Summary
CKD is a major public health concern, because of its high prevalence and strong positive association with cardiovascular diseases and osteoporosis. It is estimated that 8-16% of the total world population are affected with CKD and it is reported to be the 19th leading cause of death in the past several years. India is the second most populous country for diabetes. Moreover, CKD prevalence will become double in the near future as a consequence of the ongoing epidemics of diabetes, hypertension, and obesity, all of which are important risk factors for CKD progression.

CKD is generally regarded as a complex trait caused by multiple genes. The polygenetic effects on CKD are modulated by gene-gene and gene-environment interactions. Most investigations have revealed that candidate genes are responsible for the osteoporotic conditions among CKD patients, though the reports from different populations were still conflicting.

Single nucleotide polymorphisms play an essential regulatory role in defining the individual’s susceptibility to disease and drug response. Recent drug research and development processes mainly rely on the relationship between the genotype and phenotype through SNPs. Establishment of personalized medicine is dependent on detailed understanding of the effect of SNPs on the patient drug response. Analyzing human genetic variations gives a valuable conclusion to understand the basis of individual variations in therapeutic responses. As we are in the personalized genomics era, the knowledge of human genetic variations provides a valuable benchmark for understanding the differences in susceptibility to diseases and designing individualized therapeutic treatments.

CKD patients suffer from severe osteoporotic conditions because of the deregulated VDR level. Most of the available treatment approaches for CKD patients are targeted toward the VDR protein to manage osteoporosis. This thesis contains a
case-control study and then computational analysis of VDR SNPs reported in dbSNP. Further, the role of deleterious polymorphisms in calcitriol drug response was investigated in detail. Moreover, an effort is made to bring further information about how molecular modeling study contributed to understanding the origin of selectivity of the VDR protein towards its agonists/antagonists. Finally, potent VDR agonists were also designed that could be the future alternative drug for osteoporosis management for CKD patients.

The chapter 1 focused on the association study of the VDR gene polymorphism with CKD patients in South Indian population. The patients (n=147) and controls (n=210) were recruited from Tamilnadu, India. From this study, we identified a significant difference in the genotype frequency of “ApaI – aa” (p= 0.03, OR= 0.51, 95% CI = 0.29 – 0.91) observed in the patients Vs control subjects. The haplotype analysis of ApaI (rs7975232) and TaqI (rs731236) polymorphisms showed an increased risk (1.60-fold) in CKD patients. Further, we observed that individuals with “a/T” haplotype were at a greater risk (0.25-fold higher; 95% CI = 0.09 – 0.67).

Numerous vitamin D analogs have been synthesized and used to treat osteoporotic conditions. The drug efficacies are also influenced by several SNPs of the VDR gene. So understanding the theoretical and experimental knowledge of VDR SNPs is essential. To address this issue, in chapter 2, computational quantitative ranking of functionally significant SNPs is made to prioritize SNPs for further analysis from the pool of SNPs reported in dbSNP. We investigated the functional and structural impact of SNPs in the VDR gene using four different computational tools. This approach might be applied to bring out the significance relationship between the SNP conversation levels and epidemiological studies of disease-related VDR gene. From 609 SNPs reported in dbSNP, we report on eight SNPs (R274L, I314S, R391C, E329K,
L230V, I367M, R358H, R154W) in the coding region that may have a possible functional effect on the coding region of our comparative sequence - structure – SNP based analysis tools with a low RMSD value. Further, through MD simulation studies we identified that the major H-bond interactions were abolished due to the mutations. These observations were in correlation with the binding free energy. Moreover, R274L, I314S, and R391C and L230V mutations were already reported to cause rickets using *in vitro* experiments. We expect that the results from the current computational approach on VDR with suitable validation in the near future will assist in understanding the effect of individual drug response and also the ability to generate personalized tools for the rapid diagnosis, prognosis, and disease treatment.

The importance of the VDR active site and agonist/antagonist mechanism of VDR is explained in chapter 3a. Posner *et al.*, (2010) designed two new analogs namely CTA-091 and CTA-018, of which the CTA-018 induced VDR expression (15-fold lower than 1α,25(OH)2D3) and it is under phase II clinical trial, whereas CTA-091 was not able to induce the VDR expression (>2000 nM). So that, this compound haven’t undergone for further process. To address the molecular mechanism, the binding specificity of the newly identified analogs was extensively studied through *in silico* approaches. Moreover, through molecular dynamics simulations, we proved that the sulfonic group (O=S=O) in the side chain of CTA-018 plays an important role in the regulation of VDR agonistic activity. VDR activity has been explained from the MD simulation data, which shows the binding mode of vitamin D analogs by “open” and “closed” conformations. This is persistent with Kakuda’s (Kakuda *et al.*, 2010) suggestion that His301 and His393 are involved in the agonistic and antagonistic mechanisms of VDR respectively. The electron lone pairs of the sulfonic group that interacted with His393 lead to a putative agonistic mechanism for the VDR activity.
Non-secosteroidal ligands are well-known vitamin D receptor agonists. In chapter 3b, we described a combined QM/MM to define the protein-ligand interaction energy. Apart from the His393 and His301, two other amino acids in the hinge region, viz. Ser233 and Arg270 acted as an electron donor/acceptor specific to the agonist in the distinct ligand potency. With respect to the specific interactions formed inside the VDR active site by each of the studied compounds, we have speculated that the –OH group in non-secosteroidal ligands mimic the functionality of the hydroxyl group in 1α,25(OH)₂D₃. Further, additional substitutions in the side chain, interacted with Ser233 and Arg270 may act as electron donor/acceptor specifically to the agonists by the occurrence of specific effect. The observed enhanced potency is due to the presence of the N-methyl-1H-pyrrole-2-carboxamide and 2-methylpropan-1-ol substituent, which makes its oxygen and hydrogen atoms to form hydrogen bonds with the side chain of Ser233 and Arg270, respectively. These additional interactions explain the high potency of non-secosteroidal ligands. MESP and HOMO/LUMO analysis revealed that the pyrrole ring with 2-aminopropan-l-ol group is associated with the region where a negative potential is favorable for VDR binding. Different electronic properties such as HOMO and LUMO indicate the molecular reactivity.

The fundamental of molecular modeling is the interaction and binding to form a complex, since it explains the action of most drugs and molecular docking can search the preferred orientation of a ligand for its optimal binding to a receptor active site. Although virtual screening and molecular docking are important tools in the structure based drug discovery process, selecting an appropriate method to calculate the electrostatic potential is crucial. In chapter 4a, three \textit{ab initio} (DFT, HF, LMP2) and five semi-empirical (RM1, AM1, PM3, MNDO, MNDO/d) charges were used on the basis of their performance in predicting the docking pose using IFD and binding free energy
calculations against the VDR. The results indicate that AM1 is the best charge model for VDR analogs. Further, we generated 3D – QSAR model for 38 VDR analogs. We performed structure and pharmacophore - based QSAR as 3D-QSAR methods are highly sensitive to ligand conformation and alignment method. The results revealed that the AM1 charge based QSAR produced more accurate ligand poses. Moreover, the hydroxyl group in the side chain of the VDR analogs played an important role in the VDR antagonistic activity.

In chapter 4b, an approach combining molecular docking and E-pharmacophore filtering was applied to meet the critical challenges faced in designing efficient multitarget compounds to treat complex sHPT in CKD. Initially, sweet-lead database was docked in the VDR active site using Glide, and the compounds with better binding characteristics were selected for further study. Common e-Pharmacophore models were developed for VDR with the experimentally known compound (CTA-018). Postprocessing of the docking results with pharmacophore filtering allowed us to bypass the difficulty with scoring functions that produced false positives from the molecular docking calculations. From the sweet-lead database, we found four promising compounds that have the possible effect on the VDR active site. These could be promising compounds for the management of osteoporotic conditions among CKD patients.

Overall the results from this thesis, highlights the importance of VDR in CKD pathogenesis. From this study, we conclude that high frequency of “aa” variant of VDR is observed in patients in South Indian population. Since, SNPs of VDR are important for the association of drug response for osteoporosis among CKD patients, we identified 8 functional SNPs from dbSNP using in silico tools. Further, the role of these SNPs in calcitriol drug response was also studied in detail using MD simulation.
Studies. Apart from these, the agonist and antagonist mechanism of VDR analogs were studied in detail using MD simulation studies. Further, the role of VDR active site in drug response also studied using QM/MM studies. Finally, the best charge model for VDR analogs have identified using QSAR as an evaluating model and four promising VDR agonists were designed which could improve bone loss and could be better remedy for CKD patients.