF-κB when compared with RV.

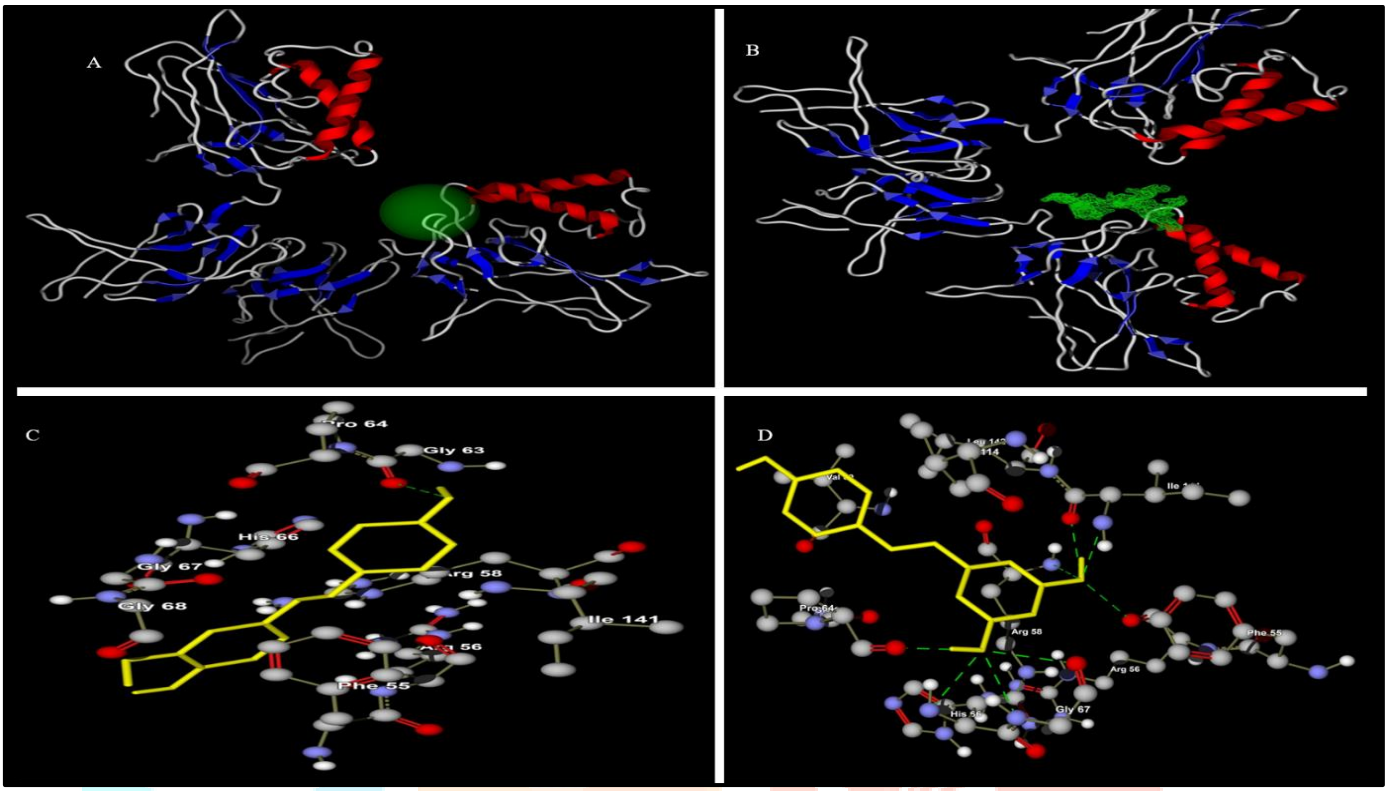

Fig 7: A) 3D back bone view of the NF-κB protein, B) active cavity of the NF-κB protein (Green mesh), C) interaction of 3E with NF-Kb one h bond with Gly63, D) interaction of RV with with seven hydrogen bonds at Pro46, two bonds with Gly67, His66 Arg56, and two with Ile141 residues indicated with green doted lines.

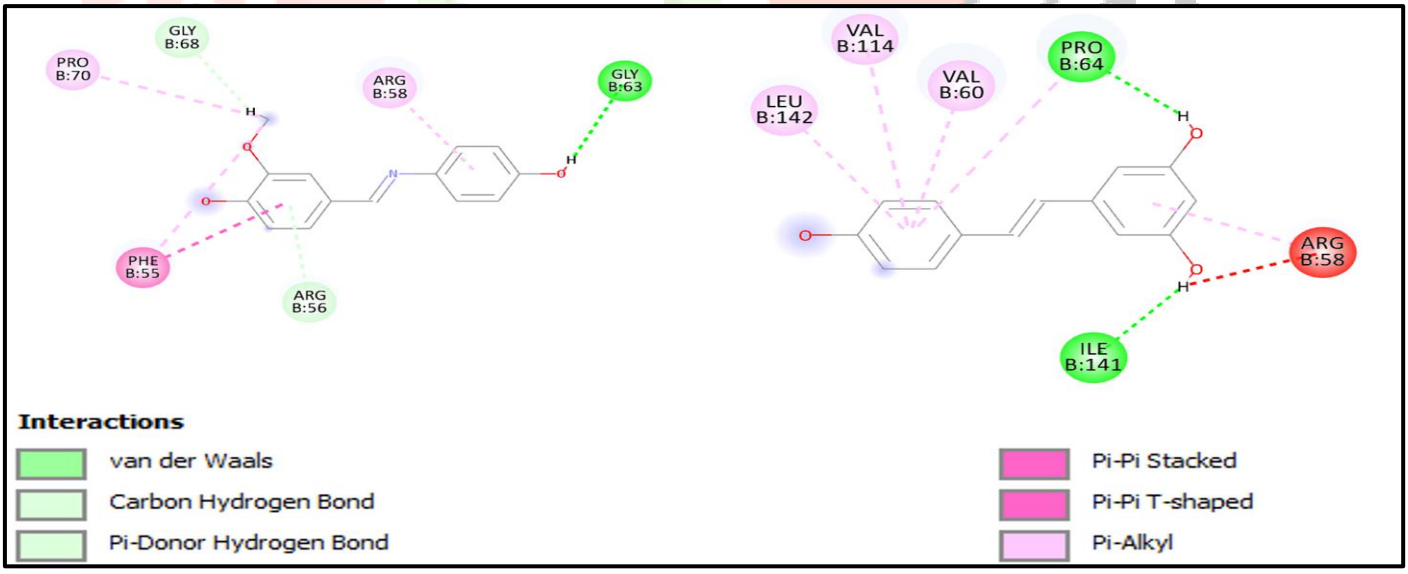

Fig 8: A 2D view of the interactions of 3E and RV with NF-Kb, residues circled in green participate in van der wall force interaction, residues circled in light green participate in Hbond interactions with green dotted lines and pink residues indicate participate in PI-PI interactions.

Among all the interactive proteins the binding scores suggest that the analog 3E when compared with RV, COX has the higher negative scores and has the higher binding affinity suggesting that the 3E could regulate the expression effectively by inhibiting transcriptional regulation of

inflammatory cytokines by COX mediated pathway.

IV. Conclusion:

The findings from insilco molecular docking demonstrated that analog 3E with efficient scores interaction and H-bonds, but within the proteins COX has a higher score, H-bond value and superior binding affinity indicating the possible physiological pathway. Hence the analog 3E could be considered ahead with the physical interactions of the relevant proteins and could perform its bioactivity in reducing proliferation, inflammation and tumorigenesis, further this can be supported by the experimental evidences.

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