Article

Study of natural naphthoquinone derivatives anticancer potential towards chemo-resistance related Never in mitosis gene A-related kinase 2 -Insilico Approach

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Abstract:

Never in mitosis gene A-related kinase 2 (NEK2) a member of serine-threonine kinase protein mainly involved in cell cycle process. Clinical studies revealed NEK2 overexpression in various tumour types, also NEK2 was reported for their association with genetic abnormalities like mitotic machinery deregulation and chromosomal instability. Besides NEK2 plays a key role in maintaining the transformed phenotype of cancer cells and chemo-resistance of several tumour types. Thus, NEK2 transcriptional profile is important for diagnosis, treatment, and prognosis stages of cancer studies. Screening of novel NEK2 inhibitor would be beneficial in developing the specific lead molecules. Our studies involved NEK2 transcriptional profile search, screening of druggable cavities in NEK2, Drug likeliness of mangrove derived naphthoquinone derivatives avicennoneA , avicennoneB , avicennoneC , avicennoneD , avicenoneE , avicennone F , and avicennone G , avicequinone A, stenocarpoquinone B , avicequinone C , avicenol A , avicenol C,brugine, apigenin, chrysin and molecular docking studies to assess MNC compounds binding efficacy towards NEK2. Mangrove derived compounds conferred the intermolecular hydrogen bond, Pi-alkyl,pi-cation interactions with NEK2 kinase domain region residues Tyr 19, Lys 37, Arg 164, Lys174. Nearly 200 kinase proteins contained this promising Cys 22 residue as its positioned in the catalytic site like NEK family proteins. Avicenol A, Avicennone G, Chrysin and Brugine formed the irreversible covalent binding with NEK2 through Cys 22, thus they can be considered as potential kinase inhibitors with limited off-target response. But these MNC compounds need to be tested further in invitro and invivo studies to propose as potent NEK2 inhibitors.

Keywords: Kinase Inhibitor;NEK2;Chemoresistance; *Avicennia marina*;Marine compounds; Molecular docking;Cancer

1. Introduction

Cancer is characterized by uncontrolled cell proliferation due to the aberrant activity of various proteins. Gynaecological cancers are among the most common women's cancers and are therefore a major public health concern. Because of the lack of awareness of cancer, variable pathology and lack of adequate screening facilities in developing countries like India, most women report in advanced stages that adversely affect prognosis and clinical outcomes[1].Cell cycle-related proteins were thought to be important in several functions, such as proliferation, invasion and drug resistance in human malignancies. Never in mitosis gene A-related kinase 2 (NEK2), a member of the cell cycle-related kinase (CCRK) family of proteins. It is categorized as serine/threonine protein kinase consists of 445 amino acids, molecular weight of 48 kD. It has an amino terminus in the kinase domain and a carboxy terminus in the uncatalyzed regulatory region [2]. NEK2, located in the centrosome, is an important centrosome protein kinase. The expression level of NEK2 is cyclically dependent. Specifically, the expression level of NEK2 is very low in G1 phase but increases rapidly by 3-4 times in G1/S phase and remains high in S and G2 phase. However, NEK2 expression immediately decreases once enter into M phase. The expression and activity

of NEK2 are highly conserved throughout the cell cycle progression. During this process, NEK2 remains inactive by dephosphorylation of protein phosphatase (PP1). NEK2 is activated by autophosphorylation until PP1 is inhibited by inhibitor-2 or phosphorylated by NEK2[2,3].Since NEK2 is involved in chromatin condensation and centrosome separation, it can affect the stability of the entire genome by regulating centrosomes and chromosomes. This can eventually promote malignant transformation of cells [4].An increasing number of studies have reported that expression of NEK2 was increased in cancer and that up-regulation of NEK2 was associated with tumor progression and poor prognosis in various types of cancer, including breast cancer[5,6]. In deng et al studies on chemoresistance to sorafenib among the HCC patients reported the role of Nek2/β-catenin complex, thus targeting NEK2 as drug target and proposing specific inhibitor would beneficial to the improve the sorafenib resistance in HCC patients [7]. Another study on revealed that over expression of NEK2 was related to cisplatin drug-resistant in ovarian cancer cells [8]. Both in vivo and invitro studies revealed that NEK2 plays a significant role in proliferation as well as cisplatin resistance. Case studies reported that NEK2 induced bortezomib based drug resistance developed mainly via activation of efflux drug pumps, meanwhile another study indicated that high NEK2 promoted the paclitaxel and doxorubicin based drug resistance through inhibiting cell apoptosis in breast cancer cell [9].Recent research strongly indicates the advantages of NEK2-targeted therapy for cancer[10]. Grove (mangal) communities represent coastal surroundings situated in tropical and semitropical seashore water zones, occurring in 112 countries and represent a potential reservoir of untapped and unique NPs with a wide range of potential industrial applications. A large number of bioactive compounds of pharmaceutical importance have also been reported from the mangrove ecosystems[11].*Verbenaceae* family sps *Avicennia marina* is an evergreen mangrove tree rich in of phytochemicals such as glycosides, tannins, terpenoids, phenolics, saponins, amino acids, sterol, alkaloids, phenolics and flavonoids [12]. Napthoquinone derivatives of *A.marina* sps namely avicennone A , avicennone B , avicennone C , avicennone D , avicenone E , avicennone F , and avicennone G , along with the known compounds avicequinone A, stenocarpoquinone B , avicequinone C , avicenol A , and avicenol C were included for our study. Studies on Polyphenol-rich *Avicennia marina* leaf extracts revealed that nearly fourteen distinct flavonoids and 7 phenolic acids. In our studies we also analysed the apigenin, chrysin were assessed for their affinity toward NEK2 [13]. In pacific region *Bruguiera gymnorrhiza* is one of the important species with broad-leaf pattern. Bioactive compounds brugine isolated from two sps of *Bruguiera namely B.Gymnorrhiza and B.Sexangula* [14,15]. Apigenin is a natural flavone isolated from mangrove plants K. candel and R. apiculata indicated the prospects of these mangroves eco system derived bioactive compounds in development of novel therapeutic agents based this compound [16].Binding affinity of MNC compounds were assessed to to screen the potential to design NEK2 specific inhibitor.

2. Results and Discusssion

NIMA-related kinases (NEK-family), were the group of serine/threonine kinases proteins comprised of eleven members and all the members shared the conserved catalytic domain as well sequence similarity. Among the group members NEKs 1, 2, 4, 6, 8, 10 and 11 were related to genomic instability. NEK 2,3,5,6,8,11 protein altered expression were studied in various cancer types. In our studies we explored the NEK2 aberrant expression in different cancers. Aberrations in the NEK2 transcriptional profile data were retrieved from TCGA and listed as **Supplementary file 1**. Impairment in signal transduction process plays a key role in molecular basis of diseases. Thus, data about NEK2 in signal transduction and their connection to disease pave the way for identifying novel drug targets. Predictions related to sequence properties, protein families, structural folds, biochemical aspects, similarity to other proteins and associated pathways of known targets were base for druggable protein screening. Web resource Human protein atlas (HPA) catalogues the maps of all the proteins in cells, tissues, and organs of *homo sapiens* using unified omics technologies namely mass spectrometry specifically proteomics, transcriptomics, and systems biology. Drug target potential of NEK2 was verified with HPA and the potential interactors were assessed using reactome database. Upstream and downstream regulators of NEK2 were explored to propose the NEK2 as promising therapeutic target.In general, mitotic inhibitors efficacy have many constraints like little rate of cell duplication in many solid tumours.So far numerous selective inhibitors were developed for CDK1, PLK1,

AURKA and AURKB, but were reported with limited anti-tumour activity. Designing the Novel mitotic kinases inhibitors have varied role in cell cycle pathways like tacking the NEK2 induced chemotherapeutic resistance. Objective of our study was identification novel marine natural compounds for developing lead compounds for NEK2 inhibition.

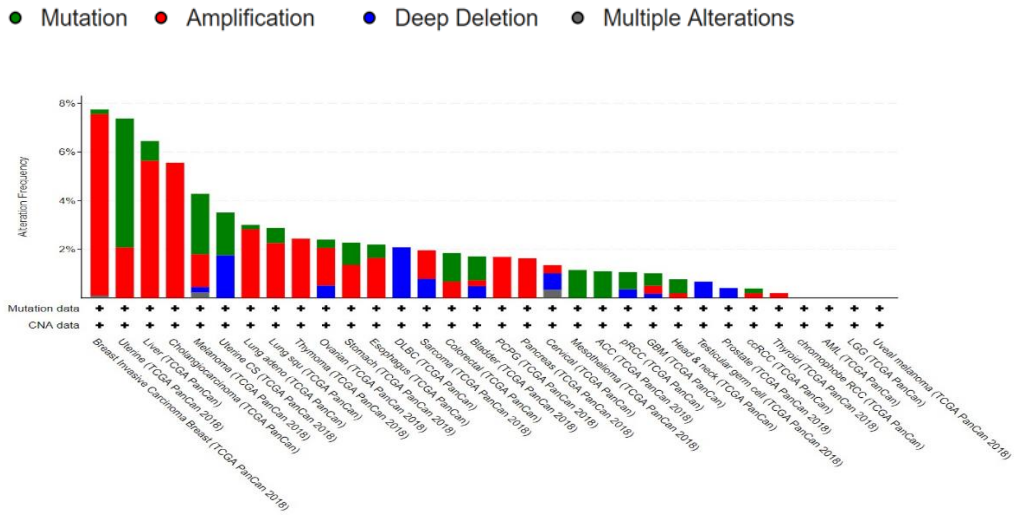


Figure 1: The above graph describes the distribution of Nek2 in various cancer TCGA database

NEK2 Interactome construction and functional enrichment analysis

Reactome based NEK2 interactome revealed the protein-protein interaction (PPI) with 104 nodes (Proteins) and 103 edges (interaction) (**Figure2 a**). NEK2 Interactome was listed in **supplementary file 1**. Pathways associated with 104 proteins were enriched with cell cycle and other major pathways related cancer induction listed **Table 1**. NEK2 interaction with CDK4 analysed in cancer gene net, this resource catalogues the two kinds of interaction layers by connecting proteins whose function were affected by cancer gene products to proteins that influence on cancer phenotypes. NEK2 role in metastasis revealed the sequence of protein activation and inhibition. (NEK2--| PPP1CC--| AXIN1--> CSNK1D--| CTNNB1--> METASTASIS). PPP1CA/B/C, the catalytic subunits of phosphatase 1(PpI), Pb1 and β-catenin were the downstream targets of NEK2.It used the Pp1 as a substrate and phosphorylation of Pp1on two c-terminal sites (Thr307,Thr318) reduces its phosphatase activity. PP1 regulates Wnt signalling by regulated dephosphorylation of Axin. Binding of axin and casein kinase (CSKND1) induces beta-catenin (CTNNB1) phosphorylation and thereby induces metastasis.NEK2/β-catenin interaction reported in many clinical studies , Shen H et al reported that NEK2 mediated carcinogenic role of NEK2 in gastric cancer[31].NEK2 dependent phosphorylation of NEK11 at the c-terminal non-catalytic region assist the elevated NEK11 kinase activity. NEK2 activation involves two main pathways namely Hippo and epidermal growth factor (EGF) signalling pathway.NEK2 physically binds to and phosphorylates both MAD2L1 and CDC20 and thereby regulates kinetochore microtubule attachment stability and. Overexpression of NEK2 enhances the ability of MAD2 to induce a delay in mitosis, in which kinase. In contrast, elimination of NEK2 by siRNA decreases the assembly of MAD2L1 to kinetochores and causes aberrant premature chromosome segregation. Thus, aberrant expression of NEK2 may promote aneuploidy by disrupting the mitotic checkpoint signalling, leading to malignant transformation [32]. Regulation of NDC80 by nek2 and Aurora B is critical for its mitotic function and cell survival. Overexpression of NDC80 was found to induce tumor formation in a mouse model by activating mitotic checkpoint. Moreover, overexpression of NDC80 was determined to be associated with poor clinical prognosis in breast cancer and other cancers [33]. Among the cancer cells, frequently it was observed that centrosome morphological features were deregulated. Centrosomal proteins α-tubulin isoform TUBA1A, TUBA4A, β-tubulin isoform TUBB, TUBB4, γ-tubulin and its isoforms namely TUBG1,

TUBG2, TUBGCP2, TUBGCP3, TUBGCP4, TUBGCP5 and TUBGCP6 interacts with NEK2 and endup in abberant expression.

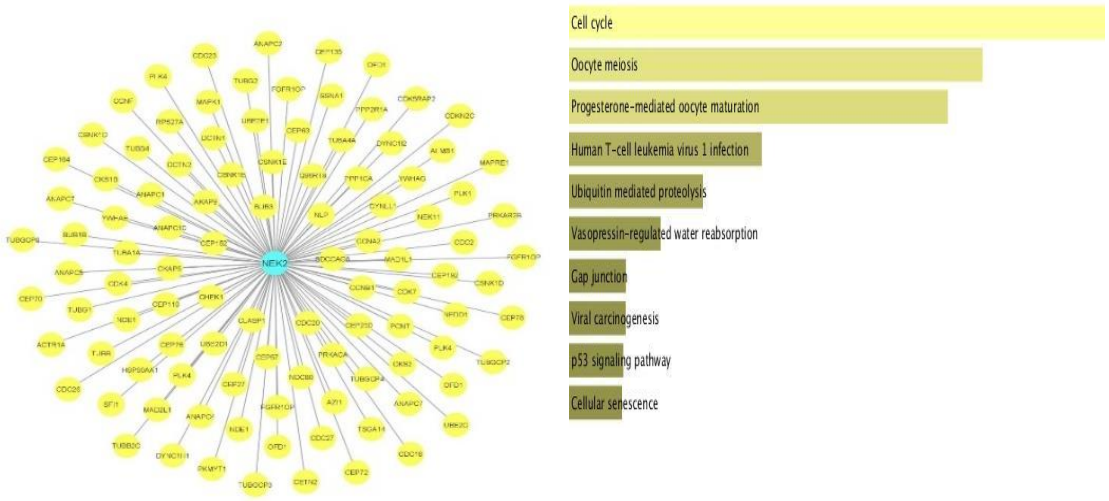


Figure 2 a) Reactome database generated NEK2 interactome b) Pathway based functional enrichment terms associated with NEK2

Functional enrichment assessment of NEK2 based Interactome:

Enrichment analysis is a computational method for inferring knowledge about an NEK2 by Bcomparing it to annotated gene sets representing prior biological knowledge (**Figure 2b**). Enrichr assessment revealed four scores to report enrichment results: p-value, q-value, rank (Z-score), and combined score for the NEK2 (**Table 1**).ClueGo was used to decipher functionally grouped gene ontology and pathway annotation networks of NEK2 and associated proteins. The node pie charts represented the biological process,molecular function and cellular component of NEK2.Most significant molecular functions mainly populated with cyclin protein serine /threonine kinase activity, gamma tubulin binding, ubiquitin enzymatic activity, telemerase activity. Biological processes of NEK2 and associated protein mainly involved in anaphase promoting complex dependent catabolic process and cell cycle phase regulation, microtubule cytoskeleton organization, polymerization (**Figure 3**)

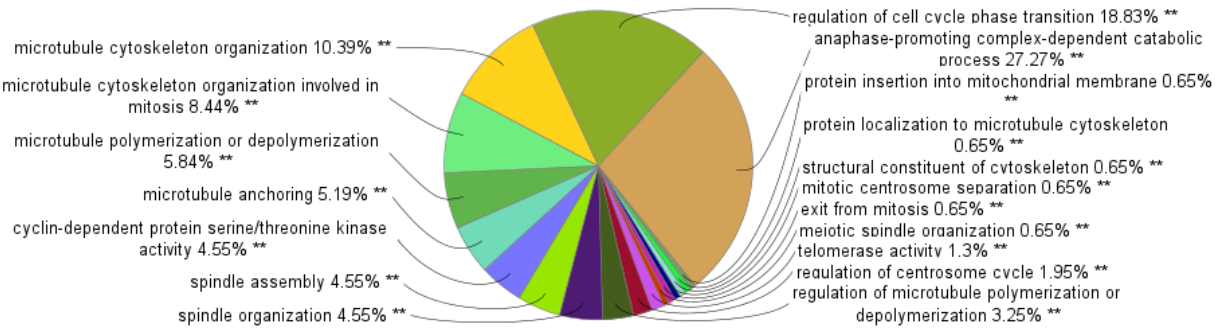


Figure 3: Functional annotation of the NEK2 associated GO terms .Listed significant GO terms at the $P < 0.01$ statistical level were indicated by double (**) asterisks and at the $P < 0.05$ level by a single (*) asterisk.

Table 1:NEK2 Interactome associated pathway and enrichment scores

Name	P-value	Adjusted p-value	Odds Ratio	Combined score
APC/C-CDC20 Complex	4.44E-36	7.63E-33	111.8	9100.69
Centriole Duplication and Separation	7.83E-29	6.74E-26	94.15	6092.78
APC/C-FZR1 Complex	8.95E-25	5.13E-22	109.75	6077.26
Spindle Assembly	8.60E-13	2.96E-10	84.95	2360.12
FOXM1 Signaling in Prostate Cancer	1.48E-09	3.64E-07	88.26	1794.44
Cell Cycle Overiew	5.04E-21	2.17E-18	30.85	1441.82
Metaphase/Anaphase Phase Transition	7.29E-06	0.0007841	72.82	861.33
HPV E4 Contribution to Life Cycle of Virus and Cell	7.29E-06	0.000738	72.82	861.33
Kinetochore Assembly	1.27E-09	3.63E-07	33.98	696.15
G1/S Phase Transition	1.93E-08	0.000004144	34.27	608.74

Drug likeness calculations

Assessment of absorption, distribution, metabolism, and excretion (ADME) is a crucial part of drug development.Here we used two servers namely SwissADME and Dicovery studio ADMET screening(**Figure 4 and Figure 5**). SwissADME is a web server for the prediction of small molecules physicochemical properties such as, but not limited to, pharmacokinetics properties and drug-likeness. It employed several predictive models and used a consensus approach for lipophilicity prediction. In this work, ADME analysis has been performed to predict if proposed MNC could reach the identified targets and it was observed all listed compounds passed the criteria for druglikeness.

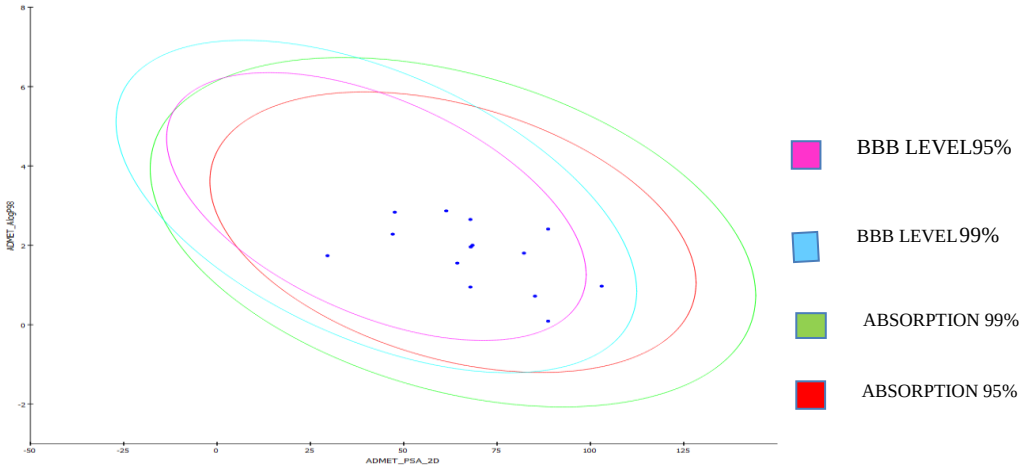
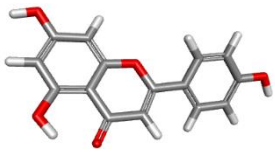
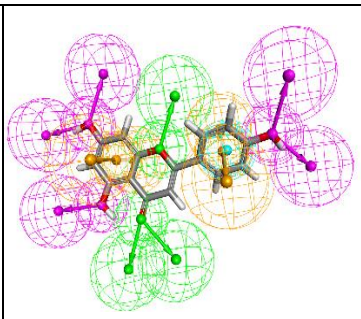
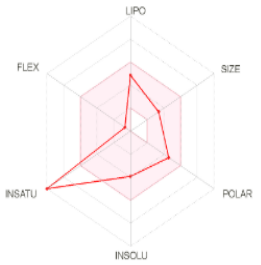
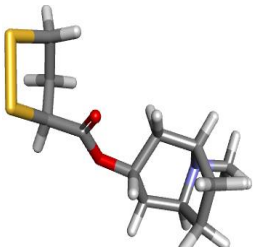
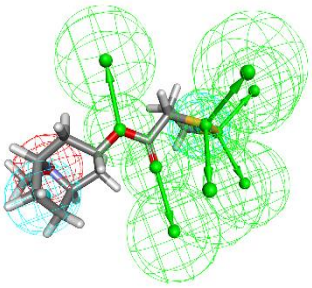
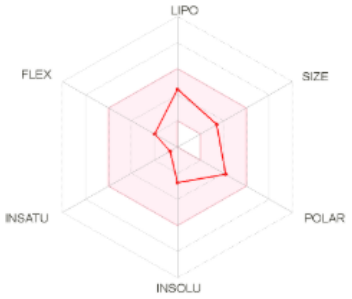
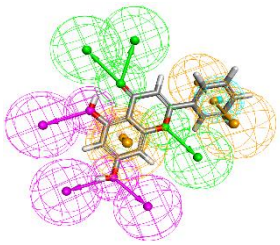
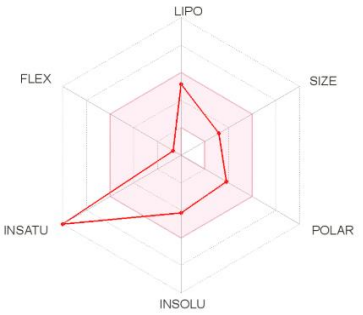
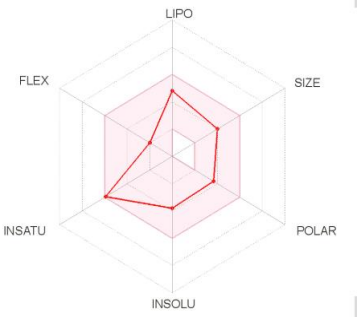
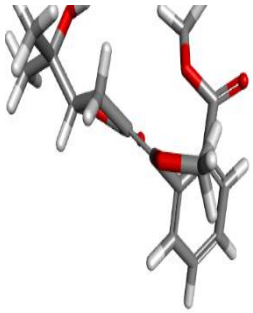
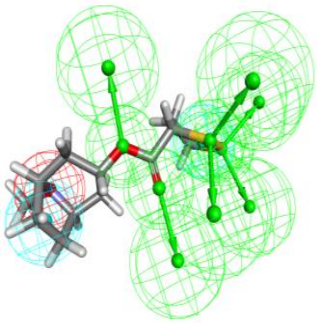
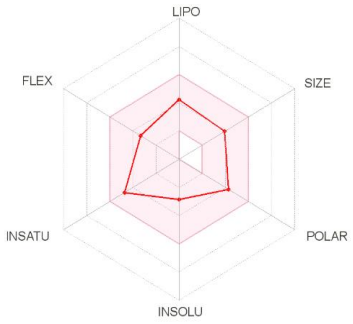
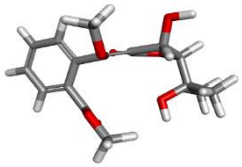
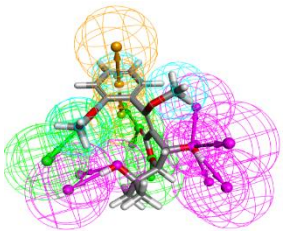
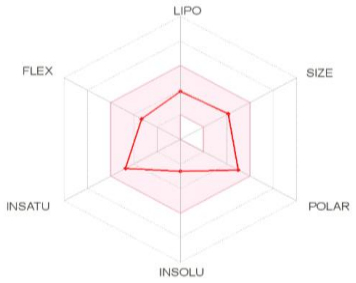
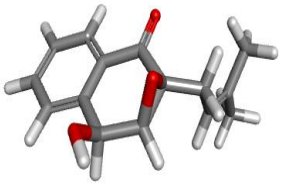
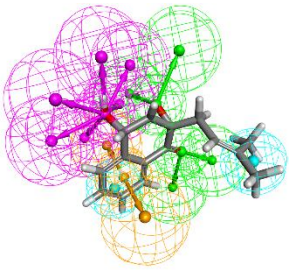
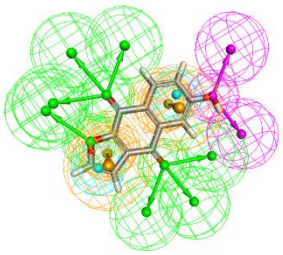
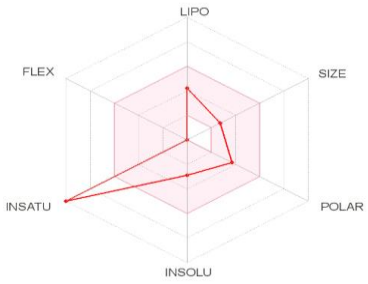
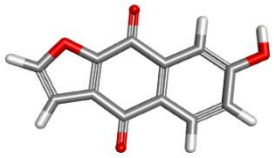
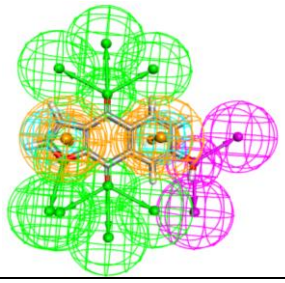
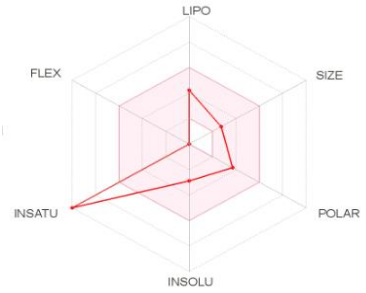
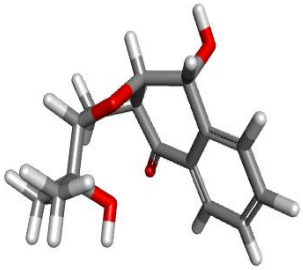
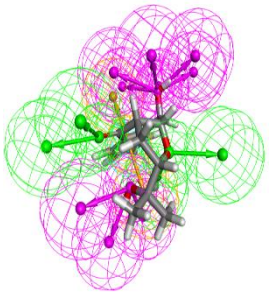
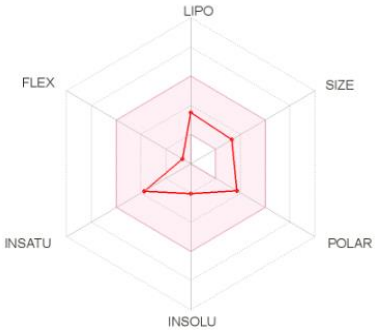
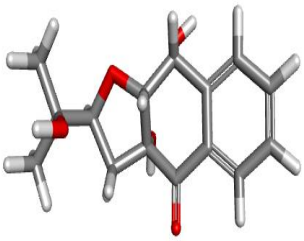
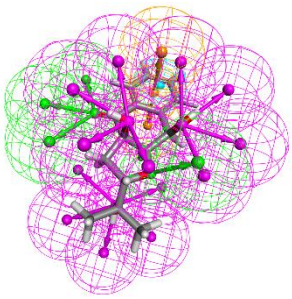
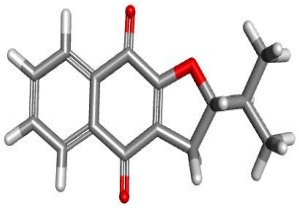
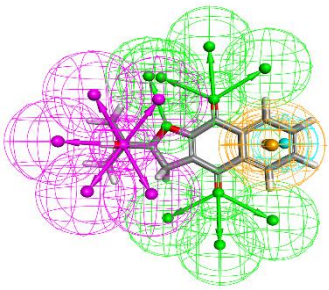
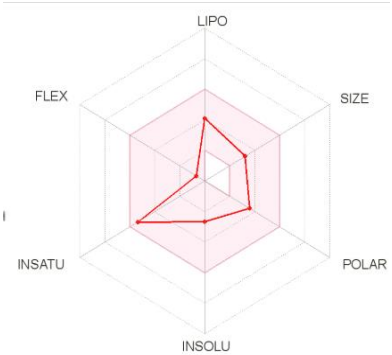


Figure 4: The above graph represents the compounds of mangrove plants satisfies all the parameters some of the compounds in BBB and Absorption 95% and 99% and others in 70% and above 30%.

Compound Name	Pharmacophore Features	Bioavailability Radar

Apigenin 		
Brugine 		
Chrysin 		
Avicennone 		

Avicennone A 		
Avicennone B 		
Avicennone C 		
Avicennone D 		

AvicennoneE 		
Avicennone  F		
Avicennone  G		
Stenocarpoquinone  B		

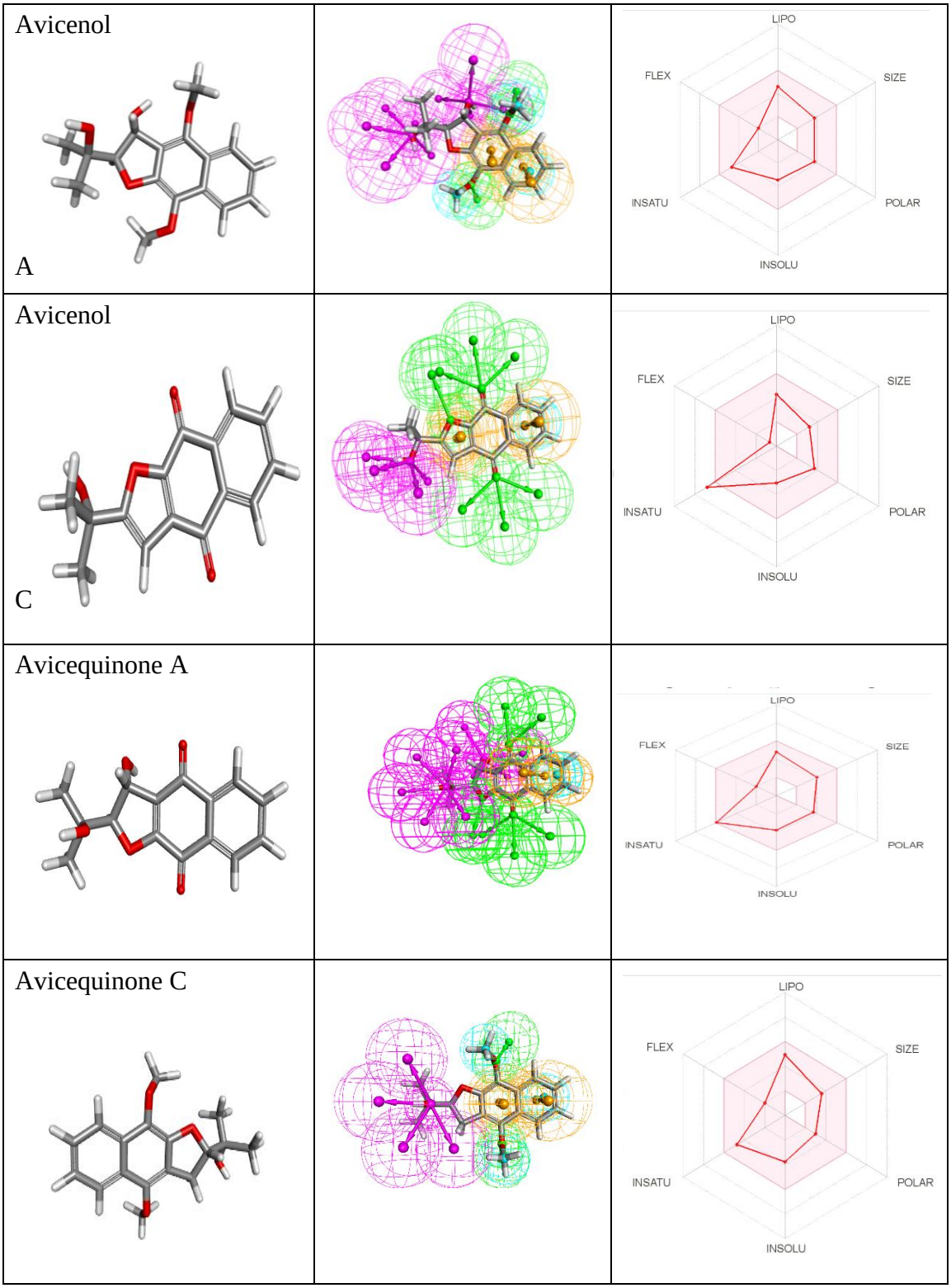


Figure 5: **Pharmacophore features** were for hydrogen bond acceptor were colour-coded with Green, Pink circles for hydrogen bond donor, brown Ring for aromatic features, cerulean blue for hydrophobic interaction ;**Bioavailabilityradar** represent the physicochemical properties Threshold for Lipophilicity:-0.7 <XLOGP3< +5.0, Size: 150 MW 500 g/mol, Polarity: 20<TPSA<130 $Å^2$, Insolubility: 0<log S<6, Instauration, Flexibility: 0.25<rotatable bonds<9

Molecular dynamic (MD) simulation, route means square fluctuation (RMSF) evaluation of the NEK2:

The receptor used in this study was NEK2, using cavity algorithm we screened the binding pocket as well as druggable cysteine residue based on the literature information.There were two druggable cavities were found and cavity one was subjected for MNC affinity screening (**Figure 6 a,b**).Molecular dynamic simulation was performed on CABSfex2.0 server by 50 ns simulation. Fluctuation plot - provides an interactive 2D plot presenting residue-wise fluctuations recorded throughout the simulation. Both graphics (svg) and numerical data (csv) were recorded for analysis.The fluctuation plot of the NEK2 was shown in Figure. RMSF value ranges from 0.05 to 3.5. Residues with higher RMSF values revealed that residue with more flexibility **(Figure 7).** In general, larger RMSF values meant for more flexibility, whereas smaller RMSF values revealed the restricted movement during simulations . Residues involved in intermolecular residues adhered to less flexibility state throughout the 10ns simulation. Residues at positions 131, 279, 152, 277, 130, 153, 238, 43, 179 were showed higher flexibility.But none of these residues conferred the intermolecular interaction with proposed MNC.

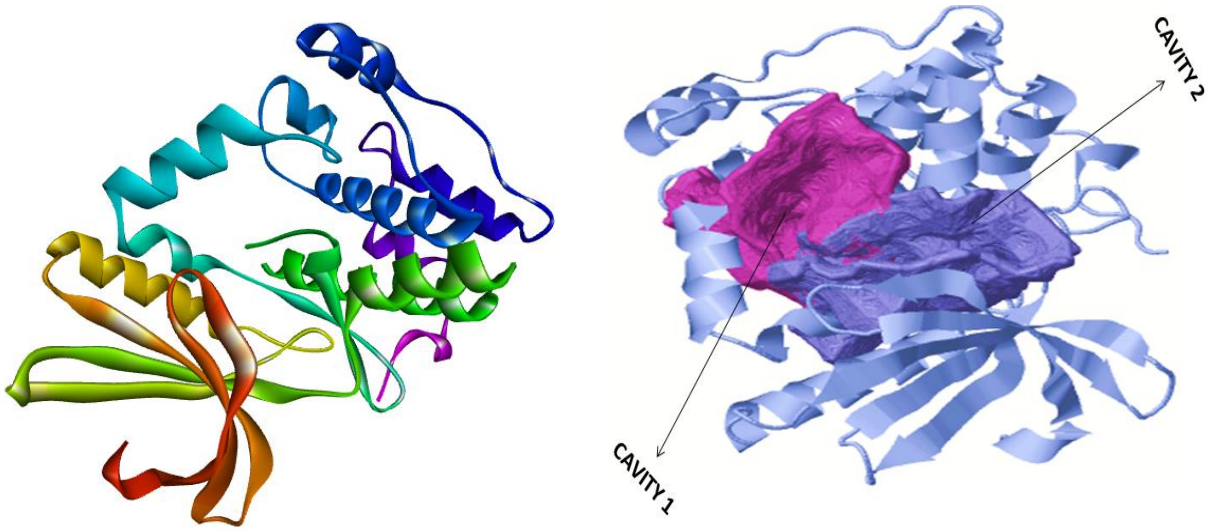


Figure 6: a) Three Dimensional structure of NEK2(PDB-Id-2W5B) b)The above picture is a snapshot of the receptor cavity binding area.

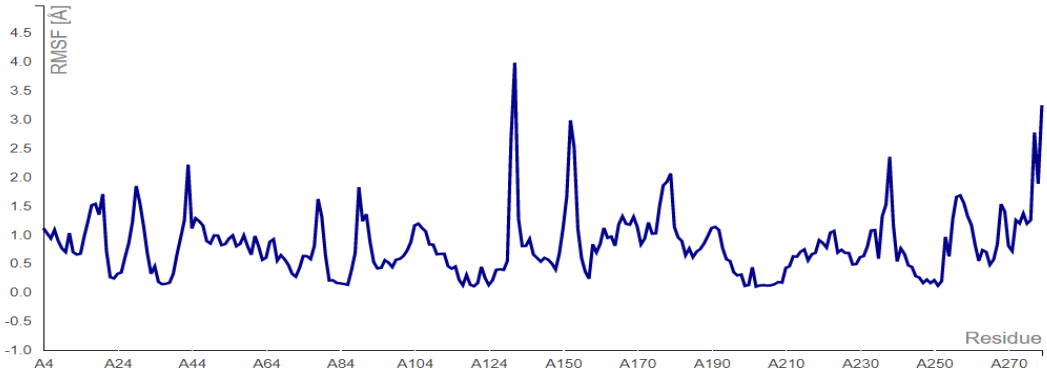
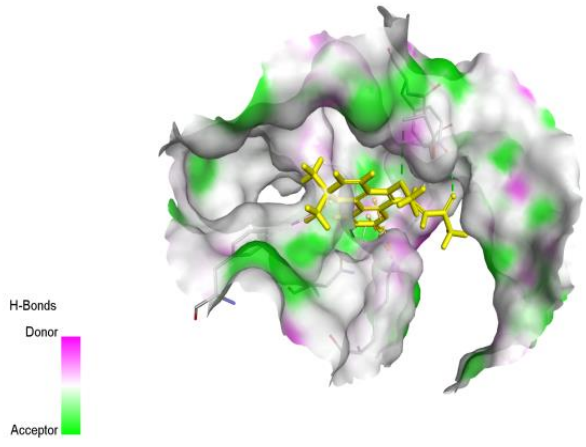
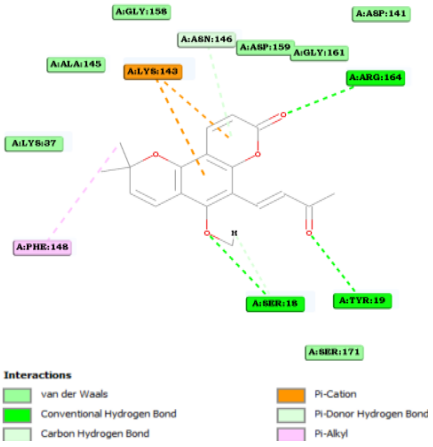


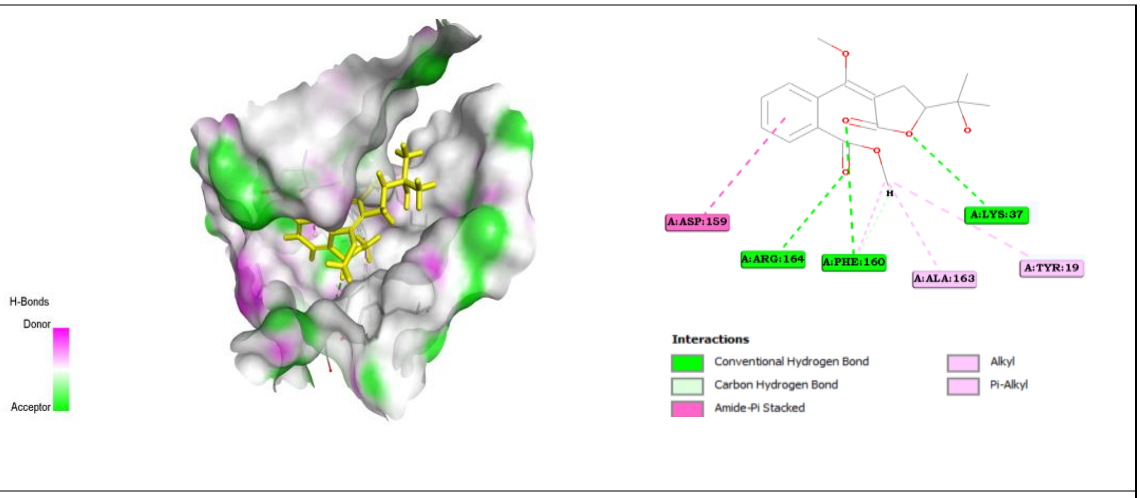
Figure 7 : Fluctuation plot of NEK2 provides an interactive 2D plot presenting to represent residue-wise fluctuations recorded throughout the 10ns simulation

Receptor–ligand interaction analysis using Molecular docking

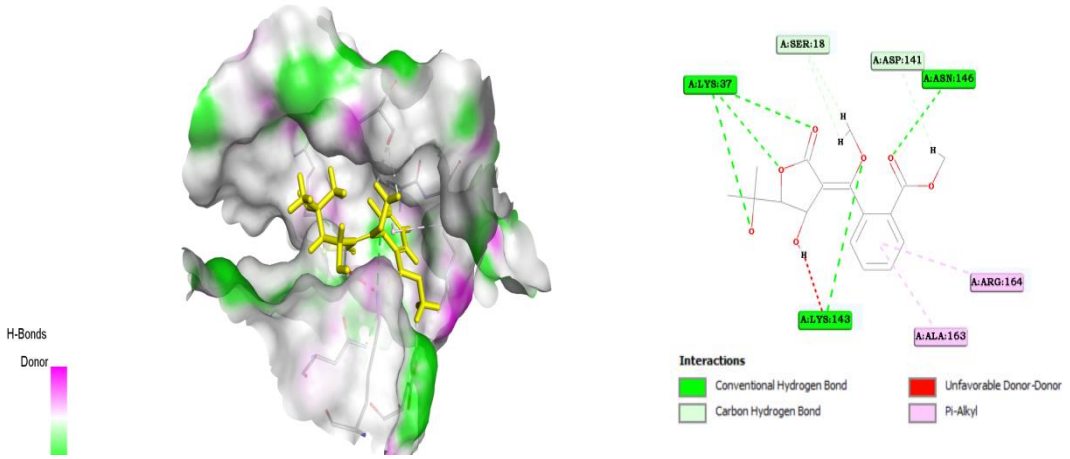
Protein kinases were prominent targets for designing the covalent inhibitors since they contribute high concentrations of ATP in cells. NEK2 kinase domain (PDB ID: 2W5B) intermolecular with compound is shown in **Figure7**. Mangrove derived compound Avicennone bound firmly at the NEK2 cavity with three conventional hydrogen bonds with residues Arg 164,Tyr 19,Ser 18 and one C-H interaction with Asn 146. Conformational stability of the Avicennone enhanced with other

Pi-stacked interaction (Phe 148) and Pi-cation interaction (Lys 143)(**Figure 8a**). Avicennone A similarly conferred the three conventional hydrogen bonds with Arg 164, Phe 160, Lys 37.Avicennone A conferred series of Pi stacked interactions with the residues Asp 159,Ala 163 and Tyr 19.NEK2 residue positions Lys 37, conferred three stable hydrogen bond with MNC(**Figure 8b**),Asn 146 and Lys 143 were the other key residues formed the hydrogen bonding with Avicennone B. Here Arg 164 and Ala 163 were involved in pi alkyl interaction.C-H bonding was seen with Ser 18 and Asp 141**(Figure 8c**). Further analysis of Avicennone C indicated the presence of a pi-alkyl interaction with Arg 164, and mediated the hydrogen bonding with the Ser 18,Lys 143,Phe 160(**Figure 8d**).Avicennone D mediated the hydrogen bonding with Phe 160,Lys 37 and Ser 18,then Pi-cation interaction with Lys 143(**Figure 7e**).Avicennone E mediated two hydrogen bonding with Arg 164 and Tyr 19.Ala 163 and Lys 37 were residues conferred the Pi-stacked interactions (**Figure 7f**).Avicennone F conferred the hydrogen bonding with Lys 37(**Figure 8g**).Avicennone G and its derivatives showed binding affinity towards the kinase pocket residues Arg 164, Phe 160, Lys 37, Tyr 19,Ser 18 , Asn 146, Asp 141 and specifically with Cys 22(Pi-staked interaction)(**Figure 8h**).Resulted data's were further supported by Cheng Huang et al work on *Avicennia marina* leaf extracts.Studies reported that xenograft MDA-MB-231 tumor growth got suppressed in nude mice on treatment with *A.marina* leaf extracts [13] (Huang C *et al*.,2016). Similarly Stenocarpoquinone B also conferred the hydrogen bonding with NEK2 residues Lys 37,Phe 160 and Ser 18(**Figure 8i**).Compounds Avicenol C, Avicenoquinone A, Avicenoquinone B, Brugine were compounds mediated the hydrogen bonding with catalytic residues(Lys 37,Ser 18,Phen 160,Arg 146) of NEK2(**Figure 8 k,l,m,n**). Avicenol A conferred the promising interaction with Cys 22 of NEK2, besides formed the Pi-stacked interaction with Gly 20, Ala 163 and Lys 37(**Figure 8j**). Chrysin another MNC compound formed the stable Pi-sulphur interaction with NEK2(**Figure 8o**).Similarly Apigenin formed the hydrogen bonding with Cys22 ,In addition to this formed two more hydrogen bonding with the residues Asp 159 and Arg 164.Pi stacked interaction(Lys 143 ,Lys 37) stabilized the interaction of apigenin with NEK2 catalytic domain(**Figure 8p**). Mentioned interacting positions were conserved among all NEK family proteins and their MNC can be tested against other NEK proteins which are mainly involved in tumorogenesis , in case of NEK2 residues located from 1 to 271 possessed the essential kinase domain and compiled with activation loop segment from 159 to 186 (**Figure 9**).Consensus dock score and bonding distance for all the reported interaction pose were listed in **Table 2.**

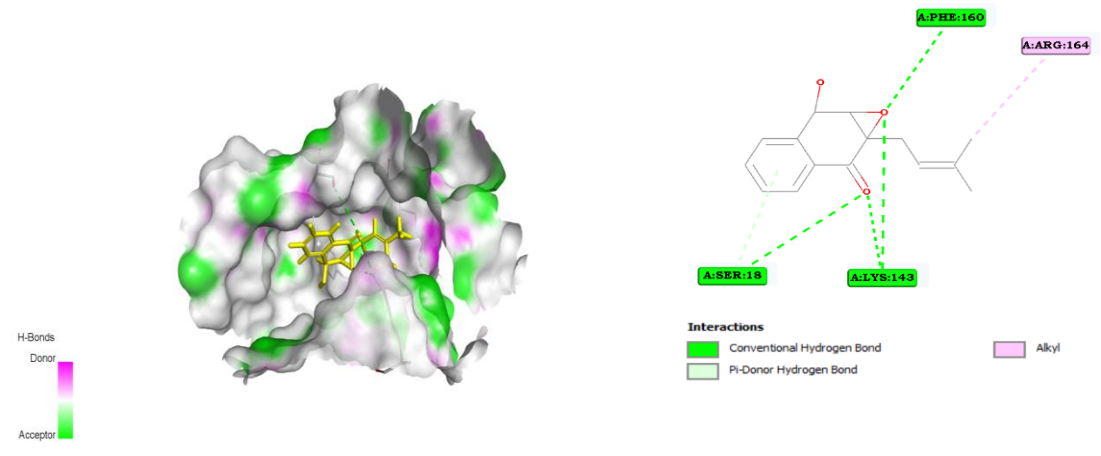
3D representation of NEK2 and Compound Interaction	2D representation of NEK2 and Compound Interaction
8a) Docked complex of NEK2 and Avicennone	
	
8b) Docked complex of NEK2 and Avicennone A	



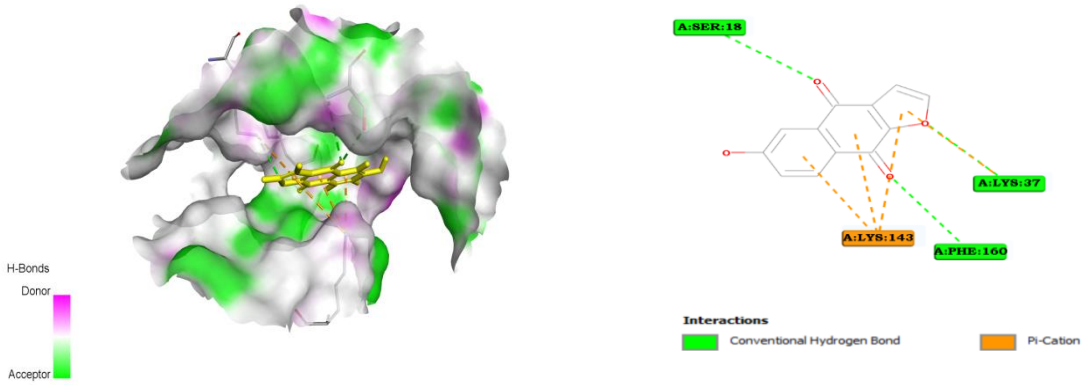
8c) Docked complex of NEK2 and Avicennone B



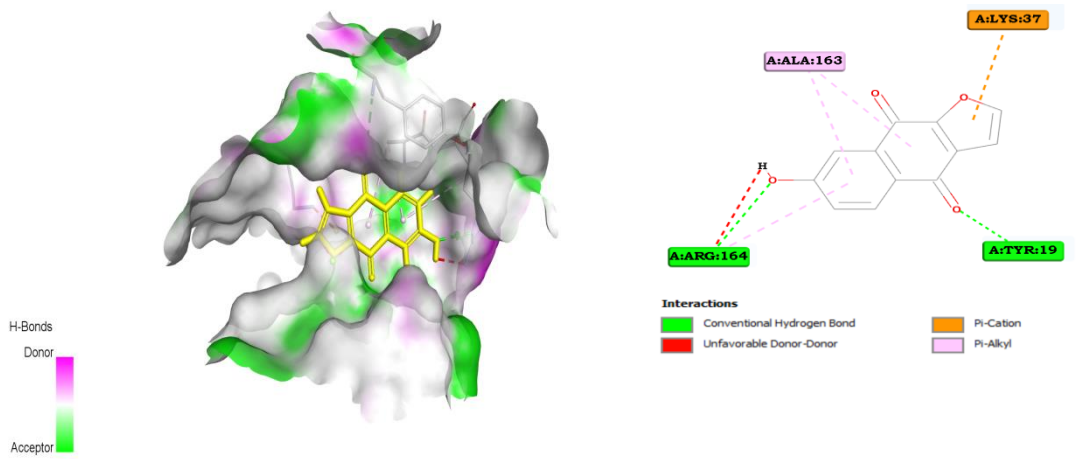
8d) Docked complex of NEK2 and Avicennone C



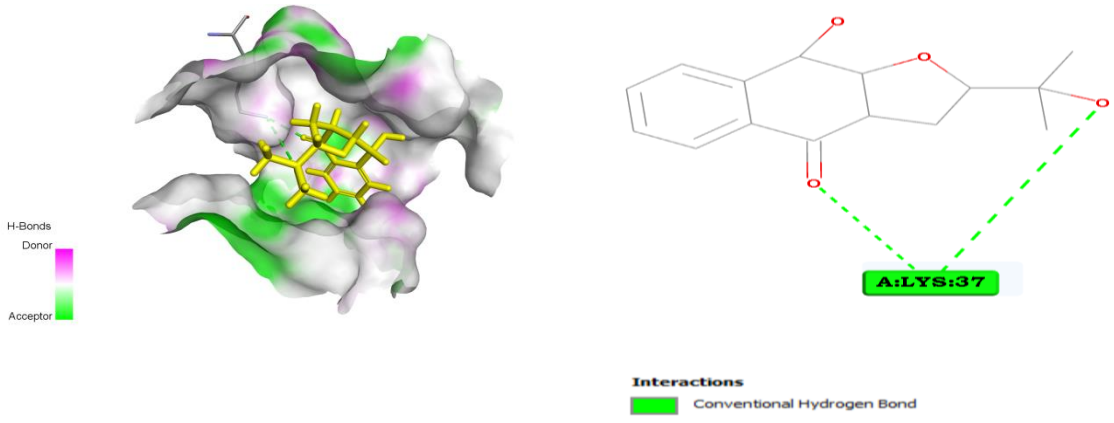
8e) Docked complex of NEK2 and Avicennone D



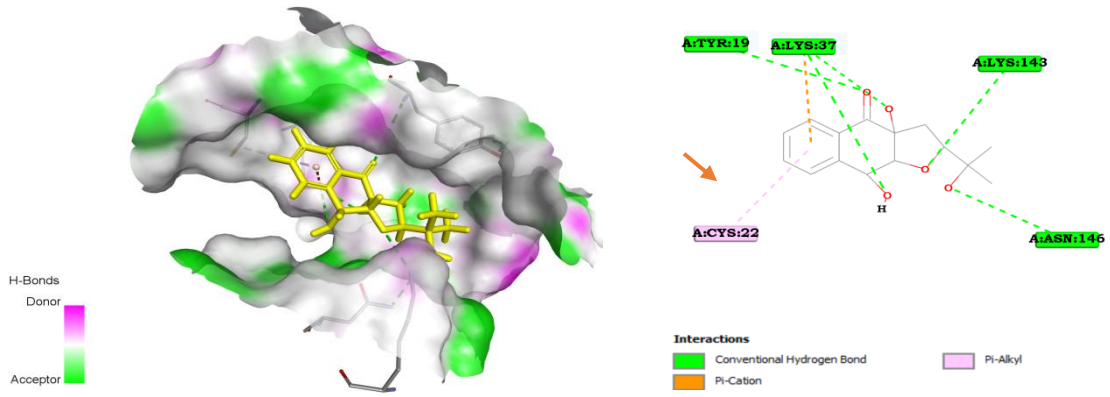
8f) Docked complex of NEK2 and Avicennone E



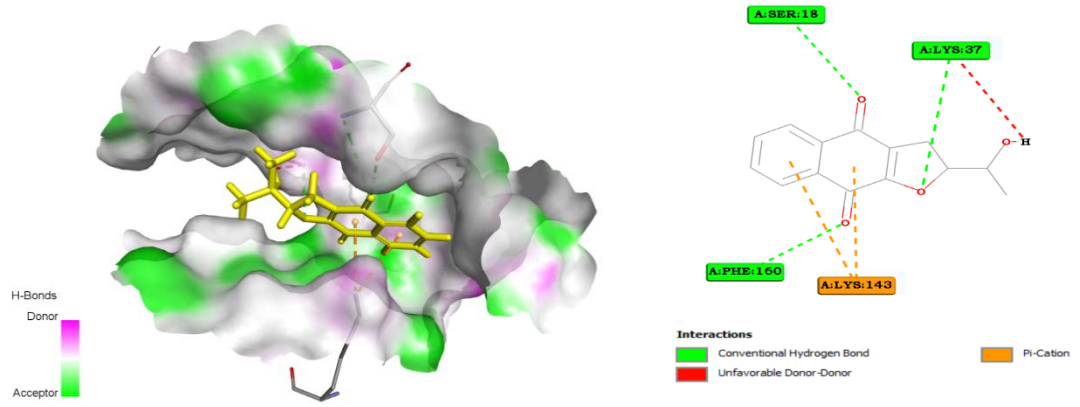
8g) Docked complex of NEK2 and Avicennone F



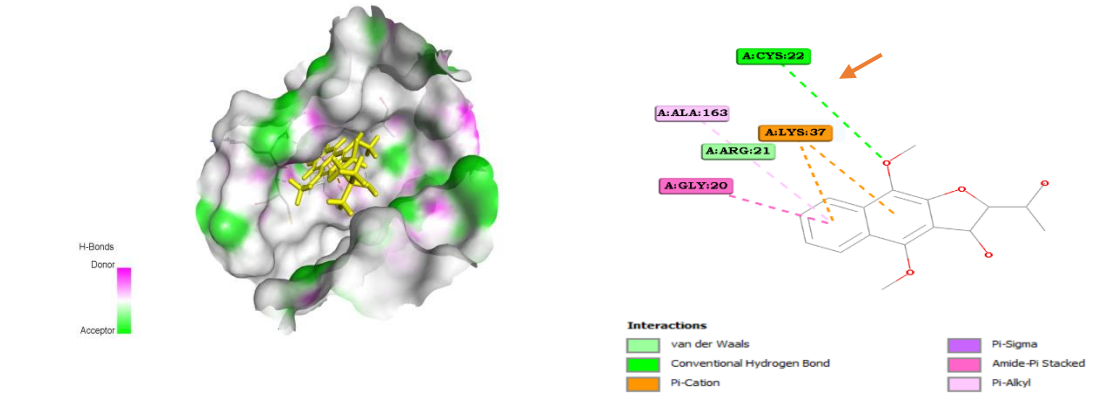
8h) Docked complex of NEK2 and Avicennone G



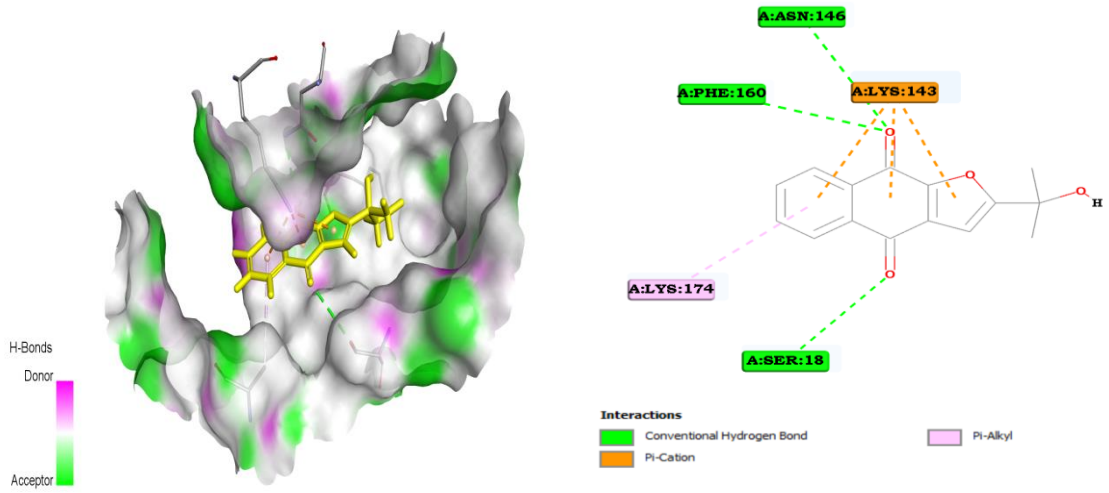
8i) Docked complex of NEK2 and Stenocarpoquinone B



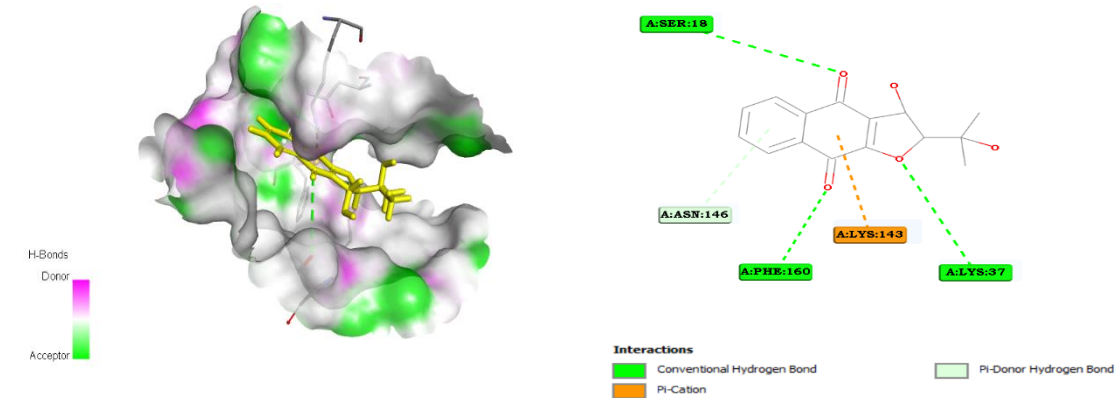
8j) Docked complex of NEK2 and Avicenol A



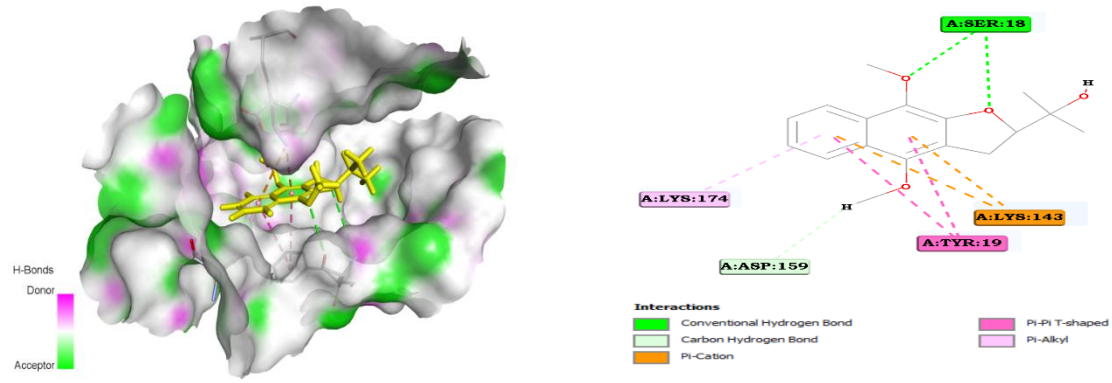
8k) Docked complex of NEK2 and Avicenol C



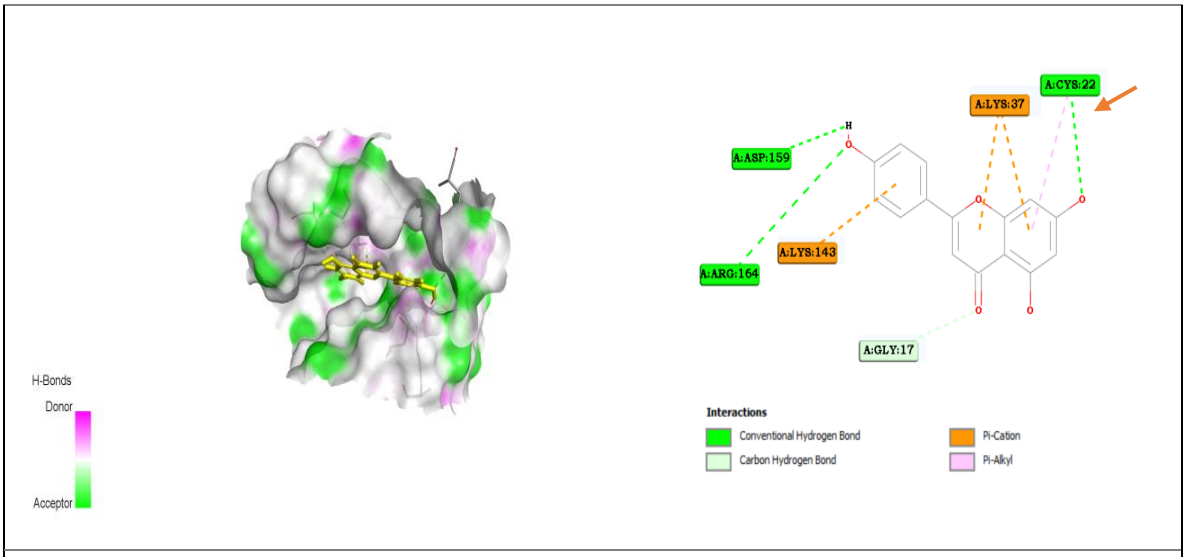
8l) Docked complex of NEK2 and Avicenoquinone A



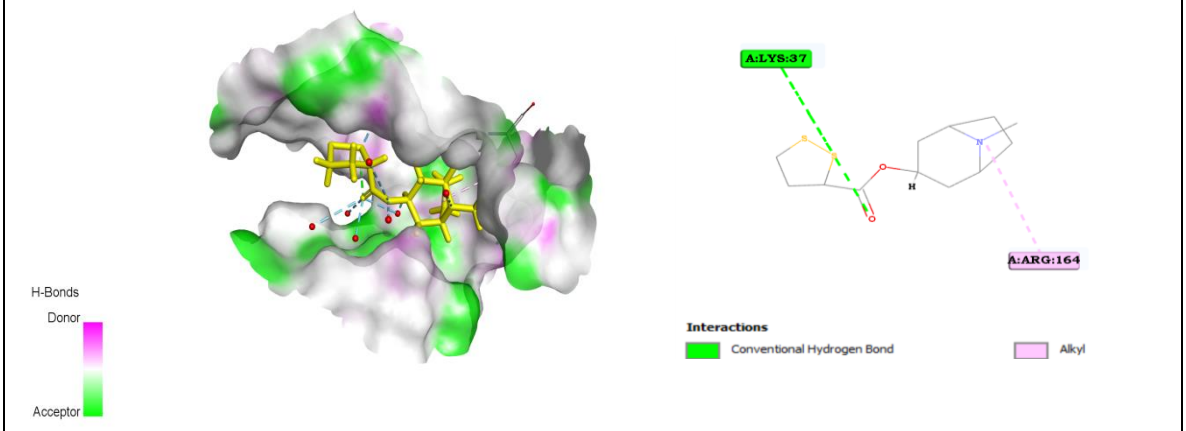
8m) Docked complex of NEK2 and Avicenoquinone B



8n) Docked complex of NEK2 and Apigenin



8o) Docked complex of NEK2 and Brugine



8p) Docked complex of NEK2 and Chrysin

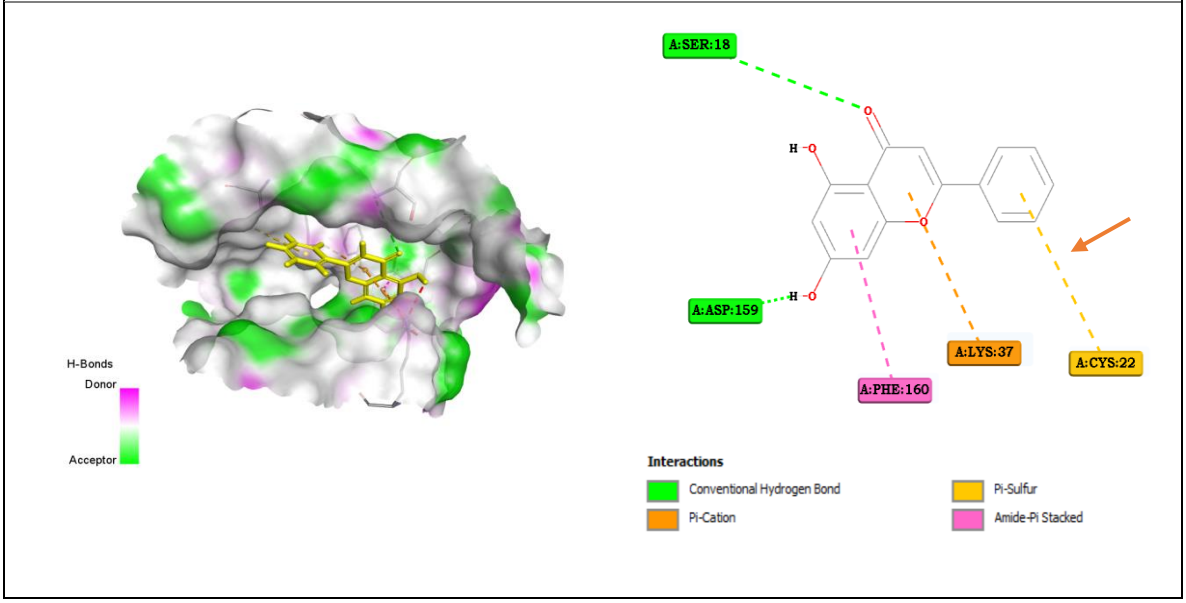


FIGURE 8: (a-p): 2D and 3D representation of MNC and NEK2 docked complexes.

(MNC: Avicennone A, Avicennone B , Avicennone C, AvicennoneD , Avicenone E , Avicennone F, Avicennone G , Avicequinone A, Stenocarpoquinone B , Avicequinone C , Avicenol A , Avicenol C, Brugine, apigenin, Chrysin)

As Nek2 contains a non-catalytic cysteine (Cys22) close to the catalytic domain that is unique to the Nek family, this residue can be targeted for irreversible binding. Nearly 200 protein kinases contain this conserved Cys22 position close to their catalytic site[34].This specific interaction significance was reported in the earlier studies by Lebraud et al on N-arylpropiolamide with NEK2, their work stated that selective against other mitotic kinases, including CDK1, PLK1, Aurora B and Mps1[35]. Henise JC *et al* proposed that, oxindole propynamide 16 compound as first NEK2 specific inhibitor by targeting the Cys 22 position [36]. This inhibitor selectively inhibited the NEK2 without causing any off-target response to the other mitotic kinases like Cdk1, Aurora B. Another recent invitro studies stated that compound 2-Arylamino-6-ethynylpurines also specifically targeting the NEK2 Cys 22 residues also conferred the similar intermolecular interaction [37]. According to Colmegna B et al chemoresistance created a bigger burden in the current cancer therapeutic regimen. Studies stated the multiple strategy of drug resistance development like mutations in the target proteins, pro survival pathway activation [38].In our studies MNC Avicenol A, Apigenin , Chrysin conferred the hydrogen bonding with Cys22 residue along with pi-sulphur, Pi-alkyl interactions with NEK2.So these compound conferred the covalent binding would ensure the prolonged duration of action and enhanced pharmacological efficacy, which allows lower dosing and reduced off-target effects.

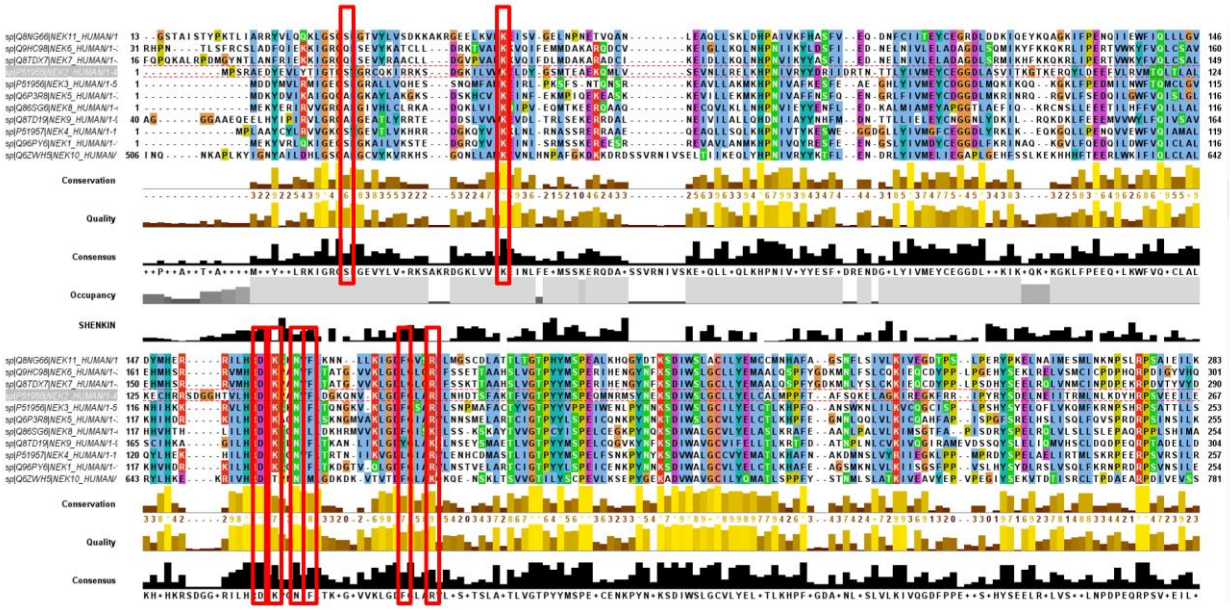


Figure 9: Ser18,Lys37,Lys143,Asn 146,Asp,159,Phe 160,Arg 164 were residues highly conserved among the NEK family proteins and MNC showed binding affinity towards these kinase domain residues.

Table 2: Molecular docking study between selected MNC along with NEK2 revealed their intermolecular docking scores presented with kind of interactions and the interacting residues.

Name	Bond Hydrogen Interaction	Amide-Pi stacked	Pi-alkyl interaction	Pi-cation	Pi- sulphur	Carbon- Hydrogen Interaction	Dock Score

Avicennone	Ser18 Tyr19 **Arg164** Asn146 **Phe148**	--	Phe148	Lys 143	--	Asn 146	301.02
Avicennone A	Lys37 **Phe160** **Arg164**	Asp159	Ala163 Tyr 19	--	--	--	308.08
Avicennone B	Lys37 Lys143 Asn146	--	Ala163 Arg164	--	--	Ser18 Asp141	282.38
Avicennone C	Ser18 Lys37 **Phe160**	--	Arg164	--	--	--	297.69
Avicennone D	Ser18 Lys37 **Phe160**	Phe160	--	Lys143	--	--	273.85
Avicennone E	Tyr18, **Arg164**	--	Ala163	Lys37	-	-	260.51
Avicennone F	Lys37	--	--	--	--	--	249.66
Avicennone G	Try19, Lys37, Lys143 Asn 146	--	Cys22	--	--	--	254.45
Avicenol A	Cys22	Gly20	Ala 163	Lys37	--	--	305.11
Avicenol C	Asn 146 **Phe 160** Ser 18	--	Lys 174	Lys143	--	--	299.15
Avicequinone A	Ser18 Lys37 **Phe160**	Asn146	--	Lys143	--	Asn146	308.12
Avicequinone B	Ser18,	Tyr 19	--	Lys174		Asp159	297.69
Stenocarpoquinone B	Ser18, Lys37, **Phe160**	--	--	Lys 143	--	--	336..99
Apigenin	Cys22 **Arg164** **Asp159**	--	--	Lys37 Lys143	--	Gly17	194.414

Brugine	Lys37	--	--	Arg164	--	--	136.26
Chrysin	Ser18 **Asp159**	Phe160	--	Lys37	Cys22	--	194.72

4. Materials and Methods

Assessment of NEK2 transcription profile transcriptional profile and associated pathway:

NEK2 expression is low in the G1 phase of the cell cycle, increasing through S and G2 to reach a peak in late G2/M, and decreasing upon cell entry into mitosis [17,18]. Similarly, NEK2 activity is stronger in the S and G2 phases compared to that in other phases. Thus, upregulation of NEK2 resulted in chromosome instability and chromosome amplification in cancer cells. CBio portal was used to retrieve the expression profile of NEK2,CBioPortal resource hold the large dataset derived from TCGA and various genomic level studies.Aberrant activation of NEK2 by its upstream proteins or overexpression of NEK2 results in the activation of its downstream proteins leading to the malfunction of NEK2 in normal cell cycle regulation, thus we constructed the protein-protein interaction network of NEK2 using reactome database [19] in the cytoscape platform [20].Since the NEK2 overexpression activated various oncogenic pathways as well as ATP-binding cassette transporters, thus, the leads to cell proliferation, invasion, and also drug resistance in patients. As NEK2 has a critical role in the progression of malignancies, as mentioned previously, it is attractive as a target for novel anticancer therapies.

Functional Enrichment assessment of NEK based Interactome:

Pathway enrichment analysis helps in gaining the mechanistic insight of given gene lists and also useful in exploring the biological pathways that are enriched in the gene list more than expected by chance. EnrichR package was used to screen the NEK2 and associated proteins functional pathways within human.The Gene Ontology (GO) based analysis is a central resource for functional-genomics research. GO term enrichment analysis was comprised of three modules namely biological process (BP), cellular component (CC), and molecular function (MF).We used ClueGo is a user friendly Cytoscape plug-in in our studies to analyze interrelations of terms and functional groups in biological networks [21].

NEK2 domain consensus comparison with other NEK family proteins

In this NEK family, 11 genes are encoding different serine/ threonine kinases, which catalytic domains have 40–45% amino acid sequence identity with NIMA's catalytic domain [22].Multiple sequence alignment was performed to find the consensus residues among the eleven members of NEK family[23]and other kinase family proteins.

Receptor NEK2 preparation:

The receptor employed in this study is a NEK2, PDB file of the receptor was downloaded from RCSB PDB 2W5B, it's a X ray diffraction structure of human with resolution of 2.40 Å [24]. Hetero atoms were removed and subjected for Discovery studio protein preparation to remove the excess water and to optimize the hydrogens. Stereochemical properties were assessed by calculating the amino acid residues that populated in the allowed regions of Ramachandran plot. Druggable binding cavities within NEK2 were screened using cavity algorithm [25]. Human protein atlas was used to verify the drug target potential of NEK2 [26]. The dynamics of protein structures defines their biological functions. Because the experimental investigation of protein flexibility is often difficult or impossible, computational approaches play a significant role in this field, NEK2 dynamics was evaluated using CABS-flex server [27].

Preparation *Avicennia marina* derived compounds as ligand:

Avicennia marina derived compounds avicennone A, avicennone B, avicennone C, avicennone D, avicenone E, avicennone F, and avicennone G, avicenol A , avicenol C, stenocarpoquinone B and apigenin, chrysin,brugine structures were downloaded from Pubchem and saved in sdf format [28].Pharmacophore features were screened for the compounds and listed in the figure **Figure 5)**.The 3D-QSAR Pharmacophore Generation (HypoGen) module in Discovery Studio 2.5 was used to screen the pharmacophore models that contain a maximum of four features of proposed compounds. Pharmacophoric features meant for hydrogen bond acceptor (green), hydrogen bond donor (magenta), hydrophobic (cyan), and ring aromatic (orange) were highlighted with mentioned colors.

Drug likeness calculations of MNC:

Open source web server SWISSADME was used to the predict the mangrove derived compounds drug likeness properties. This webserver computationally predicts ADME (absorption, distribution, metabolism, and elimination) profile and computes physicochemical parameters such as molecular weight, H-bond donor/acceptor, lipophilicity, solubility. Moreover, several drug likeness criteria based on Lipinski, Ghose, Veber, Egan and Muegge are implemented in SwissADME webserver. Bioavailability radar of the *Avicennia marina compounds* were listed in the **Figure 5**. Swiss ADME predicted results cross evaluated with Discovery studio 3.5 (Accelrys San Diego, USA) ADMET predictions are considered before designing a drug as these properties play a vital role in clinical phases and the results were depicted in the **Figure 4.**The evaluation of the interactions of CYPs with small molecules (such as drugs) constitutes a fundamental step for drug design, as well as for toxicity assessment[29].

Receptor–ligand interaction analysis using Molecular docking

The molecular docking corroborates the intermolecular interaction between the protein and ligands in the form of docking score. Proposed compounds were docked against NEK2. Docking process carried out using LIGANDFIT methodology [30]. The docking cavity was defined using the LigandFit site search utility. After characterization of the binding pockets, a Monte Carlo method was employed for the conformational search of the ligand. Meanwhile A short rigid body minimization was then performed (steepest descent and Broyden Fletcher Goldfarb Shanno (BFGS) minimizations). After a new conformation was generated, the fitting was carried out using the following procedure: the shape of the ligand was compared to the shape of the active site and if acceptable able, a dock energy (Dock Score) was computed between the protein and the ligand trial conformation. Top ten poses were saved for each ligand after docking and 100 steps of BFGS rigid body minimization were then carried out. Scoring was performed with six scoring functions: Dock Score, LigScore1,

LigScore2, PLP1, PLP2, and PMF, CFF and CHARMM force field was used for LigScore calculations. Here we assumed that the bioactive orientation would be among the top 10 poses ranked by Dock Score. Each of the 10 poses saved after docking were rescored with each scoring function and the best score for each molecule was retained to compute enrichment factors. Recent version of Discovery Studio visualization tool is used to analyse the top hit conformations and the score were listed in **Table 2 .**LigScore1 is relatively efficient regardless of the binding site properties (polar, hydrophobic, open, closed).PMF uses pair wise potentials to estimate contacts, not the classical Lennard-Jones potential as in LigScore1, Ligscore2, and Dock Score, and could be less sensitive to clashes. This observation could also apply to PLP1 and PLP2.Consensus scoring (combination of a knowledge-based function, PMF, an empirical scheme, the JAIN scoring function, and a force field-based scoring scheme, Dock Score.Besides we used C Docker programme to assess the intermolecular interaction between MNC and NEK2 and confirmed the ligand fit generated results.

4. Conclusions

Serine-threonine kinase protein NEK2 overexpression and associated errors in cell cycle were reported in several studies. NEK2 plays a key role in chromosome instability and causes the tumorigenesis environment and reported for causing drug resistance in cancer patients. Thus, NEK2 is considered as a promising target cancer therapy. Naphthoquinone derivatives molecular interactions involved in both the hydrophobic interactions and hydrogen bonding with the NEK2.Avicenol A, Avicennone G ,Chrysin and Brugine compounds intermolecular interaction with residue like Cys22, a potent residue in the catalytic site ,besides few positively charged residues of the kinase domain formed the stable hydrogen bond interaction with the proposed compounds. In addition, numerous van der Waals and Pi-interactions contributed to the stabilization of the binding structures. NEK 2 Kinase inhibitor design would be beneficial to regulate the NEK2 expression in various cancer and thereby suppress the chemoresistance and enhance the survival rate. Further naphthoquinone derivatives effect needs to be tested through invitro and invivo studies to warrant their therapeutic potential against cancer.

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Conflicts of Interest: The authors declare no conflict of interest"

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