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Research Article

A CHEMICAL SYSTEMS BIOLOGY APPROACH FOR IDENTIFYING OFF-TARGETS OF CALCITRIOL

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ABSTRACT

Drugs having off-targets other than their intended targets often result in side effects. Therefore the identification of possible off-targets during the process of drug design can prevent possible side-effects and rejections of the drugs during chemical trials thereby improving therapeutic actions of drugs. Calcitriol, an FDA approved therapeutic agent, is a Vitamin D3 Receptor inhibitor that is used for the treatment of psoriasis and to prevent low levels of calcium in bone disease. However, this drug has been reported to have certain side effects and complications such as bone pain, slow growth, heart rate changes (fast, slow, or uneven). Present *in silico* based study focuses on the identification of off-targets of Calcitriol using a computational biology approach. The crystal structure of VDR-Calcitriol complex (PDB ID:2HC4) was used for binding pocket similarity search to identify the putative off-targets of Calcitriol. These were further investigated using molecular docking approach. Analogs of Calcitriol were then investigated to make a more efficient drug with fewer side effects.

Keywords: *Drug Off-Targets, Pocket Similarity Search, Molecular Docking.*

1. INTRODUCTION

Drug discovery process is an expensive and bewildering challenge for researchers all over world. Even after overcoming these vast challenges, one-third of the drugs are rejected during clinical trials or even after they have reached market [1]. This often results due to side effects of drugs which are caused due to off-target effects or due lack of systems level understanding of drug response. Earlier identification of off-targets can thus emerge out as a boon to drug discovery, thus reducing drug failures and increasing success probability. For many years the objectives of drug design have been to create target specific drugs that will affect a particular specified target [2]. But still it has been widely accepted that drug may have many physiological targets. Drug polypharmacology has stimulated efforts to predict and potray drug target associations. Some systems biology approaches have been applied to targets in lead and drug discovery within the pharmaceutical industry. Thus these approaches highlight the usefulness of *in silico* techniques to accentuate the need for collaboration between the areas of disease research and computational science.

Unintended 'off-targets' cannot be determined using pragmatic methods alone. Drugs often act on several other structurally similar protein targets which might have some implicated side-effects [3]. These side effects due to off-target interactions can limit the use of otherwise effective drugs. Torcetrapib is an example of drug which was withdrawn from phase III clinical trial due to off-target effect [4]. Thus to prevent such catastrophic drug toxicities, which are often detected only after failures in the clinical trials, prediction of unknown off-target drug interactions is required at earlier stages of development. Methods for systematic prediction of off-targets, and their correlation with side-effects, have thus engrossed severe interest, frequently in the form informatics approaches. Whereas the informatics methods have never been tested systematically on a large scale, in principle they can be deployed against thousands of targets. Current drug discovery assays are not able to detect these weak off-target interactions. Thus, improvement in the early identification of off-target interactions is critical to reduce the drug attrition rates related to toxicities.

Calcitriol or 1, 25-dihydroxycholecalciferol is the active form of vitamin D found in the body. Calcitriol is used to treat and prevent calcium deficiency and bone disease in patients whose kidneys or parathyroid glands are not working normally [5]. But Calcitriol is reported to have side-effects such as hypercalcaemia [6] that may result in

bone pain, irregular heartbeat [7], slow growth [8] etc. These side-effects may be due to certain off-target interactions yet no off-targets of Calcitriol have been reported in literature. In the present study, Calcitriol was investigated for identifying its off-targets to understand the underlying causes of its reported side-effects.

2. MATERIAL AND METHODS

2.1. Retrieval of Calcitriol information

The information about the intended target of Calcitriol and other information related to drug was retrieved from DrugBank (URL: https://www.drugbank.ca/).The DrugBank database is a freely available bioinformatics and chemoinformatics resource that contains unique information about drugs and their target [9].

2.2. Target structure retrieval

The structure of target*i.e.*Vitamin D3 Receptor complexed with Calcitriol was retrieved from PDB (PDB ID: 2HC4)(URL: http://www.rcsb.org/).PDB is repository for the 3-D structure of large number of macromolecules such as proteins, nucleic acids, carbohydrates etc. maintained by Research Collaboratory for structural bioinformatics [10].

2.3. Identifying off-targets

Off-targets of Calcitriol were retrieved from PoSSuM, a pocket similarity search tool (URL: http://possum.cbrc.jp/PoSSuM/) [11]. A total of 231 hits were obtained from PoSSuM, but most of the hits were repetitive with different PDB ID's. Some of the off-target hits had no connection with the reported side-effects, so those were not further investigated. Thus, seven off-target hits were studied.

2.4. Docking

Docking of Calcitriol with Vitamin D3 receptor (PDB ID: 2HC4) and its off-targets was done using SwissDock (URL: http://www.swissdock.ch/).

2.5. Retrieval of Structural Analogs of Calcitriol

PubChem database was searched using substructure search to identify Analogs of Calcitriol. Two thousand sixteen Analogs of Calcitriol were present in PubChem with 66 Analogs having reported bioactivity. Only 15 Analogs of Calcitriol were further investigated since they conformed with the Lipinski rule of five and 51 Analogs were not studied as their molecular weight was greater than 500 Daltons. These 15 Analogs were also docked using SwissDock.

3. RESULTS AND DISCUSSION

In the present study the off-targets of Calcitriol, a drug for hypocalcemia, osteoporosis and treatment and prevention of bone disease are being investigated to understand the causes of its reported side-effects which are bone pain, irregular heart beat and slow growth.

Drug target information was retrieved from DrugBank, which gave Vitamin D3 Receptor as the intended target for Calcitriol. In addition it gave information about chemical formula of drug and its SMILE key.

DrugBankDB00136

(1R,3S,5Z)-5-{2-[(1R,3aS,4E,7aR)-1-[(2R)-6-hydroxy-6-methylheptan-2-yl]-7a-methyl-octahydro-1H-inden-4-ylidene]ethylidene}-4-methylidenecyclohexane-1,3-diol

SMILES

C[C@H](CCCC(C)(C)O)[C@@]1([H])CC[C@@]2([H])\C(CCC[C@]12C)=C\C=C1\C[C@@H](O)C[C@H](O)C1=C

Seven off-targets retrieved from PoSSuM are enlisted in Table 1. In addition to this, PoSSuM also gave binding pocket residues of Calcitriol in actual and off-targets given in Table 2. An online docking server, Swissdock was used for docking of Calcitriol with its actual target (PDB ID: 2HC4) and off-targets retrieved from PoSSuM (Fig 1) visualized in UCSF Chimera. The docking scores are summarized in the Table 3.

Table 1: Hits obtained for Off-targets of Calcitriol using PoSSuM

PDB ID	Protein Name	P value	RMSD
1N46	Thyroid Hormone Receptor beta	0.059986	2.06
2I4J	Peroxisome proliferator-activated receptor gamma	0.045877	1.91
3GD2	Farsenoid X Receptor	0.023321	1.69
4JYG	Retinoic Acid Receptor beta	0.050554	1.73
3E7C	Glucocorticoid Receptor	0.001484	1.93
4UDB	Mineralocorticoid Receptor	0.058295	2.29
2Q6J	Estrogen receptor	0.053953	1.97

From the results of docking it was observed that the binding energies of off-targets of Calcitriol were comparable with the binding energy of actual target Vitamin D3 Receptor. This non-specific binding of Calcitriol with its off-targets may be responsible for its

observed side-effects in patients prescribed Calcitriol to treat and prevent calcium deficiency and bone disease.

Binding pocket residue analysis (Fig. 2) indicated that some residues were identical in the binding pocket of actual target (PDB ID: 2HC4) and off-targets (PDB ID's: 2I4J, 3GD2 & 4UDB) such as Alanine, Arginine, Leucine, Methionine, Phenylalanine and Serine. The off-targets Farsenoid X receptor, Peroxisome proliferator-activated receptor gamma and Mineralocorticoid receptor due to their interaction with Calcitriol may result in the reported side-effects observed following Calcitriol intake.

Table 2: Binding pocket residues in the binding sites of Calcitriol

	Target	Binding Pocket
Actual target	2HC4	tyr175, tyr179, phe182, leu255, leu258, leu261, val262, ser265, iso296, iso299, met300, **arg302, ser303**, ser306, trp314, cys316, tyr323, val328, ala331, his333, leu337, 338,341, his423, tyr427, leu430,440, val444, phe448
Off-targets	2I4J	phe226, phe282, cys285, **arg288, ser289**, ala292, his323, ile326, tyr327, met329, leu330, leu333, val339, ile341, met348, leu353, met364, lys367, his449, leu453, leu465, leu469, tyr473
	3GD2	arg264, met265, thr270, phe284, leu287, met290, ala291, his294, val325, met328, phe329, **arg331, ser332**, ile335, gly343, leu348, ile352, ile357, tyr361, met365, his447, trp454, phe461, leu465, trp469
	4UDB	leu766, leu769, leu772, ala773, glu776, met807, leu810, ser811, leu814, arg817, phe829, met840, met845, leu848, cys849, met852, leu938, cys942, thr945, phe956, leu960
	1N46	asn233, phe269, phe272, iso275, ile276, ala279, met310, met313, ser314, arg316, ala317, arg320, thr329, gly332, leu341, gly344, gly345, leu346, iso353, his435, met442, phe455
	4JYG	phe192, trp218, phe221, leu224, ala225, cys228, leu259, leu269, ile263, arg265, ile266, arg269, thr278, phe279, phe295, leu298, val302, gly384, val388, leu391, met399, ile403, met407
	3E7C	met560, leu563, leu566, gly567, gln570, met601, met604, ala605, ala607, leu608, arg611, cys622, phe623, met639, gln642, met646, leu732, cys736, thr739, phe749, leu753, ile757
	2Q6J	met343, leu346, leu349, ala350, glu353, leu384, leu387, met388, leu391, arg394, phe425, leu428, gly521, leu525, met528, leu540

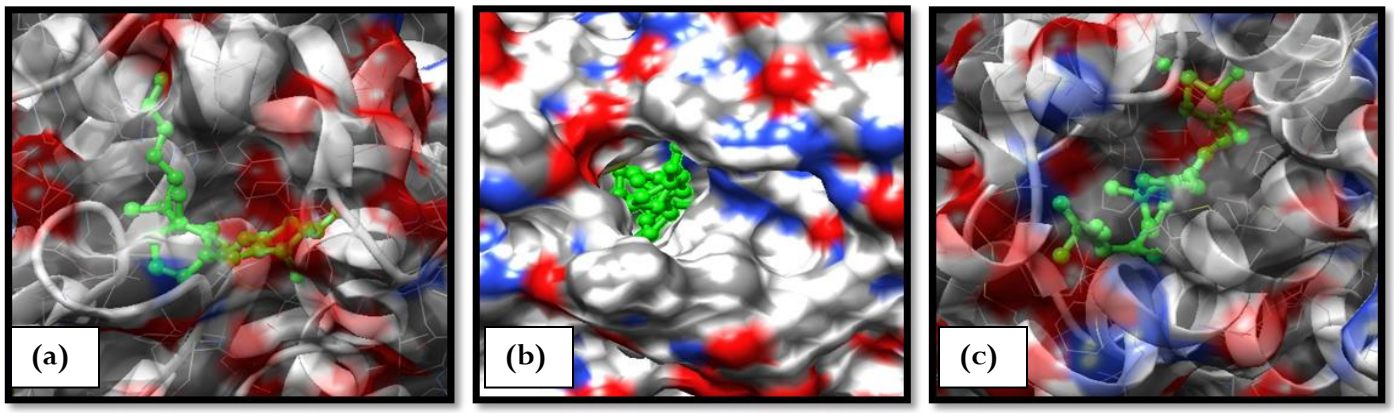


Fig. 1: Docking of Calcitriol with its (a) actual target i.e. Vitamin D3 Receptor (PDB ID: 2HC4) and off-targets (b) Farsenoid X Receptor (PDB ID:3GD2) and (c) Mineralocorticoid receptor (PDB ID:4UDB).

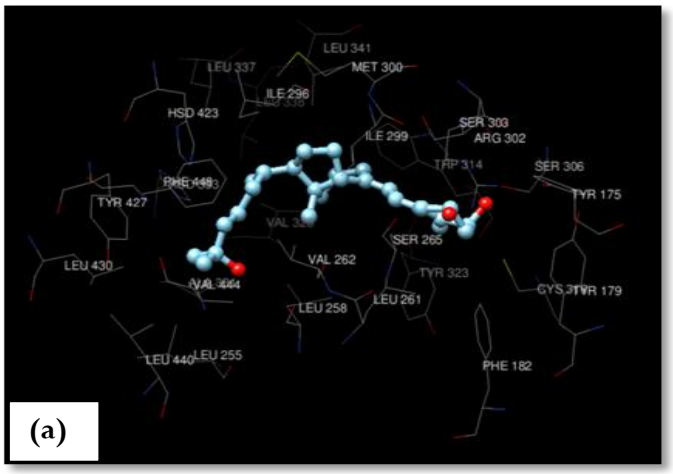
Farsenoid X (Bile acid) receptor (PDB ID: 3GD2): In 2011, Khurana S *et al*. reported that bile acids regulate heart rate by affecting channels in cardiomyocytes and also regulates vascular tone [12]. Therefore prescription of Calcitriol to prevent calcium deficiency and bone disease may be responsible for changes in heart rate, reported as a side effect following Calcitriol prescription.

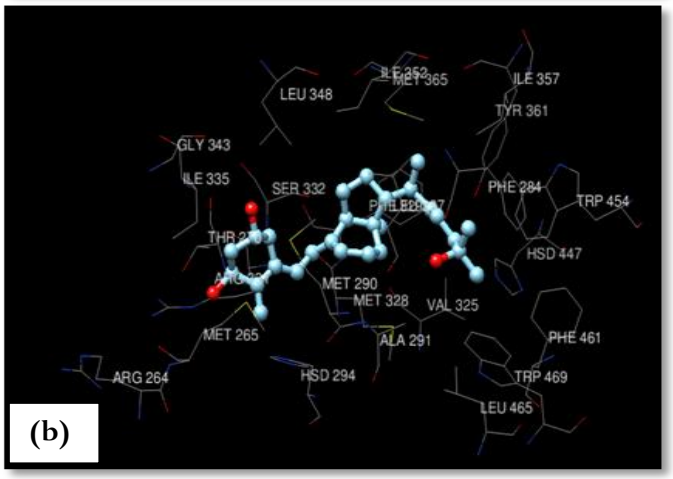
Mineralocorticoid receptor (PDB ID: 4UDB) is another off-target that is involved in controlling cellular calcium homeostasis. It has been reported that cellular

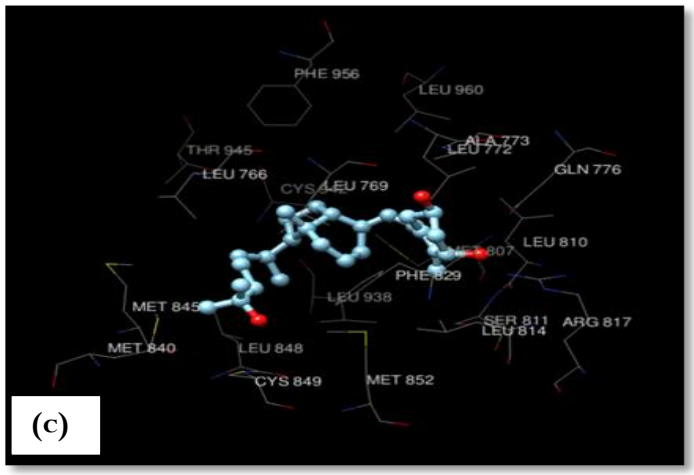
calcium homeostasis, sarcoplasmic reticulum diastolic leaks and extension in action potential are controlled by activation of cardiac mineralocorticoid receptor, which results in rhythm disorders [13] and may be responsible for irregular heart rate following Calcitriol intake.

Table 3: Docking scores of Calcitriol with its actual and off-targets.

Receptor	Full fitness (kcal/mol)	Free energy (ΔG) (kcal/mol)
Target		
2HC4	**-1528.18**	**-10.77**
Off-targets		
3GD2	**-1388.49**	**-10.66**
4UDB	**-1432.08**	**-9.92**
2I4J	**-1792.25**	**-9.12**
1N46	**-1522.11**	**-8.23**
4JYG	**-1302.06**	**-8.06**
3E7C	**-1232.51**	**-7.42**
2Q6J	**-1094.24**	**-7.03**







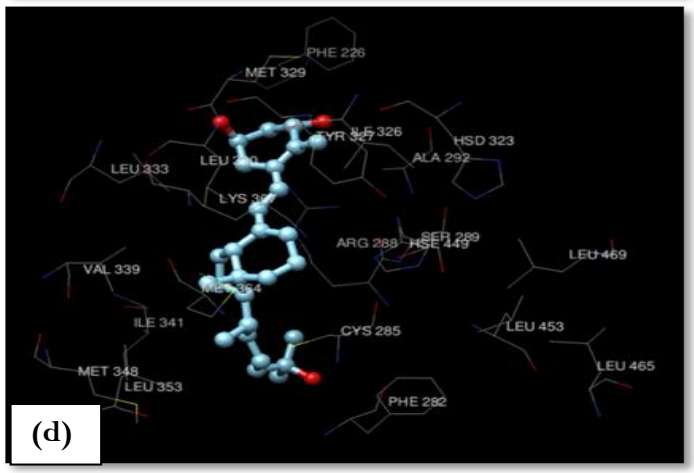


Fig. 2: Binding site residues of (a) Vitamin D3 Receptor (PDB ID: 2HC4), (b)Farsenoid X Receptor (PDB ID: 3GD2) and (c) Mineralocorticoid receptor (PDB ID:4UDB) and (d) Peroxisomeproliferator-activated receptor gamma (PDB ID: 2I4J) respectively.

Peroxisome proliferator activated receptors (PPARs) (PDB ID: 2I4J) is yet another off-target identified in the present study. These nuclear transcription factors subtypes have been identified in osteoblasts and osteoclasts [14] and thus may result in the side effect of bone pain.

The docking sites of other four off-targets obtained from PoSSuM (Table 1) namely, Thyroid hormone receptor (PDB ID: 1N46), Retinoic acid receptor (PDB ID: 4JYG), Glucocorticoid receptor (PDB ID: 3E7C) and Estrogen receptor (PDB ID: 2Q6J) were different from the binding pockets predicted by PoSSuM. Yet Calcitriol binds efficiently at these off-targets as seen from docking results.

The importance of the thyroid hormone in the regulation of skeletal growth and bone metabolism has been well established. Studies suggest that thyroid hormone

regulates a number of signaling pathways including insulin-like growth factor-I, parathyroid hormone related protein, fibroblast growth factor to influence skeletal growth [15]. Therefore, the observed side effect of bone pain and slow growth may be due to the binding of Calcitriol to the thyroid hormone receptor (PDB ID: 1N46) thereby inhibiting it, disrupting its function since binding of thyroid receptor to its thyroid hormone regulates bone cell functions and growth.

Retinoic acid (RA), which acts through retinoid receptors, is involved in signal transduction pathways. Retinoic acid is important for the development of the heart. Targeted gene deletion of retinoic acid receptors suggests its importance during early cardiovascular morphogenesis. Specific cardiovascular targets of retinoid action include effects on the specification of cardiovascular tissues during early development, anteroposterior patterning of the early heart, left/right decisions and cardiac situs, endocardial cushion formation, and in particular, the neural crest [16]. Therefore off-target interactions of Calcitriol with retinoic acid receptor (PDB ID: 4JYG) may also be responsible for the irregular heart function following Calcitriol regimes.

Glucocorticoids are steroid hormones which act through the glucocorticoid receptor. They regulate a wide variety of biological processes. Glucocorticoids results in a decrease in cytosolic Ca^{2+} concentration in neurons and astrocytes [17]. Due to the role of glucocorticoid receptor in Ca^{2+} signaling may be responsible for the side effect of bone pain following Calcitriol prescription due to its off-target interaction of glucocorticoid receptor (PDB ID: 3E7C).

Estrogen receptor is yet another off-target of Calcitriol which has been identified in the present study. Estrogen plays a significant role in regulation of intestinal calcium absorption and its deficiency results in postmenopausal osteoporosis and also results directly in calcium malabsorption which is the major cause of bone loss [18]. Thus estrogen receptor (PDB ID: 2Q6J) might also be responsible for the side effect of bone pain after Calcitriol intake.

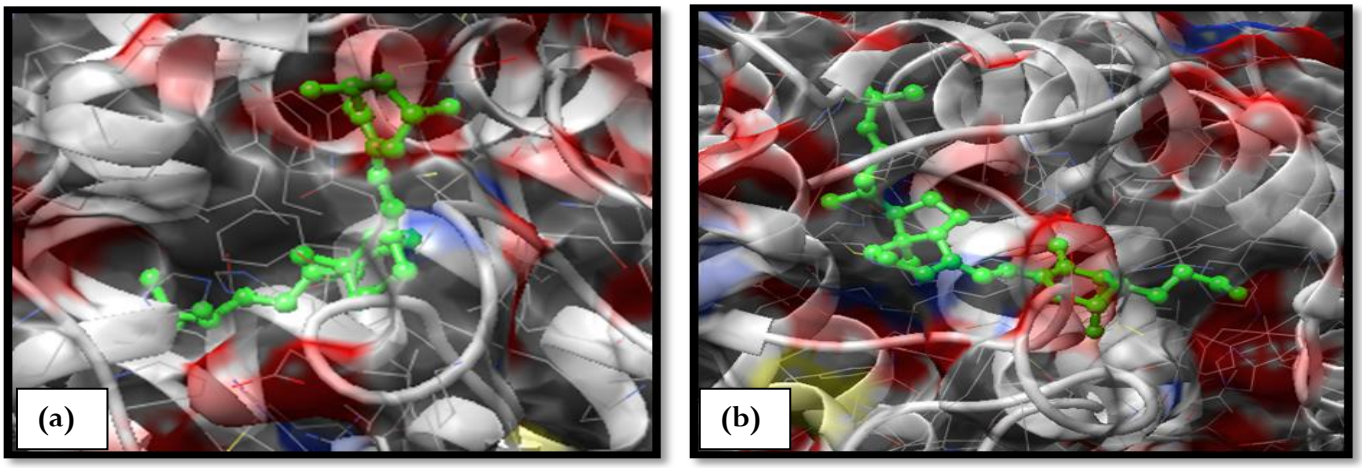


Fig. 3: Docking of (a) Analog 2 and (b) Analog 11 with Vitamin D3 Receptor.

Table 4: Docking scores of structural Analogs of Calcitriol with Vitamin D3 Receptor using Swissdock

Ligand	Full Fitness (kcal/mol)	Free energy (△G) (kcal/mol)	Ligand	Full Fitness (kcal/mol)	Free energy (△G) (kcal/mol)
Calcitriol	-1528.17	-10.77	**Analog 8**	-1475.31	-10.35
Analog 1	-1515.19	-09.16	**Analog 9**	-1528.13	-10.49
Analog 2	-1508.67	-11.31	**Analog 10**	-1528.79	-10.72
Analog 3	-1500.08	-10.41	**Analog 11**	-1523.88	-11.31
Analog 4	-1526.35	-10.04	**Analog 12**	-1495.95	-10.33
Analog 5	-1496.83	-09.87	**Analog 13**	-1503.45	-10.02
Analog 6	-1504.82	-10.50	**Analog 14**	-1495.44	-10.16
Analog 7	-1510.88	-10.22	**Analog 15**	-1479.59	-10.08

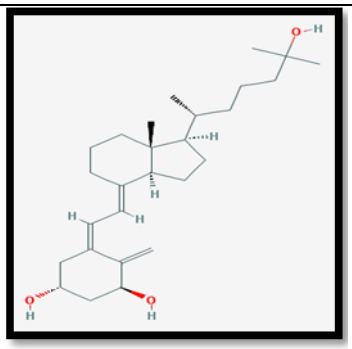
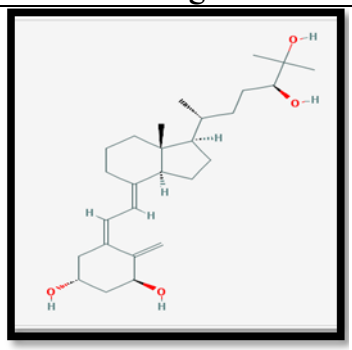
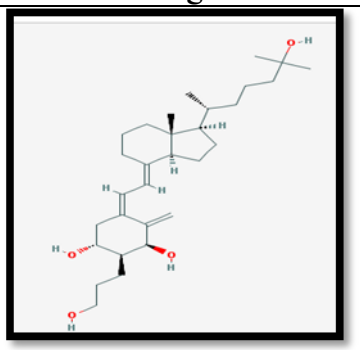
Docking of Analogs from PubChem was done using SwissDock (Fig 3)and docking scores are given in Table 4.

From the 15 Analogs investigated, only two Analogs *i.e.* Analog 2 and Analog 11 had free energy of binding more negative than Calcitriol binding with vitamin D3 receptor (Table 4). Both these Analogs *i.e.* Analog 2 and Analog 11 were then docked with all the seven off-targets obtained from PoSSuM. The docking scores are enlisted in Table 5.

Table 5: Docking scores of Analog 2 and Analog 11 of Calcitriol with off-targets of Calcitriol using SwissDock

Receptor	Analog 2		Analog 11	
	Free energy (ΔG) (kcal/mol)	Full Fitness (kcal/mol)	Free energy (ΔG) (kcal/mol)	Full Fitness (kcal/mol)
2HC4	-11.31	-1508.62	-11.31	-1523.88
3GD2	-10.72	-1363.44	-10.35	-1357.31
4UDB	-07.47	-1441.18	-08.19	-1451.65
2I4J	-09.14	-1769.05	-09.07	-1781.48
1N46	-08.80	-1508.62	-08.17	-1512.06
4JYG	-08.08	-1272.71	-07.57	-1451.65
3E7C	-08.77	-1188.08	-05.60	-1095.88
2Q6J	-06.88	-1070.87	-07.79	-1090.21

Table 6: Drug properties predicted using DataWarrior

Properties	Calcitriol	Analog 2	Analog 11
Structure			
Total weight	416.643	432.642	474.723
cLogP	5.9646	4.9428	6.216
cLogS	-5.146	-4.747	-5.339
H-Acceptors	3	4	4
H-Donors	3	4	4
Polar surface area	60.69	80.92	80.92
Druglikeness	-5.1086	-6.007	-9.7383
Mutagenic	None	None	None
Tumorigenic	None	None	None
Reproductive effective	None	None	None
Irritant	None	None	None

From the docking studies it was observed that there was no appreciable change in the binding energies of the off-targets with the Analogs as compared to Calcitriol, though the Analogs were bound to actual target *i.e.* Vitamin D3 Receptor more strongly. Therefore, it can be concluded that the Analog 2 and Analog 11 have higher specificity for Vitamin D3 receptor as compared to off-targets and therefore may be considered to replace

Calcitriol in treatment of disorder where Calcitriol is usually prescribed. Both Analog 2 and Analog 11 have similar free energy of binding with the vitamin D3 receptor but the full fitness score of Analog 11 is slightly more negative than Analog 2 suggesting that it might be more suitable than Calcitriol and Analog 2.

Drug properties of Calcitriol, Analog 2 and Analog 11 (Table 6) were predicted using DataWarrior. Table 6 gives the drug properties such as molecular weight, cLogP, cLogS, H-Acceptors, H-Donors and druglikeness. From the Table 6 it can be concluded that Analog 2 is better than Analog 11, due to its more favorable drug like properties. The cLogP value of Analog 11 is more than 5 which is against Lipinski's rule of five, while for Analog 2 it is less than five and thus making it a better substitute for Calcitriol.

4. CONCLUSION

From the present study we concluded that the side-effects observed after Calcitriol intake are due to certain off-target interactions with Farsenoid X Receptor, mineralocorticoid receptor, peroxisome proliferator receptor gamma, thyroid hormone receptor and others and thus to reduce these side-effects due to certain off-target interactions, the drug is required to be made more specific for actual receptor which is Vitamin D3 Receptor. Therefore, out of 15 Analogs of Calcitriol identified using PubChem, Analog 2 might perform better than Calcitriol and appear as a better substitute to minimize side-effects.

5. REFERENCES

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