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E-Pharmacophore mapping and docking studies on Vitamin D receptor (VDR)

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Abstract:
Small structured chemical molecules are importance in the field of molecular medicine since their pharmacokinetic and pharmacodynamic properties are predictable and because of its ability to bind with target molecules and execute biological function. In this study, we engaged computer-aided methodology in combination with molecular docking and pharmacophore filtering to identify chemical compounds that can increase the synthesis of vitamin D receptor (VDR) since its lower expression leads to calcium phosphate metabolic disorders in Chronic Kidney Disease. Energy-optimized pharmacophore was mapped using available agonists for VDR. Based on the e-pharmacophore, we propose the pharmacophore features that should present in VDR agonists. The resulting pharmacophore model contains one hydrogen bond acceptor (A), one hydrogen bond donor (D) and two hydrophobic regions (H). Using these features pharmacophore had been made and screened against large public library of compounds (Asinex, TOSLab, Binding and Zinc database) to find potential lead compounds. The compounds which yield fitness score of more than 1.0 were further subjected to Glide HTVS, SP and XP. Glide docking results revealed five hits (*BD_230, BD_12938, BD_18601, BD_19517 and BD_19584*) were identified as potential lead molecules against calcium phosphate metabolic disorders.

Keywords: Chronic Kidney Disease, VDR agonists, E-Pharmacophore mapping and Molecular docking

Background:
Chronic Kidney Disease (CKD) is an international public health problem affecting 5–10% of the world population **[1]**. Individuals who are suffering from this also experience secondary health problems like osteoporosis, a disease that is characterised by low bone mass and poor bone quality. Reports have shown that CKD patients show high level of bone fracture than the normal population and it is increasing with progressive decline in renal function. Almost 50% of the CKD patients undergo dialysis show severe osteoporotic conditions. One way to reduce the incidence of osteoporosis in CKD patients is to stimulate new bone formation **[2]**.

To improve bone mass and control renal problems in patients with CKD, several phosphate binding drugs in combination with vitamin D therapy have been developed. However, most of these approaches are becoming expensive and less effective **[3]**. Therefore, there is a need in search of alternate molecules to reduce the pathogenesis of CKD and incidence of osteoporosis in humans. To address this concern, we used E-Pharmacophore mapping and Glide High Throughput Virtual Screening (HTVS) to look for possible candidates to increase vitamin D synthesis by binding to Vitamin D receptor (VDR) **[4]**. We collected the VDR agonists from the literature and docking analyses were performed with the VDR protein. The docked complexes were given as input for E-Pharmacophore mapping. Based on this pharmacophore we screened large collection of databases.

Finally we have identified five potential lead molecules based on docking score and ADME/T properties. Among these five compounds, compound *BD_230, BD_12938* show an excellent agonistic activity against VDR protein. Further *in vitro* and *in vivo* studies will determine if any of these five identified

molecules have the ability to synthesize Vitamin D and regulating phosphate-calcium metabolism. Such findings will pave the way for preclinical studies and eventually develop into a therapeutic molecule to treat osteoporotic conditions in CKD patients and improve the quality of life in these individuals.

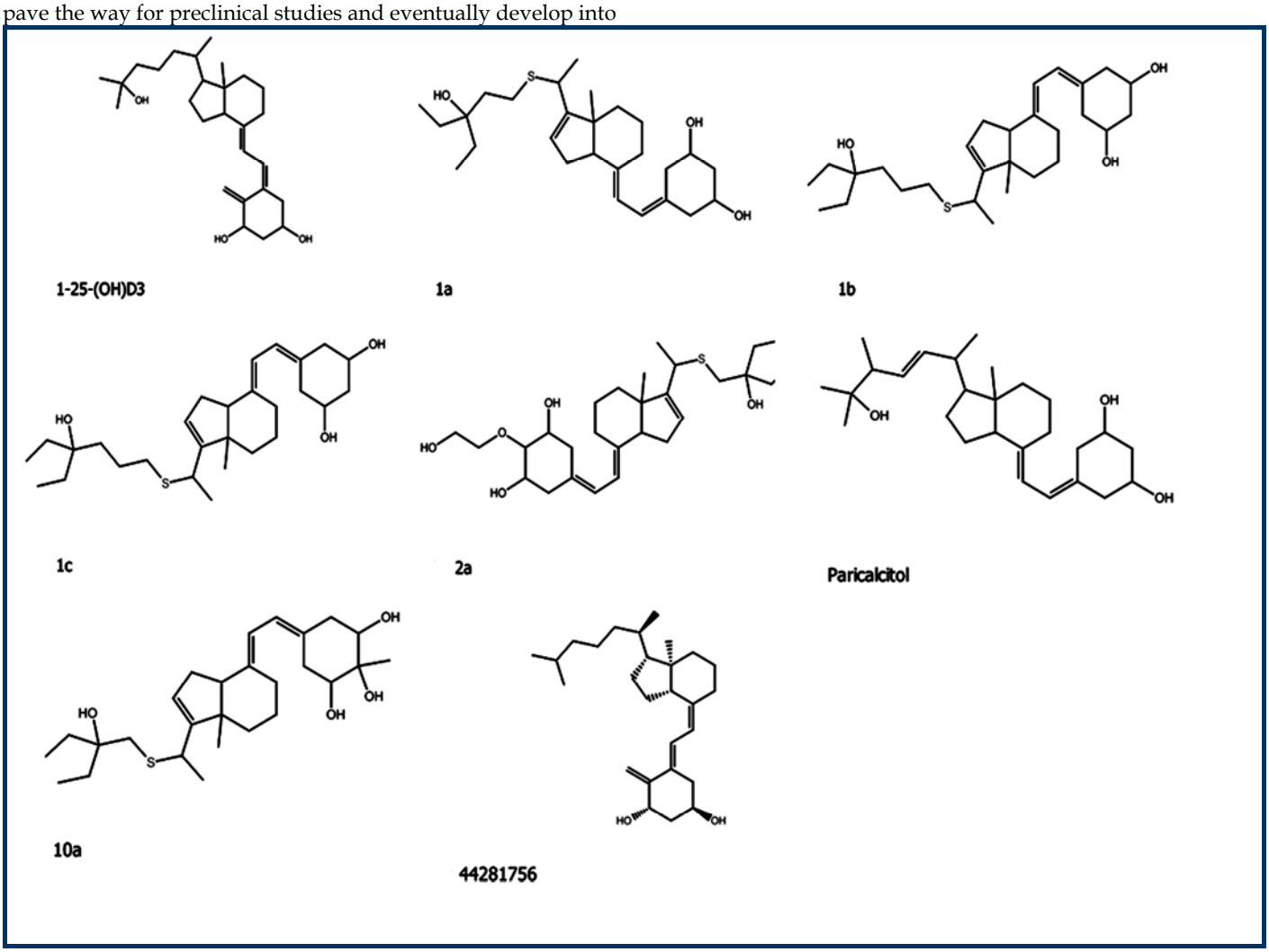

Figure 1: The 2D structures of the experimentally proved VDR agonists

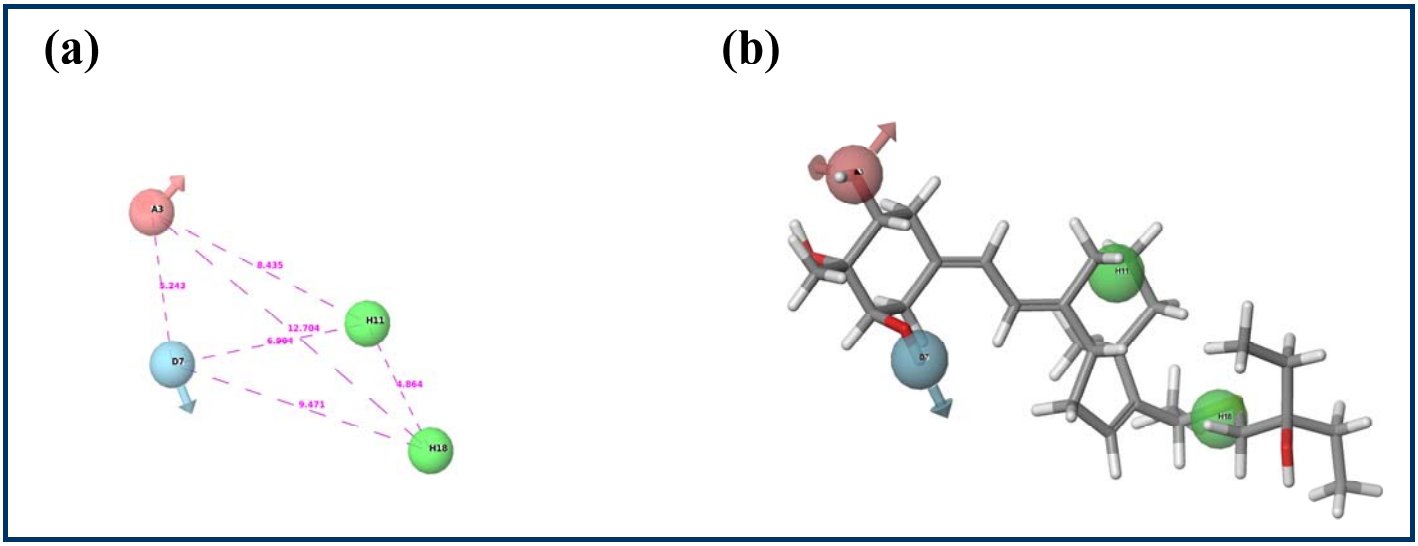

Figure 2: (a) Generated pharmacophore model for VDR along with its inter feature distance **(b)** Overlay of the most active compound in the training set of VDR.

Methodology:

Protein preparation of the VDR

Docking studies were conducted on the three dimensional (3D) structure of the VDR (PDB ID: 3A3Z) which was obtained from

protein data bank **[5]**. Before performing docking, hydrogen atoms and charges were added to this crystal structure of 3A3Z. The complex was submitted to a series of restrained, partial minimizations using the optimized potential for liquid simulations-all atom (OPLS_2005) force field **[6]**. The 3D structures were processed by use of the 'Protein Preparation module' with the 'preparation and refinement' option before docking. Hydrogen atoms were added and all unwanted water molecules were removed from the structure. Partial charges were assigned according to OPLS_2005 force field.

Ligand structure Preparation

The Vitamin D_3 analogues (8 molecules) were retrieved from the literature **[7]** and the chemical structures of these molecules were drawn by using ChemSketch version 11.01 (http://www.acdlabs.com). All these eight ligands were prepared for docking by using LigPrep, version 2.3 **[8]**. The tautomers for each of these ligands were generated and optimized. Partial atomic charges were computed using the OPLS_2005 force field.

Molecular docking and E-Pharmacophore generation

The eight different vitamin D_3 analogues were docked in the active site of VDR using Glide extra-precision (XP), version 5.5 **[9]**. The grid box enclosed at the centroid of the VDR co-crystallized ligand. All reasonable conformations for each low-energy conformer in the designated binding site were determined by Glide XP mode. We used Glide XP score for finding the best conformer with optimum binding affinity. The best conformer of the each compound along with the binding pose was given as input for E-pharmacophore mapping. E-Pharmacophores were constructed by mapping the energetic terms from Glide XP scoring function onto atom centres. Protein-ligand complexes were given as input for generating pharmacophore sites. The constructed e-pharmacophore was used as query for virtual screening **[10]**. Phase **[11]** module of schrodinger was used for pharmacophore generation using default set of six chemical features: hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic region (H), positive ionizable region (P), negative ionizable region (N) and aromatic ring (R).

E-Pharmacophore based database screening

Explicit matching must required for the most energetically favourable site in the e-pharmacophore approach. Screening molecules should match at least 3 sites for a hypothesis with 4 sites. Different databases namely Asinex (http://www.asinex.com), TOSLab (http://www.toslab.com), Binding database (http://www.bindingdb.org/bind/index.jsp) and zinc database (http://zinc.docking.org) were used for compound screening. The fitness score is a measurement of how well the aligned ligand conformer matches the hypothesis based on RMSD site matching, vector alignments and volume terms. Database hits were ranked based on this fitness score. The ligand with the best fitness score were docked into the active site of the VDR protein.

Result & Discussion:

Molecular docking results for VDR agonists

A total of eight Vitamin D_3 analogues having agonistic activity against VDR were collected from the literature **[7]** and docked with the VDR enzyme. The docked complexes were given as input for e-pharmacophore mapping. The 2D structures of these eight agonist molecules are depicted in **(Figure 1)**.

E-Pharmacophore generation and database screening

E-Pharmacophore generation is the combined aspects of ligand-based and structure-based approaches to enhance enrichments rather than ligand information alone. The method presented here attempts to take a step beyond than simple contact scoring since it incorporates structural and energetic information using the scoring function in Glide XP. Six pharmacophore features were predicted, but only four pharmacophore sites have been chosen based on the site score. The final hypothesis consists of one hydrogen bond acceptor (A), one hydrogen bond donor (D) and two hydrophobic regions (H) and their distances are shown in **(Figure 2)**. These energetically favourable sites encompass the specific interactions of vitamin D_3 analogues and the VDR protein, and this information will be helpful in the development of new VDR agonists. Compounds were screened against Asinex, TOSLab, Binding database, etc.: Molecular docking is a computational approach which samples conformations of small compounds at protein-binding sites; the best complements of the protein-binding sites are assessed by scoring functions. The quality of docking method is assessed two main aspects: (i) docking accuracy (ii) screening enrichment.

Thirty compounds were selected from the HTVS for further Glide XP docking study based on the Glide score. Here, we report five compounds from the Glide XP docking study with the best Glide scores (-9.24 to -15.57) and Glide energies (-69.95 to -91.85), which suggest strong enzyme-ligand interactions. The chemical names of the five lead compounds and their corresponding database id numbers are: *BD_230* - 5-{[(5-{[(2S)-3-carboxy-1-[5-(2,6-dichloro phenyl)-1,3-oxazol-2-yl]-1-oxopropan-2-yl] carbamoyl} thiophen-2-yl) methyl] sulfamoyl}-2-hydroxy benzoic acid *BD_12938*-({4-[(2S)-2-carbamoyl-2-[(2S)-2-(2-{4[difluoro (phosphono) methyl] phenyl} acet amido)-3-phenyl propanamido] ethyl] phenyl} difluoromethyl) phosphonic acid, *BD_18601* - (R)-{[(S)-{[(2R,3S,4R,5R)-5-(6-amino -9H- purin-9- yl)- 3,4-dihydroxyoxolan-2-yl] methoxy}(hydroxy) phosphoryl]methyl}[2-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl) ethoxy] phosphinic acid, *BD_19517* -4-[(2S)- 2-{[(2S)-1-[(2S)-2-{[(1S)-3-carbamoyl-1-{[(1S,2S)-1-carbamoyl-2- hydroxy propyl] carbamoyl}propyl] carbamoyl} pyrrolidin-1-yl]-4-methyl-1-oxopentan-2-yl] carbamoyl} -2-(phenyl formamido) ethyl] phenoxyphosphonic acid and *BD_19584* - (4S)-4-{[(1S,2S)-1-{[(1S)-1-carbamoyl-2-methylpropyl]carbamoyl}-2-hydroxy propyl]carbamoyl}-4-{[(2S)-1-[(2S) -2-[(2S)-2-acetamido-3-[4-(phosphonooxy)phenyl]propanamido]-4-methyl pentanoyl]pyrrolidin-2-yl]formamido}butanoic acid. The chemical structures of these lead compounds are illustrated in **(Figure 3)** and **(Figure 4)** represented the binding modes of these five lead molecules and their interacting residues. The docking results for the nine compounds have shown in **Table 1 (see supplementary material)**.

***Binding mode of the best five compounds*:**

Binding mode analysis of the compound_230

The hydrogen atom from the –OH group is interacted with side chain oxygen atom in the Tyr 236. The oxygen atom from P=O group interacted with hydrogen atom in the amino group of Asp 144. The hydrogen atom from the two different group of C-

O interacted with two different group of backbone NH in Arg 274.

Binding mode analysis of the compound_12938
The three oxygen atoms in the side chain PO_3 group are well interacted with hydrogen atom from NH group of three different atoms of Asp 144, Lys 240 and Arg274. The oxygen atom from the C=O group of ligand interacted with hydrogen atom from OH group of two different amino acids namely Ser 275 and Ser 278.

Binding mode analysis of the compound_18601
The compound_18601 shows two interactions with VDR. The hydrogen atoms from two different OH group of the ligand interacted with backbone oxygen atoms of Ser 278.

Binding mode analysis of the compound_19517
The oxygen atom from the ligand molecule interacted with two different hydrogen atoms in the amino group (NH_2) of Arg 274. Another oxygen atom of the ligand molecule interacted with the hydrogen atom of the OH group in Tyr 143. The hydrogen atom from the OH group in Asp 144 interacted with oxygen atom in the ligand molecule.

Binding mode analysis of the compound_19584
The hydrogen atom (NH) from the ligand molecule interacted with the oxygen atom (C-O) of Arg 274. The oxygen atom (C-O) from the ligand molecule interacted with two different hydrogen atom (NH_2) of Arg 274. The oxygen atom from the imidizole ring has been interacted with hydrogen atom of (NH) Asp 144.

ADME/T or pharmacokinetics prediction of the lead molecules
QikProp settings determine which molecules are flagged as being dissimilar to other 95% of the known drugs. Predicted significant ADME/T properties such as permeability through MDCK cells (QPlogMDCK), QikProp predicted log IC_{50} value for blockage of K+ channels (QPlogHERG), QikProp predicted gut-blood barrier (QPPCaco) and violations of the Lipinski's rule of five (LROF) were reported in **Table 2 (see supplementary material)**. The number of stars indicates the deviations from the 95% of the known drugs. In accordance with Lipinski's rule of five, QikProp was used to evaluate the drug-likeness of the lead molecules by assessing their physicochemical properties. These properties are well within the acceptable range of the Lipinski rule for drug-like molecules. These compounds were further evaluated for their drug-like behavior through analysis of pharmacokinetic parameters required for absorption, distribution, metabolism, excretion and toxicity (ADME/T) by use of QikProp. For the five lead compounds, the partition coefficient (QPlogPo/w) and water solubility (QPlogS), critical for estimation of absorption and distribution of drugs within the body, ranged between ~ -1.98 and ~ -6.83, cell permeability (QPPCaco), a key factor governing drug metabolism and its access to biological membranes, ranged from 0.00 to 63.54. All these pharmacokinetic parameters are within the acceptable range defined for human use, thereby indicating the selected three drug-like compounds their potential as drug- like molecules could be a potential agonists for VDR protein and further analysis can be performed through various experimental studies.

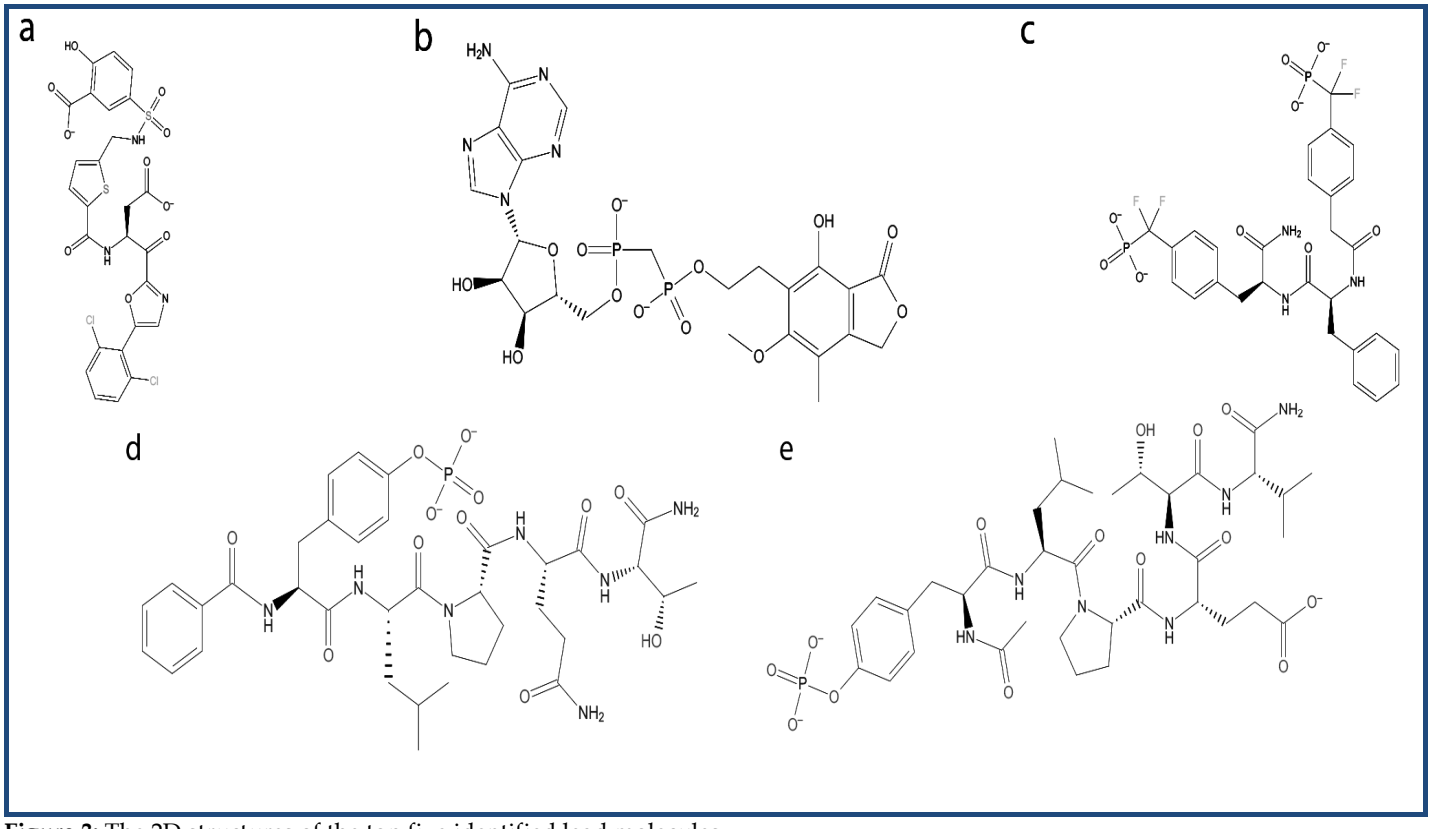

Figure 3: The 2D structures of the top five identified lead molecules

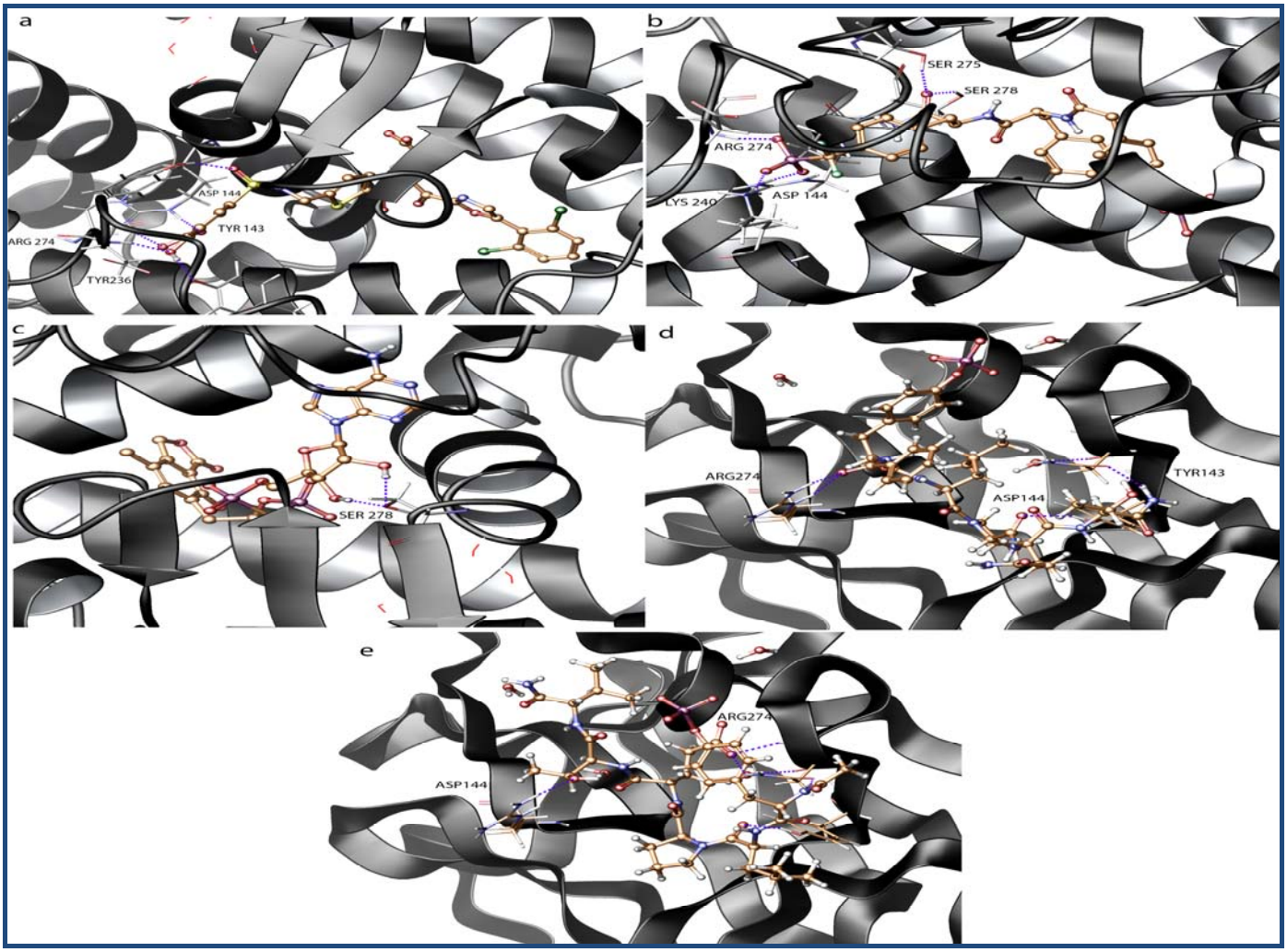

Figure 4: Binding mode analyses of the top five identified compounds with the target protein VDR

Conclusion:

In summary, a new approach combining molecular docking and pharmacophore filtering have been employed to meet the critical challenges faced in designing efficient VDR agonists to treat calcium phosphate metabolism disorders. Primarily, eight experimentally proven VDR agonists were docked into VDR active site by using molecular docking program glide, and the compounds were selected for additional study. Based on the protein-ligand complexes common pharmacophore model has been created for VDR agonists. Pharmacophore based virtual screening retrieved nine compounds from different databases. Based on glide score, glide energy and ADME/T properties five lead molecules are identified as potent lead molecules for VDR agonism. Further study using *in vitro* and *in vivo* approach will delineate the therapeutic utility of this molecule in improving bone mass and reducing renal abnormal function in patients with Chronic Kidney Disease.

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Supplementary material:

Table 1: Glide docking score and docking energies of final lead molecules identified in this study

Compound	VDR binding	
	Glide docking score	Glide energy (kcal/mol)
BD_230	-15.577	-91.850
BD_12938	-16.741	-90.882
BD_18601	-9.576	-68.864
BD_19517	-9.452	-65.231
BD_19584	-9.246	-69.953
BD_19535	-8.457	-66.458
BD_19537	-8.245	-68.243
BD_15028	-7.874	-55.326
BD_18602	-6.214	-50.321

Table 2: ADME/T properties for the best five compounds

S. No	Compound[a]	QPlog MDCK[b]	QPlog HERG[c]	QPP Caco[d]	rule of five[e]	stars[f]	QPlog Po/w[g]	QPlogS[h]
1.	BD_230	65	-3.81	26.48	2	5	3.24	-6.79
2.	BD_12938	50.63	-4.78	54.26	3	5	1.37	-2.02
3.	BD_18601	87.21	-2.54	47.47	3	5	-0.66	-2.90
4.	BD_19517	63.02	-5.40	26.84	3	5	-1.72	-3.68
5.	BD_19584	45.01	-8.96	63.54	3	5	-1.29	-2.99
6.	BD_19535	0.04	7.26	0.01	3	5	-1.71	-2.41
7.	BD_19537	0.03	6.33	0.00	3	5	-1.68	-1.98
8.	BD_15028	50.21	2.75	5.21	3	5	41.90	-4.88
9.	BD_18602	0.05	-3.20	0.13	3	5	0.84	-6.83

[a]Ligand IDs from zinc database
[b]Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood brain barrier. (<25 poor >500 great)
[c]Predicted IC50 value for blockage of HERG K+ channels. (Concern below -5)
[d]Predicted Caco-2 cell permeability in nm/s – (<25 poor >500 great)
[e]mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB ≤ 10. (Maximum is 4)
Predicted apparent MDCK cell permeability in nm/sec (acceptable range: < 25 are poor, >500 are high).
[f]Number of property or descriptor values that fall outside the 95% range of similar values for known drugs. (0-5)
[g]Predicted octanol/water partition coefficient. (-2.0 -6.5)
[h]Predicted aqueous solubility, log S. S in mol dm^{-3} is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid. (-6.5-0.5)