International Journal of Bone and Rheumatology Research (IJBRR)
ISSN: 2470-4520

In silico Pharmacological Screening of Nitrogen containing Bisphosphonate Conjugate with Hydroxyapatite Active Constituents in Finding Potent Inhibitors for Bone Related Diseases

Research Article

Kalyani A[1*], Bharath Srinivasan[1], Parasuraman Pavadai[2]

[1] Department of Pharmaceutics, Faculty of Pharmacy, M.S Ramaiah University of Applied Sciences, Bangalore, Karnataka, India.
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, M.S Ramaiah University of Applied Sciences, Bangalore, Karnataka, India.

Abstract

Osteoporosis is a skeletal disease-causing bone fragility by disturbing micro architecture of the bone leading to osteoclasts mediated bone loss. Inhibition of the Farnesyl Pyrophosphate Synthase (FPPS) of mevalonate pathway and Receptor Activator of Nuclear factor kappa-B ligand (RANKL/OPG) complex by using anti resorptive drugs like Nitrogen containing Bisphosphonates aid in the effective treatment of osteoporosis. The in-silico docking analysis of newly synthesized Ibandronate–hydroxyapatite conjugate has shown as a most powerful binary to HMG-COA Reductase, Farnesyl pyrophosphate synthase (FPPS), Human Geranyl Geranyl pyrophosphate synthase (GPPS) & RANKL/OPG in contrast to pure Ibandronate. The docking score of Ibandronate hydroxyapatite (IBA-HAP) was found to be -6.12, -6.75, -5.33 and -6.49 as against standard pure Ibandronate of -1.28, -2.07, -1.97 and -3.23 kcal/mol. Also, ΔG binding energy and pIC_{50} values showed promising potential anti-osteoporotic effect for Ibandronate-hydroxyapatite conjugate.

Keywords: N-Bisphosphonates; Mevalonate pathway; Ibandronate Hydroxyapatite Conjugate; Anti-Osteoporotic Activity; Molecular Modelling.

Introduction

Osteoporosis (OP) is a skeletal disease which thins bone and weakens it making porous, fragile and finally leading to bone fracture easily. Majorly low bone mass is seen deterioration of the micro architectural of bone leading to pain and disability [4].

The concept behind bone becoming weaker and thinner with aging and disease is due to FPPS, a key regulatory enzyme in the mevalonate pathway which is responsible for the bone metastases and osteoclasts mediated bone loss. Anti-resorptive agents are used in various clinical conditions like hypercalcemia, Paget's disease, cancer treatment apart from metabolic bone diseases [10]. Bisphosphonates are the analogues of pyrophosphate and are widely used in osteoclasts mediated bone loss due to induced long term therapy of glucocorticosteroids and also in post-menopausal women [5]. A step by step deterioration in bone mass that effects both sexes and due to menopause in women, where women begin to extremely lose bone mass [11].

Bisphosphonates (BPs) are regarded as most potent drug, widely used in patients with osteoporosis on osteoclasts suppression [9]. It inhibits FPPS in mevalonate pathway which in turn prevents osteoclasts by acting on biosynthesis of farnesylation and geranylgeranylation post translational of signalling proteins where by inhibiting the activity of osteoclasts and reducing bone resorption and increasing bone turn over and increase in net bone mass [6]. These BP's are having high binding affinity towards hydroxyapatite indicating high potency and a long duration of action. Nearly 70% in weight, 50% in volume of human bone contains hydroxyapatite, commonly known as bone mineral. It contains excellent biocompatibility and bioactivity with stoichiometric Ca/P ratio of 1.67 with a hexagonal structure. It is having unique properties like Osteo conductivity, biocompatibility, bioactivity, non-toxicity and non-inflammatory nature [12].

BPs, are the bone mineralization endogenous regulators and were alternatively binding to bone sites for active osteoclasts bone re-

***Corresponding Author**:
Kalyani A,
Research Scholar, Department of Pharmaceutics, Faculty of Pharmacy, M.S Ramaiah University of Applied Sciences, Bangalore, Karnataka, India.
E-mail: kalyani6009@gmail.com

Received: March 10, 2020
Accepted: July 22, 2020
Published: July 28, 2020

Citation: Kalyani A, Bharath Srinivasan, Parasuraman Pavadai. *In silico* Pharmacological Screening of Nitrogen containing Bisphosphonate Conjugate with Hydroxyapatite Active Constituents in Finding Potent Inhibitors for Bone Related Diseases. *Int J Bone Rheumatol Res.* 2020;5(1):77-84. doi: http://dx.doi.org/10.19070/2470-4520-2000016

sorption, which held by osteoclasts and sequentially inhibit bone resorption [13]. Cholesterol synthesis of mevalonate pathway is a bio synthetic pathway responsible for osteoclasts inactivation which is crucial in achieving the therapeutic target [1] by inhibiting some of the isoprenoid lipids like HMG CO-A synthase, HMG CO-A reductase, mevalonate kinase, Human Geranyl Geranyl pyrophosphate synthase which bound to Geranyl Geranyl pyrophosphate (GGPP) that are essential for the activation of prenylation of small GTPase isoprenoids to prenylation GTPase signalling protein that helps in normal functioning of proteins [7]. Another protein target Cytokine receptor triad Human RANKL/OPG a bone morphogenic protein (BMP-2) that crucially controls the terminal differentiation step of osteoblasts and osteoclasts also binds to a protein secreted by the cells of osteoblasts linage known as Osteoprotegerin [2].

In the present paper, the comparative docking analysis of newly synthesized Ibandronate–hydroxyapatite conjugate and pure Ibandronate (N-Bisphosphonate drug) ligand with target proteins like mevalonate pathway enzymes and RANKL/OPG have been discussed.

Materials and Method

Autodock works on Monte Carlo simulated annealing and Lamarckian genetic algorithm (LGA) to create a set of probable conformations. LGA method was used as a global optimizer and energy minimizer. The docking studies were carried out by using Autodock 4.2 suite to predict binding of molecules to the target protein structure [8]. Apart from generating the binding energies of the docked molecules, nature and the position of ligand in the binding site can be visualized. In this docking simulation, semi-flexible docking protocols have been used in which the target proteins were set aside as rigid. The drug substrate to be docked were kept flexible, in order to discover random number of torsional degrees of freedom in addition to the six spatial degrees of freedom spanned by the translational and rotational parameters.

Ligand preparation

The ligand molecular structure of Ibandronate and Ibandronate-hydroxyapatite conjugate were drawn using chem draw Ultra 7.0.4. (Figure 1 & 2).

Protein preparation

The crystal structure of protein receptors with PBD ID's – 3SQZ (HMG_CoA synthase), 1DQ9 (Human HMG-COA Reductase), 2F7M (Human Farnesyl Pyrophosphate synthase (FPPS))[13], 2HFU (Mevalonate kinase), 2Q80 (Human Geranyl Geranyl pyrophosphate synthase bound to GGPP), of Mevalonate pathway and 3URF (Human RANKL/OPG complex), solved using X- ray diffraction method obtained from RSCB protein data bank (PDB) (http://www.rcsb.org/). Protein structure were subjected for protein preparation using BOVIA Discovery Studio 2017 R2 Client software. He-atoms and water molecules were removed from the protein structure and subjected to applied force field in order to minimize the energy of the atoms present in the structure and made them stable to react with the ligands. Hydrogen atoms were added, Gasteiger chargers were assigned by merging non polar bonds, followed by assigning of AD4 type to the protein receptor.

Docking Protocol

Ligands were defined for rigid roots of Ibandronate and Ibandronate conjugate hydroxyapatite automatically. The grid box size was set at 90, 90, and 90 A° (X, Y, and Z), depending on the protein size. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. The size of the population was

Figure 1. Two-dimensional Structure of Ibandronate ligand.

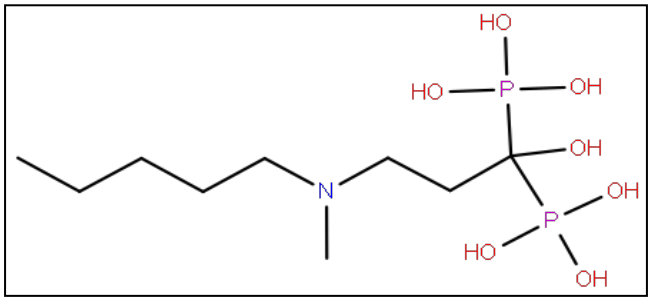
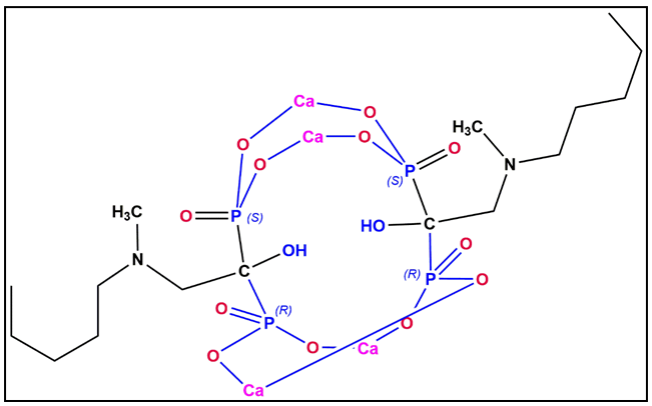


Figure 2. Two-dimensional Structure of Ibandronate drug conjugate with Hydroxyapatite ligand.



set to 100 and the individuals were initialized arbitrarily. Energy estimation was set to 500000, maximum number of generations 1000, maximum number of top individual that automatically survived was set to 1to 10 LGA runs were performed [3].

Grid generation

Receptor grid was generated to determine the size of the active site. The most probable orientation of the ligands in the binding pocket is identified and a scoring function is used to quantify the strength ofthe interaction in a molecule can make in a particular orientation. In this the grid box is prepared by taking X, Y, Z dimensions as 90, 90, 90 respectively centred the grid box on macromolecule. The precision was favoured over the standard mode sequentially give a better correlation between good poses and good scores.

Results and Discussion

The docking analysis was done for the ligands with the target proteins RANKL/OPG, Mevalonate pathway enzymes using the docking software Autodock and the docked images are shown. The structures dockedby Autodock are generally ranked according to the Binding energy function & pIC_{50} values. Autodock program scoring function is presented in the kcal/mol form. The method of evaluating the accuracy of a docking procedure is todetermine how closely the lowest energy pose (binding conformation) predicted by the object scoring function.

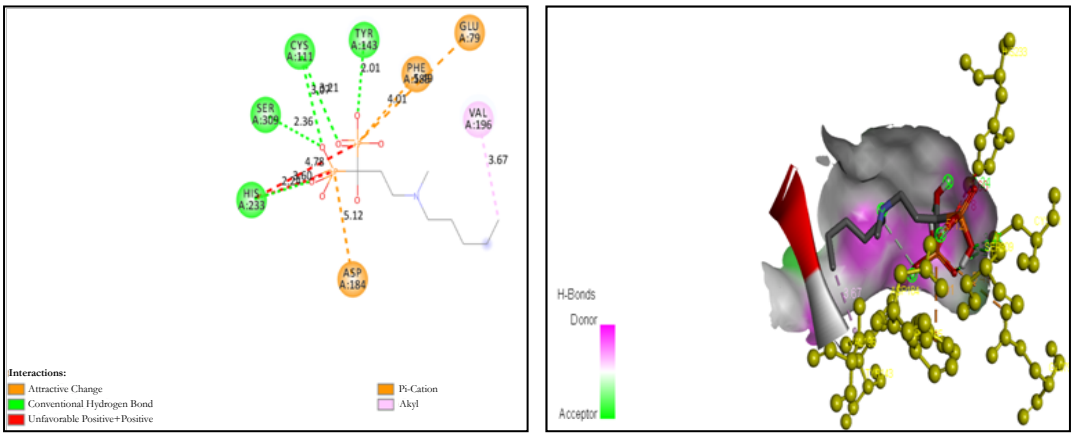
To study the binding interaction and binding affinity of the Ibandronate and Ibandronate hydroxyapatite conjugate to HMG-CoA synthase, Human HMG-COA Reductase, FPPS, Mevalonate kinase, GGPS of Mevalonate pathway and Human RANKL/OPG complex, both the ligands were docked into theactive site ofproteins. The docking result of these ligands is given in Tables 1 and 2. The interaction energywere calculated for each complex.

The docking score by using Autodock has shown -1.84 kcal/mol, -0.16 kcal/mol for Ibandronate ligand and Ibandronate hydroxyapatite conjugate ligand with HMG COA synthase(PDB ID: 3SQZ) shown the phosphate groups of the parent Ibandronate molecule had formed conventional hydrogen bonding with HIS233, SER309, CYS111, TYR143 of A-chain amino acid residues; the alkyl group had formed alkyl bonding with VAL196 of A chain amino acid residues; the oxygen atom of phosphate group had undergone attractive charges with PHE185, GLU79, ASP184 of A-chain amino acid residues (Figure 3), whereas the interactions of Ibandronate hydroxyapatite conjugate molecule with the protein receptor of HMG co A synthase shown, the oxygen atoms of phosphate groups had formed an unfavourable positive-positive interactions with SER309, CYS111, PHE185, HIS233, ASN206, TYR278, PRO235, PHE236, VAL196, SER201; the phosphate atom of the molecule had undergone attractive charges and pi-cation interactions with GLU79, PHE185, ASP184 and a conventional hydrogen bonding was found with another oxygen atom of the molecule with THR152 of A-Chain amino acid residues (Figure 4).

Table 1. Docking results of the parent and final drug molecules with various protein targets.

Sl. No.	Protein (PDB ID)	Binding Energy (Kcal/mol)		pIC_{50} values	
		Pure Ibandronate (Parent)	IBA-HAP Conjugate	Pure Ibandronate (Parent)	IBA-HAP Conjugate
1	3SQZ	-1.84	-0.16	44.58mM	17.47mM
2	1DQ9	-1.28	-6.12	25.12mM	32.49 µM
3	2F7M	-2.07	-6.75	30.31mM	11.37µM
4	2HFU	-2.98	-1.67	6.51mM	7.85mM
5	2Q80	-1.97	-5.33	35.88mM	123.93µM
6	3URF	-3.23	-6.49	4.3nM	17.47 µM

Figure 3. 2D & 3D surface interactions of 3SQZ with Pure Ibandronate drug molecule.



Ibandronate ligand and Ibandronate hydroxyapatite conjugate ligand with Human HMG-COA Reductase (PDB ID: 1DQ9) shown docking score -1.28 kcal/mol & -6.12 kcal/mol, the phosphate groups of the parent molecule Ibandronate had formed conventional hydrogen bonding with VAL772, ASN776 of B-chain amino acid residues; the alkyl group had formed alkyl bonding with PRO693 of A chain and PRO693 of B-chain amino acid residues; the oxygen atom of phosphate group had undergone carbon hydrogen bonding with GLY773 of B-chain amino acid residues (Figure 5), the interactions of conjugate molecule with the protein receptor shown the oxygen atoms of phosphate groups had undergone conventional hydrogen bonding with ASN771, ASN750, CYS777, ALA694 of D-Chain amino acid residues; the alkyl side chain had undergone alkyl bonding with PRO693 of D-chain amino acid residue and PRO693, ALA695, ILE746 of C- Chain amino acid residues and covalent bonding with PRO693 of C- Chain amino acid residue and GLY773, ASN776, VAL772, SER775 OF D-Chain amino acid residues (Figure 6).

The docking score shown -2.07 kcal/mol, -6.75 kcal/mol for Ibandronate ligand and Ibandronate hydroxyapatite conjugate ligand with Human Farnesyl Pyrophosphate synthase (FPPS) (PDB ID: 2F7M) shows, the phosphate groups of the parent molecule had formed conventional hydrogen bonding with THR201, LYS200, LYS266 of F-chain amino acid residues; the alkyl chain of parent molecule had formed carbon hydrogen bonding with ASP247, ASP261, ASP244 of F-chain amino acid residues; the phosphorus atom of phosphate group had undergone attractive charges with ASP174 of F-chain amino acid (Figure 7) the interactions of conjugate with the protein receptor shown, the alkyl side chain had undergone pi-alkyl interactions with TYR193 of F-chain amino residue; the oxygen atoms of phosphate groups had undergone conventional hydrogen bonding with ARG 112, GLN96, ARG60, SER205, LYS200 of F-chain amino acid residues; the dihydro pyridine of conjugate had undergone attractive charge interaction with ASP243 of F-chain amino acid residue (Figure 8).

The docking score shown -2.98 kcal/mol, -1.67 kcal/mol for Ibandronate ligand and Ibandronate hydroxyapatite conjugate ligand with protein receptor Mevalonate kinase (PDB ID: 2HFU) shows the phosphate groups of the Ibandronate molecule had formed conventional hydrogen bonding with GLN229, HIS225 of A-chain amino acid residues and GLN229, GLN244 of B-chain amino acid residues; the alkyl chain of parent molecule had formed alkyl bonding with ALA248 of A chain and ALA248 of B-chain amino acids (Figure 9) The interactions of Ibandronate hydroxyapatite conjugate shows, the oxygen atoms of phosphate groups had undergone unfavourable positive-positive interactions with GLN244, ALA248, LEU245, GLN244, HIS225, GLN229, HIS225, LEU245 of A&B-chain amino acid residues respectively; another oxygen atom of phosphate group had undergone carbon hydrogen bonding with ILE226, ARG241 of A-chain amino acid residue; the hydroxyl group had undergone conventional hydrogen bonding with GLN229 of B-chain amino acid residue (Figure 10).

The interactions of Ibandronate molecule with the protein receptor with PDB ID: 2Q80 (Human Geranyl Geranyl pyrophosphate synthase bound to GGPP), shows the oxygen atom of phosphate groups of the Ibandronate molecule had formed conventional hydrogen bonding with GLN236, GLU239, ARG237, THR238 amino acids (Figure 11) with the docking score -1.97 kcal/mol and -5.33 kcal/mol for the Ibandronate Hydroxyapatite conjugate and the interactions are, the oxygen atoms of phosphate groups had undergone conventional hydrogen bonding with ILE85 of

Figure 4. 2D &3D surface interactions of 3SQZ with Ibandronate -Hydroxyapatite conjugate.

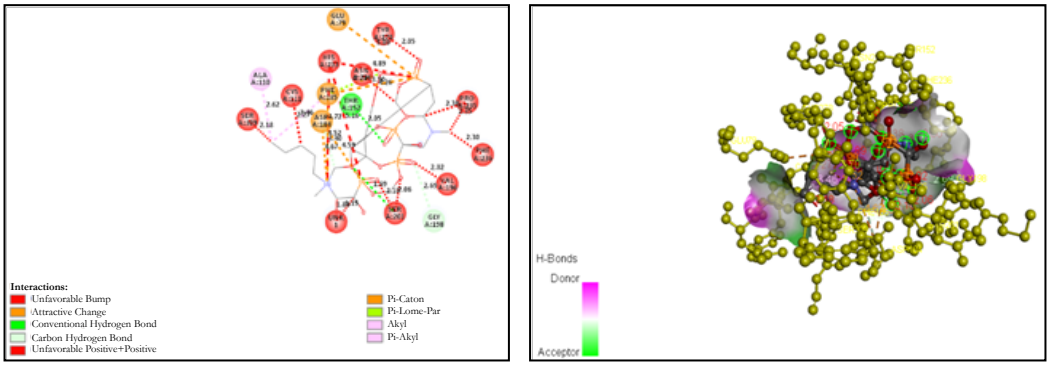


Figure 5. 2D & 3D surface interactions of 1DQ9 with Pure Ibandronate drug molecule.

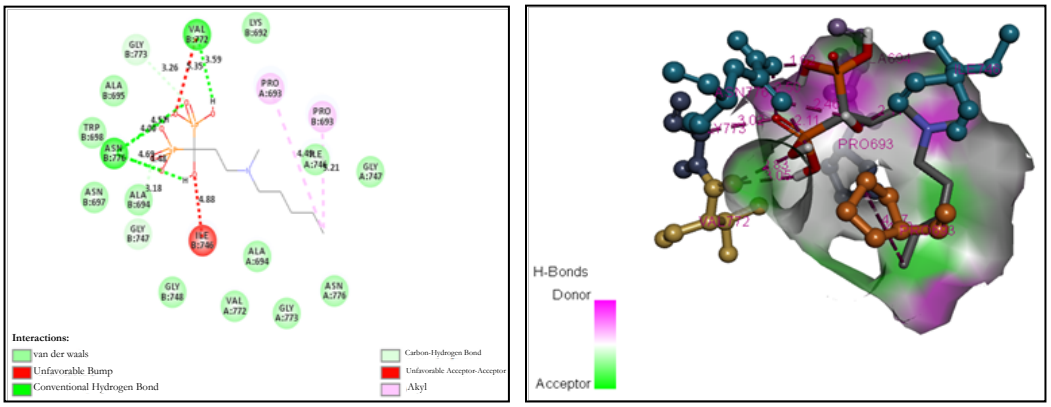


Figure 6. 2D & 3D surface interactions of 1DQ9 with Ibandronate hydroxyapatite conjugate.

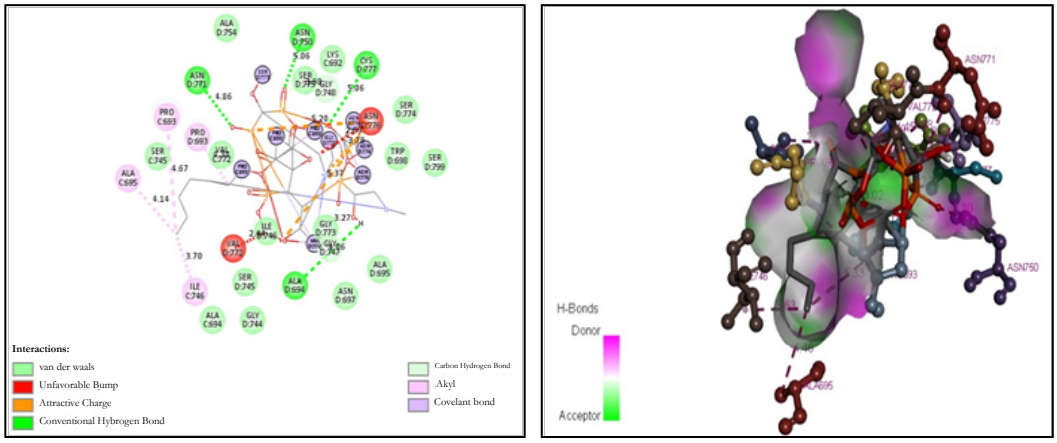


Figure 7. 2D& 3D surface interactions of 2F7M with Pure Ibandronate drug molecule.

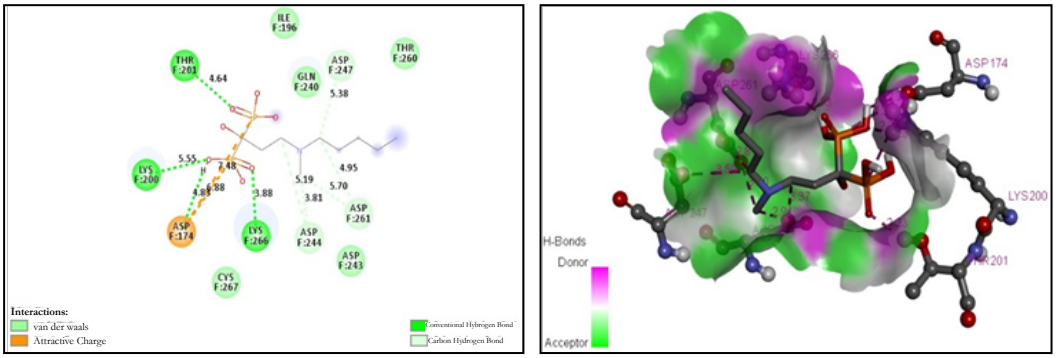


Figure 8. 2D &3D surface interactions of 2F7M with Ibandronate -Hydroxyapatite.

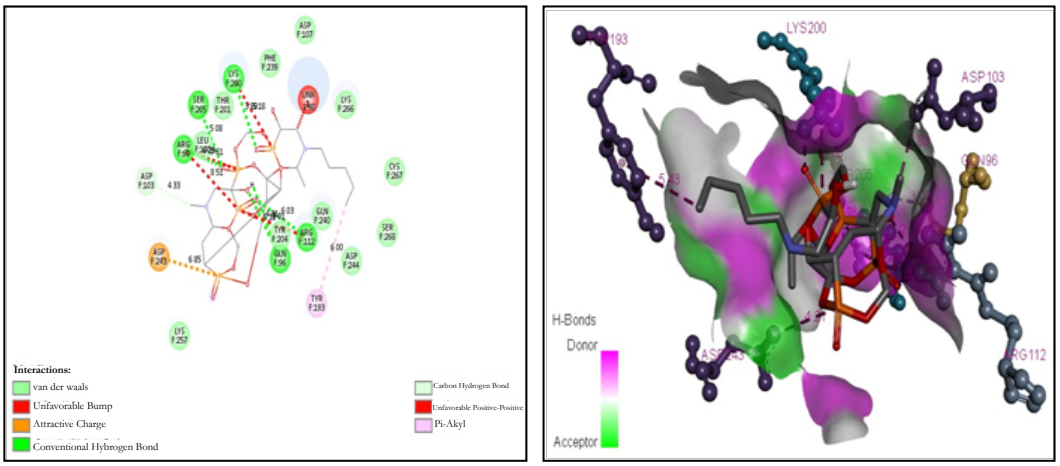


Figure 9. 2D & 3D surface interactions of 2HFU with Pure Ibandronate drug molecule.

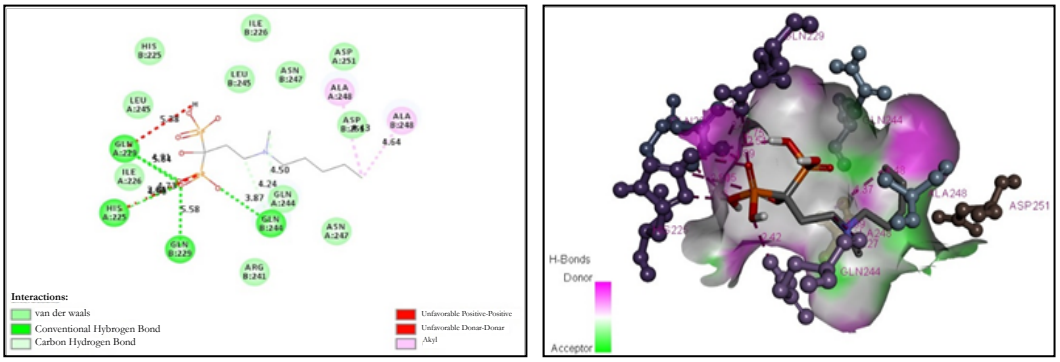


Figure 10. 2D & 3D surface interactions of 2HFU with Ibandronate -Hydroxyapatite.

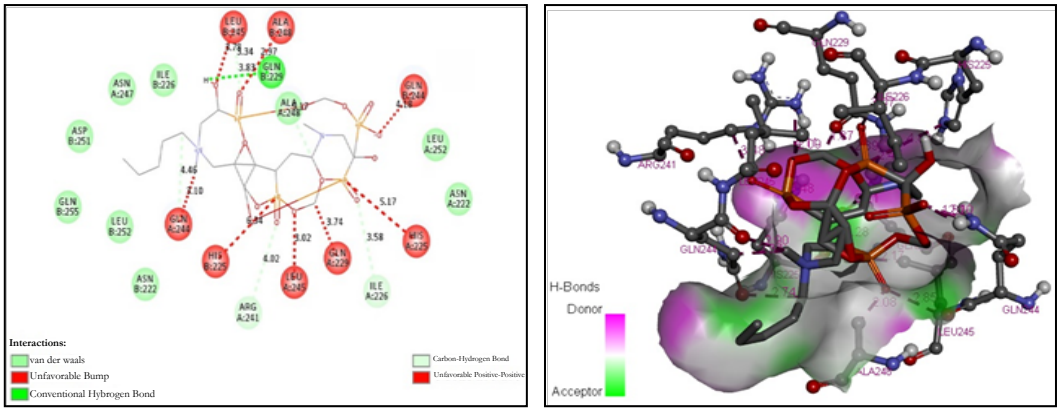


Figure 11. 2D & 3D surface interactions of 2Q80 with Pure Ibandronate drug molecule.

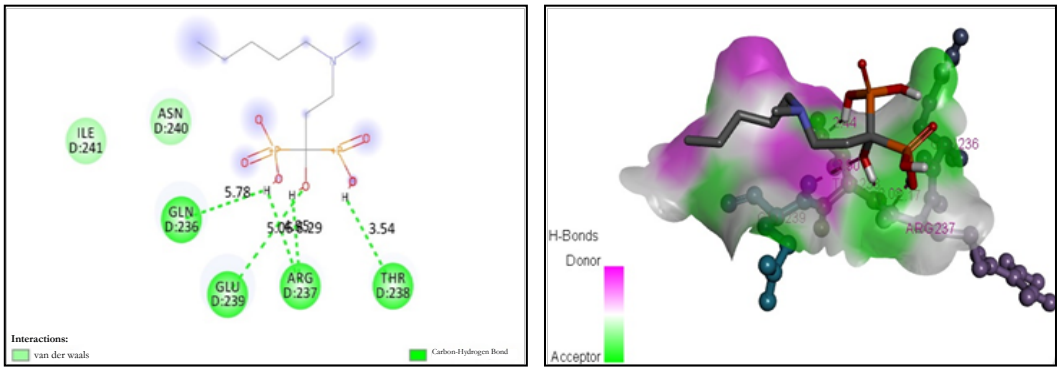


Figure 12. 2D & 3D surface interactions of 2Q80 with Ibandronate -Hydroxyapatite.

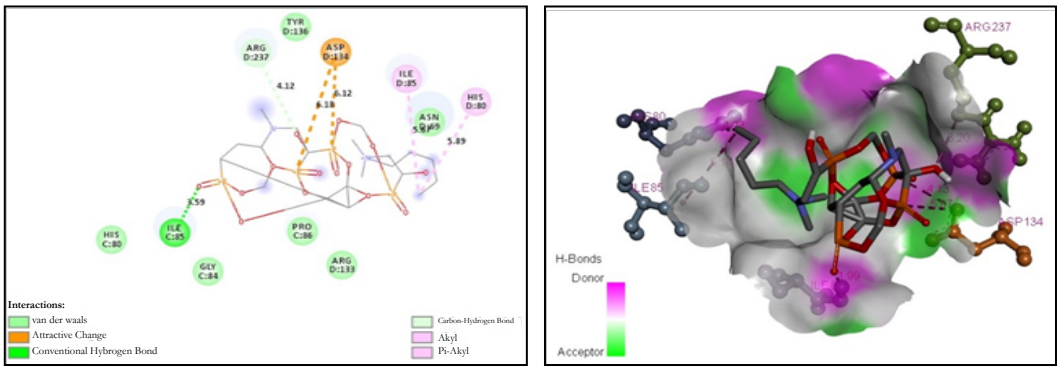
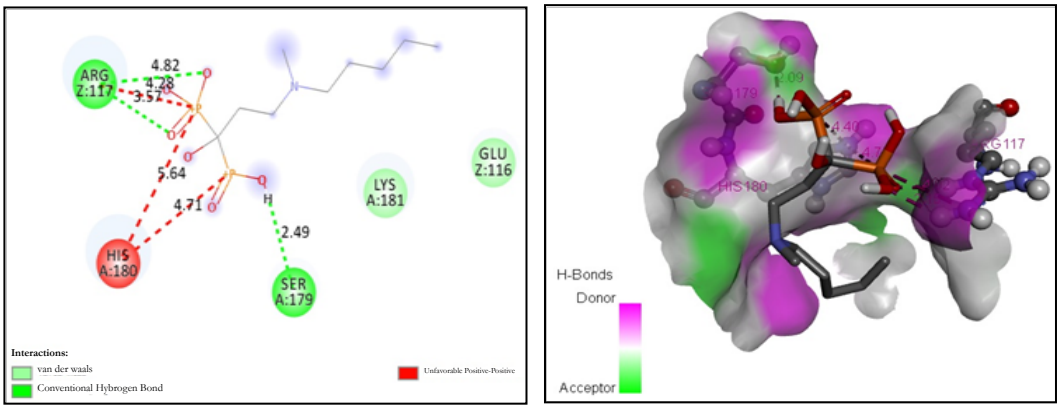


Figure 13. 2D & 3D surface interactions of 3URF with Pure Ibandronate drug molecule.



C-chain amino acid residues; another oxygen atom of phosphate group of conjugate had undergone carbon hydrogen bonding with ARG237 of D-chain amino acid residue; the phosphate group of final molecule had undergone attractive charge interaction with ASP134 of D-chain amino acid residue; the alkyl side chain had undergone alkyl and pi-alkyl interactions with HIS80, ILE85 of D-chain amino acid residues (Figure 12).

The docking score shown -3.23 kcal/mol, -6.49 kcal/mol for Ibandronate ligand and Ibandronate hydroxyapatite conju-

Figure 14. 3D & 3D surface interactions of 3URF with Ibandronate - Hydroxyapatite.

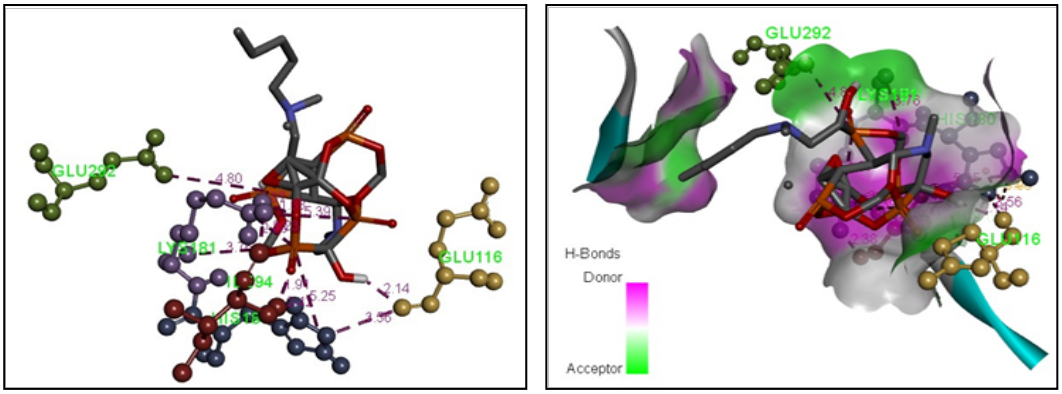


Table 2. Interaction of parent and final drug molecules with various protein targets.

Sl. No.	Protein (PDB ID)	Binding Interactions	
		Pure Ibandronate	Ibandronate -Hydroxyapatite conjugate
1	3SQZ	A-Chain: GLU79, CYS111, TYR143, ASP184, PHE 185, VAL196, HIS233, SER309	A-Chain: THR152, VAL196, HIS233, ASN206, TYR278, GLU79, PRO235, PHE236, VAL196, SER201, UNK1, CYS111, ASP184, PHE 185, ALA110, SER309
2	1DQ9	A-Chain: PRO693, ILE746, GLY747, ASN776, GLY773, VAL772, ALA694	C- Chain: PRO693, ALA695, ILE746
		B-Chain: LYS692, VAL772, GLY773, ALA695, TRP698, ASN697, ALA694, GLY747, GLY748, ILE746, ASN776	D-Chain: ASN771, ASN750, CYS777, ALA694, PRO693, ALA695, PRO693 GLY773, ASN776, VAL772, SER775
3	2F7M	F-Chain: THR201, LYS200, ASP174, LYS266, ASP244, ASP261, ASP243, CYS267, ILE196, GLN240, ASP247, THR260	F-Chain: ASP107, LYS266, CYS267, SER268, ASP244, TYR193, GLN240, ARG112, GLN96, TYR204, ASP243, LYS257, ASP103, ARG60, LEU100, SER205, THR201, LYS200, PHE239
4	2HFU	A-Chain: GLN229, ILE226, HIS225, GLN244, ASN247, ALA248, ASP251, LEU245	A-Chain: ASN247, ARG241, HIS225, ASN222, LEU252, ALA248, LEU245, GLN229, ILE226, GLN244
		B-Chain: HIS225, GLN229, ARG241, GLN244, ASP251, ASN247, LEU245, ILE226	B-Chain: ASP251, GLN255, LEU252, ASN222, GLN229, LEU245, ALA248, ILE226, GLN244
5	2Q80	D-Chain: ASN240, ILE241, GLN236, GLU239, ARG237, THR238	C-Chain: HIS85, HIS80, GLY84, PRO86
			D-Chain: ARG237, TYR136, ASP134, ILE85, ASN69, HIS80, ARG133
6	3URF	A-Chain: HIS180, SER179, LYS181	A- Chain: GLU 292, LYS 181, HIS180
		Z-Chain: ARG117, GLU116	Z Chain: ILE 94, GLU 116

gate ligand with protein receptor 3URF. The interactions of Ibandronate molecule with the protein receptor with Human RANKL/OPG complex shown, the oxygen atom of phosphate groups of the parent molecule had formed conventional hydrogen bonding with SER179, ARG117 of A&Z-chain amino acid residues respectively; another oxygen atom of phosphate group had undergone unfavourable positive-positive interactions with HIS180 of A-chain amino acid residues (Figure 13). The interactions of Ibandronate hydroxyapatite conjugate with the protein receptor with PDB ID: 3URF are, the Ibandronate hydroxyapatite conjugate had undergone bonding with GLU292, LYS181, HIS180 of A - chain amino acid residues and ILE94, GLU116 of Z - chain amino acid residues respectively. The 3D interactions and 3D surface interactions (Figure 14).

Docking simulation at the active sites with proteins 3SQZ, 1DQ9, 2F7M, 2HFU, 2Q80, 3URF with respect to pure Ibandronate & Ibandronate-hydroxyapatite conjugate docking simulation score and pIC_{50} values were recorded in Table 1. Docking studies performed by Autodock 4.2 was confirmed that above inhibitors fit into the binding pocket of the HMG_CoA synthase, Human HMG-COA Reductase, FPPS, Mevalonate kinase, GPPS of Mevalonate pathway and Human RANKL/OPG complex, receptors are shown in the Figures: 4-15. We observe successful docking, intermolecular hydrogen bonding and lipophilic interactions between the ligand and receptor. Mostly the oxygen atoms of phosphate groups had undergone conventional hydrogen bonding with Amino acid residues of targets. On the basis of minimum

Table 3. Molecular description and ADME parameters of Ibandronate and Ibandronate hydroxyapatite conjugate.

Properties	Parent molecule (IBA)	Final molecule (IBA-HAP conjugate)
Total Mol. weight	319.23	766.686
cLogP	-6.673	-12.118
cLogS	1.605	1.335
H-Acceptors	8	8
H-Donors	5	1
Total Surface Area	220.58	211.54
Relative PSA	0.467	0.510
Polar Surface Area	158.15	169.47
Drug likeness	-10.533	-12.658
Mutagenic	none	None
Tumorigenic	none	None
Reproductive Effective	none	None
Irritant	none	None
Nasty Functions	-	-
Shape Index	0.579	0.556
Molecular Flexibility	0.760	0.798
Molecular Complexity	0.677	0.692
Rotatable Bonds	9	8

energy concept, it can be concluded that ΔG binding energy and pIC_{50} values shows promising anti-osteoporotic effect for Ibandronate-Hydroxyapatite conjugate and it can act as a most powerful to inhibit FPPS, GPPS & RANKL/OPG in contrast to pure Ibandronate. Data warrior the drug - conjugate also complied with Lipinski's rule without any compliances for mutagenicity, irritability and tumorigenicity (Table 3).

Conclusion

The newly synthesized drug molecule IBA-HAP has shown high binding and powerful inhibitory activity towards Mevalonate pathway enzymes like Human HMG-COA Reductase, FPPS, GGPS and HUMAN RANKL/OPG proteins as compared to Pure Ibandronate. Based on the in - silico studies, the results support in designing of new drugs to treat osteoporosis in which potential inhibitors will interact strongly with residues.

Acknowledgments

The authors are thankful to the management of M.S. Ramaiah University of Applied Sciences for providing required support to carry out the research work.

References

[1]. Amin D, Cornell SA, Perrone MH, Bilder GE. 1-Hydroxy-3-(methylpentylamino)-propylidene-1,1-bisphosphonic acid as a potent inhibitor of squalene synthase. Arzneimittelf orschung. 1996;46(8):759-762. Pubmed PMID:9125274.

[2]. Çankaya M, Cizmeci Şenel F, Kadioglu Duman M, Muci E, Dayisoylu EH, Balaban F. The effects of chronic zoledronate usage on the jaw and long bones evaluated using RANKL and osteoprotegerin levels in an animal model. Int J Oral Maxillofac Surg. 2013;42(9):1134-1139. Pubmed PMID:23522850.

[3]. Jagyasi A, Choubey J, Patel A, Verma MK. Molecular Modeling and Docking Analysis of Novel Drug like Compounds for NDM-1. Int J Chem Anal Sci. 2013;1:47-54.

[4]. Dang L, Liu J, Li F, Wang L, Li D, Guo B, et al. Targeted delivery systems for molecular therapy in skeletal disorders. Int J Mol Sci.2016 Mar;17(3):428. Pubmed PMID:27011176.

[5]. Dunford JE, Thompson K, Coxon FP, Luckman SP, Hahn FM, Poulter CD,, et al. Structure-activity relationships for inhibition of farnesyl diphosphate synthase in vitro and inhibition of bone resorption in vivo by nitrogen-containing bisphosphonates. J Pharmacol Exp Ther. 2001;296(2):235-242. Pubmed PMID:11160603.

[6]. Frith JC, Mönkkönen J, Auriola S, Mönkkönen H, Rogers MJ. The molecular mechanism of action of the antiresorptive and antiinflammatory drug clodronate: evidence for the formation in vivo of a metabolite that inhibits bone resorption and causes osteoclast and macrophage apoptosis. Arthritis Rheum. 2001;44(9):2201-2210.Pubmed PMID:11592386.

[7]. Luckman SP, Hughes DE, Coxon FP, Graham R, Russell G, Rogers MJ. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. J Bone Miner Res. 1998;13(4):581-589.Pubmed PMID:9556058.

[8]. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of computational chemistry. 1998 Nov 15;19(14):1639-62.

[9]. Nancollas GH, Tang R, Phipps RJ, Henneman Z, Gulde S, Wu W,,et al. Novel insights into actions of bisphosphonates on bone: differences in interactions with hydroxyapatite. Bone. 2006;38(5):617-627.Pubmed PMID:16046206.

[10]. Rodan GA, Fleisch HA. Bisphoshonates:Mechanism of action.J Clin Invest.1996; 97:2692-2693.

[11]. Rodan GA, Martin TJ. Therapeutic approaches to bone diseases. Science. 2000 Sep 1;289(5484):1508-14.

[12]. Russell RG, Watts NB, Ebetino FH, Rogers MJ. Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. Osteoporos Int. 2008;19(6):733-759.Pubmed PMID:18214569.

[13]. Widler L, Jaeggi KA, Glatt M, Müller K, Bachmann R, Bisping M, et al. Highly potent geminal bisphosphonates. From pamidronate disodium (Aredia) to zoledronic acid (Zometa). J Med Chem. 2002;45(17):3721-3738. Pubmed PMID:12166945.