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Article: **Pharmacophore-based Drug Designing against COL7A1: Causative Protein of Dystrophic Epidermolysis Bullosa (DEB)**

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Research Article

Pharmacophore-based Drug Designing against COL7A1: Causative Protein of Dystrophic Epidermolysis Bullosa (DEB)

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Keywords

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Abstract

Epidermolysis Bullosa (EB) is a rare genetic disorder that causes skin fragility, trauma induced dissociation of the skin and painful wound growth. More than 20 types of genes are involved in causing EB as it is a polygenic disease and each gene is involved in causing a different subtype of EB. Dystrophic Epidermolysis Bullosa (DEB) is a subtype of EB caused by mutations in the COL7A1 (Collagen Type VII Alpha 1 Chain) gene and it affects people from all racial backgrounds. No drug is available for the treatment of DEB in the market. So, it is the need of the hour to come up with a potential inhibitor that may inhibit the faulty protein of COL7A1 gene. Different exons of COL7A1 were analyzed and exons 70-75 were selected. These exons are important from a mutational point of view. The mutations in them were identified and verified using various *In silico* tools. The 3D structure of the protein was retrieved using specific exons (which were edited) and mutations were introduced in it. Moreover, the protein was further analyzed for its stability, toxicity and solubility. The inhibitors of COL7A1 were designed using CAAD techniques (pharmacophore modeling) and the best inhibitor of COL7A1 was further checked to determine its drug-likeness, solubility, toxicity, and various physiochemical properties. The constructed inhibitor was found to have the best docking results and good ADMET properties. The developed inhibitor construct showed promising results *In silico* and it is also expected to show good results if it would be tested *In vitro* and *In vivo*. Thus, it would be a breakthrough to treat DEB using this inhibitor, if it is synthesized *In vitro* and further tested *In vivo*.

1. Introduction

Epidermolysis Bullosa (EB) is a skin fragility which is clinically and genetically heterozygous. It is characterized by trauma-induced dissociation and painful injury development. EB affects people of all races and ethnicity [1]. Mutations in 20 genes are known to be associated with over 30 subtypes of clinical EB [2]. There are four key forms of EB which depend on the ultra-structural fraction. These are SIMPLEX, Dystrophic Epidermolysis Bullosa (DEB), Kindler's Syndrome, and

Junction Epidermolysis Bullosa [3].

DEB is a very well-known EB subtype. Its molecular foundation, that is, collagen VII defects have been known for nearly twenty years. Moreover, several hundred separate gene mutations have so far been revealed of both recessive and dominant types of DEB in the VII collagens, that is, COL7A1 (Collagen Type VII Alpha 1 Chain) [4]. Many other studies addressed genotyping-phonotypic associations, indicating that the scope of biological and clinical phenotypes is much wider than expected. Nevertheless, the mechanisms of cellular and molecular conditions are relatively well established.

DEB blistering is due to the structural and functional changes in the anchoring fibrils of collagen VII polymers (AF), which bind to the epidermal dermis basement membrane. In the electron microscope typical Afs appear as centro-symmetrically cross-banded fibrils with frayed ends, emanating into the dermal connective tissues from the lamina dense of the basement membrane. Collagen VII polymerizes in a highly coordinated fashion to ensure the dermal / epidermal adherence to the fibrils stabilized by transglutaminase and also binds covalently to the dermal collagen fiber of the DEB skin [1]. There are various bioinformatics-based approaches have been introduced for the drug and vaccine design with recent advancements [5].

Mutations exist in all types of DEB gene including COL7A1. Their range is large with several families having their own mutations. Heterozygosity is normal in recessive DEB compounds (for instance, the patient has two different mutations, one inherited from the father and one from the mother). Both COL7A1 mutations are responsible for the premature termination of codons (PTCs) in many patients with the recessive DEB gene COL7A1. They are also responsible for the premature termination of codons (PTCs) in many patients with recessive DEB [6].

The current study aims to demonstrate the cause of this disease and the development of its drug using computational *In silico* techniques. These techniques increase the probability of success in identifying the polymorphisms of drug targets, drug-metabolizing enzymes, and other genes that influence the drug response, as well as new disease susceptibility genes and pathways that are important. For the future, there remains the possibility to come up with novel drugs using the same techniques for other types of EB.

2. Materials and Methods

The study was carried out using bioinformatics-based approaches and the following materials and methods were employed.

2.1. Sequence Retrieval

National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) was used for retrieval of C0L7A1 protein carrying mutations that cause EB [7].

2.2. Physiochemical Properties

Physiochemical properties of the selected protein were calculated using ExPASy-Protpram (https://web.expasy.org/protparam/) online tool from ExPASy bioinformatics resource portal.

2.3. Secondary Structure Prediction

Secondary structure was predicted by PsiPred (http://bioinf.cs.ucl.ac.uk/psipred/). It is a well-cited tool used for the analysis of the secondary structures of protein [8]. Secondary structure was analyzed based on confidence scores ranging from 0-9. A score greater than 5 was considered a good score in the secondary structure analysis via PsiPred.

2.4. Tertiary Structure Prediction

Three-dimensional (3D) structure prediction of selected proteins was performed using I-TASSER, which is primarily a Zhong Labs tool and is considered as among the finest tools for predicting the tertiary structure of protein (https://zhanglab.ccmb.med.umich.edu/I-TASSER). On a scale of C-score of -5 to 2, the best five models were generated. The highest C-score model has more authenticity. Moreover, Tm-Score and Z-Score were also reported for the current study [9].

2.5. 3D Structure Refinement

The Galaxy Refine web server, freely available at (http://galaxy.seoklab.org/refine), is based on a method of refinement tested in CASP10. It rebuilds and repacks the side chains which eventually relaxes the entire system by simulating molecular dynamics. It utilizes the structure which gets better after performing the 22 refinements [10].

2.6. Model Stability Analysis and Validation

The RAMPAGE (http://legacy.ccp4.ac.uk/html/rampage.html) Ramachandran plot evaluation was carried out for checking the model stability of the constructed vaccine. The stability of the predicted protein model is considered a point standard for research. It is a well-utilized technique used for evaluating protein stability, is mainly quoted in literature and analyzed using PROCHECK (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) by Ramachandran Plot Assesment[11].

2.7. Pharmacophore Construction

Pharmit (https://pharmit.csb.pitt.edu/) was used to identify all pharmacophore features present in a provided ligand structure. Pharmacophores are spatial arrangements of features required for interaction with specific receptors. PharmaGhist (https://bioinfo3d.cs.tau.ac.il/PharmaGist/) is an online free tool available for pharmacophore generation. The input ligand was given to PharmaGhist which was closely related to our wild protein and the output structure was candidate database screening [12].

2.8. Virtual Screening of Pharmacophore

Virtual screening of constructed pharmacophores was carried out through molecular docking. Patchdock (https://bioinfo3d.cs.tau.ac.il/PatchDock/) was used for this purpose [13]. The pharmacophore model with the best binding affinity, the lowest ACE score and the highest number of clusters was selected for further analysis.

2.9. Molecular Docking Protocol

Docking was performed using Patchdock (https://bioinfo3d.cs.tau.ac.il/PatchDock/) [13]. The 3D structure-based function prediction of the protein COL7A1 was done through I-Tasser.. Mutation in the normal sequence of COL7A1 sequence was identified and verified (which was important from a mutational point of view, containing specific exonic region) using mutation verification tools. Then, deleterious mutations (which were verified by Polyphen, SIFT, MuPro) were introduced in the COL7A1 gene sequence with the help of BioEdit software. Finally, I-Tasser was used to retrieve the 3D structure of COL7A1 protein for further studies. The hit molecule obtained through virtual screening was docked with the active site of the COL7A1 protein, which was obtained via I-Tasser. The top three compounds were obtained by means of the docking analysis [13].

2.10. ADMET Analysis

Swiss-ADME (http://www.swissadme.ch/) was applied to check whether the compounds had the properties of aqueous solubility, blood-brain barrier penetration,

hepatoxicity, human intestinal absorption, physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness. The ADMET properties of the three compounds which had reasonable docking results are mentioned in the results [14]. Chemical absorption, delivery, metabolism, excretion, and toxicity (ADMET) play important roles in drug exploration and development. A high-quality drug candidate should not only have adequate efficacy against the therapeutic target but should also display acceptable ADMET properties at the time of therapeutic dosage. A lot of *In silico* models are thus built for the prediction of chemical ADMET properties. However, it is not a straightforward matter to determine the drug-likeness of compounds in terms of too many similar ADMET properties. In this research, a scoring feature called the ADMET-score is suggested to determine the drug-likeness of a compound [14].

3. Results

3.1. Sequence Retrieval

The complete gene sequence of COL7A1 was retrieved from the NCBI GenBank with the accession no NC_000003.12. The sequence was selected based on the previous studies which were important from a mutational point of view. Mutations were identified with the help of blast and inserted in the sequence with the help of BioEdit.

3.2. Physiochemical Analysis

The analysis of the physiochemical properties was done using the tool ExPASy-Protpram. The instability index (II) was calculated to be 115.42 and the aliphatic index was found to be 120.86. The grand average of hydropathicity (GRAVY) was -0.739. Protparam is a tool available online to check protein stability and to determine the functions of protein to see if the protein is stable enough to work with (https://web.expasy.org/protparam). This method is used to calculate the physical and chemical parameters of the specified protein stored in Swiss-Prot or TrEMBL or for the protein sequence entered by the user. The parameters measured included the molecular weight, theoretical pI, the composition of amino acids, atomic composition, the coefficient of extinction, predicted half-life, instability index, and aliphatic index.

3.3. Protein 2-D Structural Analysis

Structural analysis of COL7A1 protein was done using an online tool PSIPRED. The results obtained from the PSIPRED is shown in Figure 1(a). The amino acid composition of the model is defined in Table 1.

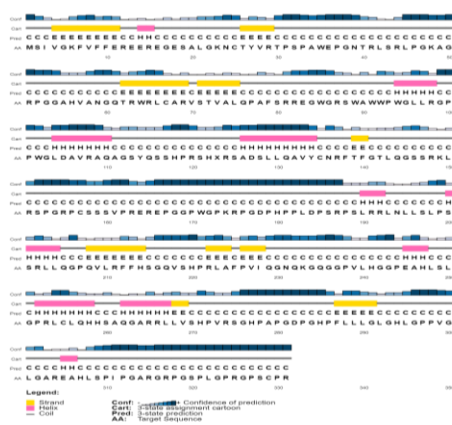
Table 1. Amino Acid Composition as The Percentage of COL7A1

Amino Acid Composition			% Composition		
Ala (A)	22	6.6%	Trp (W)	9	2.7%
Arg (R)	38	11.5%	Tyr (Y)	3	0.9%
Asn (N)	6	1.8%	Val (V)	16	4.8%
Asp (D)	5	1.5%	Pyl (O)	0	0.0%
Cys (C)	6	1.8%	Sec (U)	0	0.0%
Gln (Q)	14	4.2%	Leu (L)	37	11.2%
Glu (E)	11	3.3%	Phe (F)	10	3.0%
Gly (G)	45	13.6%	Pro (P)	43	13.0%
His (H)	15	4.5%	Ser (S)	33	10.0%
Ile (I)	3	0.9%	Thr (T)	7	2.1%
Lys (K)	6	1.8%	Met (M)	1	0.3%

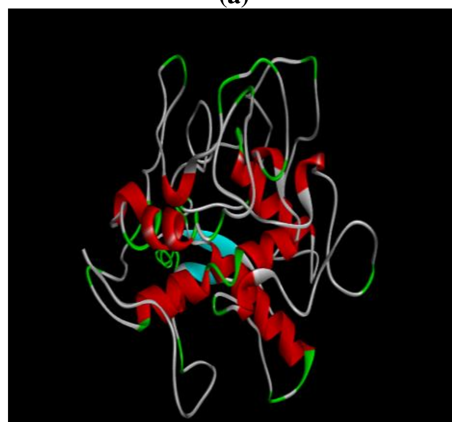
3.4. Protein 3D Structural Analysis

Protein tertiary structure was predicted using I-Tasser (Iterative Threading Assembly Refinement Tool), which is considered to be among the finest online structure modelling tools. Wild protein of COL7A1 with an exonic region of 70 to 75 was used and mutations in it were amended by BioEdit. I-Tasser was utilized to retrieve the 3D structure of COL7A1 protein. The model with the highest C-score value was selected. The secondary

and tertiary structures of protein are shown in Figure 1(b).



(a)



(b)

Figure 1. (a): Secondary structure of COL7A1 protein predicted by PsiPred **(b):** Tertiary structure of COL7A1 protein predicted by I-Tasser

3.5. Tertiary Structure Refinement

GalaxyRefine was used to refine the 3D structure of the protein for improved results based on a method of refinement tested in CASP1. Its algorithm rebuilds and repacks the side chains and eventually relaxes the entire system by simulating molecular dynamics. It basically utilizes molecular dynamic simulation methods for structure refinement and for the validation of the structure of wild protein. Table 2 shows model refinement by GalaxyRefine. The structure was improved after performing the 22 refinements when it was again validated using the Ramachandran plot, which gave a better score than the previous one. The refined model is shown in Figure 3.

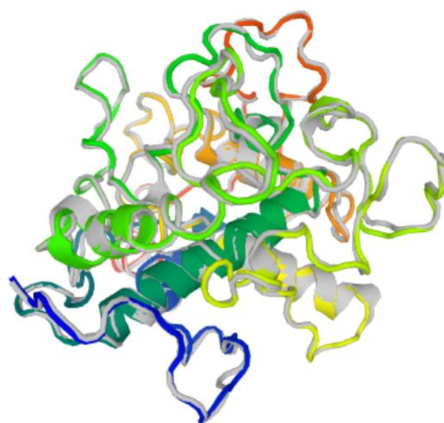


Figure 3. Tertiary structure of protein refinement by GalaxyRefine

3.6. Model Validation and Refinement

Possibly, the best predictor of consistency in the experimental determination of three-dimensional protein coordinates is the Ramachandran plot. Precheck analyses the plot of a PDB file for Ramachandran and compares it to the plots of 400 high-resolution representative structures solved by Ramachandran. I-Tasser structure was validated using RC plotting. RC plot monitors the corners of the residual amino acid in a protein. The 3D structure of the wild protein of COL7A1 was retrieved after inserting mutations in it with the help of the BioEdit software. Then, the 3D structure was refined using GalaxyRefine. It was further refined using RC plotting. The constructed Ramachandran plot is shown in Figure 2.

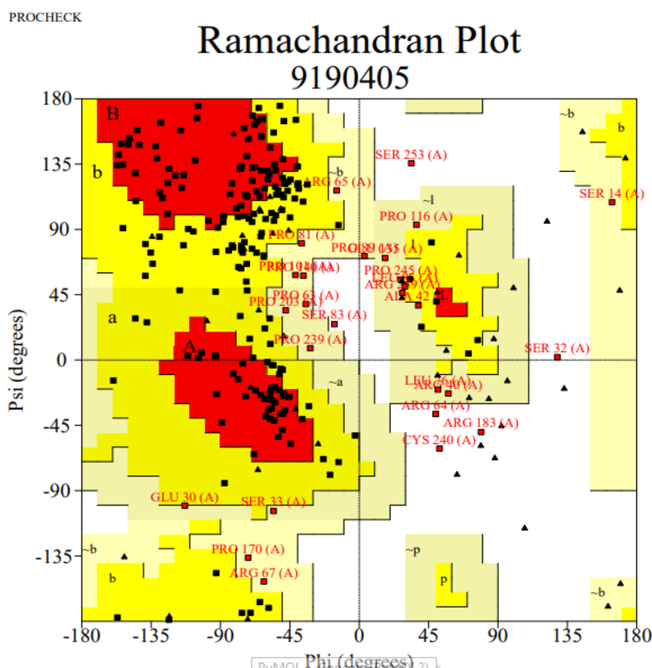


Figure 2. Ramachandran plot for the wild protein of COL7A1 by ProCheck

3.7. Pharmacophore Construction

Pharmit identifies all pharmacophore features present in a given ligand structure. Pharmacophores comprise the spatial arrangements of features required for specific receptor interaction. PharmaGhist was utilized to prepare the pharmacophores.

Pharmghist is an online tool available for free and used for pharmacophore generation. The employed method is ligand-based. The input ligand was given to PharmaGhist. It was closely related to our wild protein and the output structures were candidate pharmacophores computed by a mutable and flexible alignment of the input ligand. The input ligand was downloaded from a pubchem named Phenytoin. Phenytoin is used as the ligand having the most resemblance with wild protein. It is mainly used as a drug to cure disorders related to the nervous system. The constructed pharmacophores are shown in Figure 4.

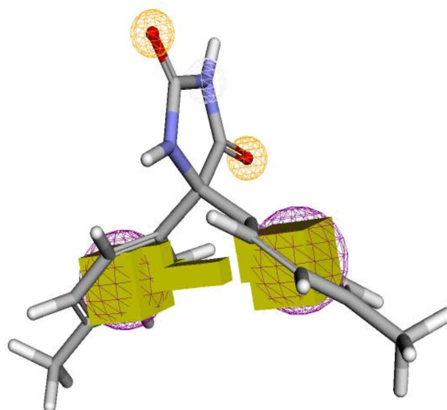


Figure 4. Pharmacophore model designed by PharmaGhist

Pharmacophores with their given ligand interacted with the wild protein of EB. Pharmacophores are the sites where the wild protein can bind and lessen the effect of disease and act as an inhibitor. Pharmit is an online tool used for database screening and the best inhibitor was constructed by it based on a low RMSD value. The results were sorted in a decreasing order based on RMSD (for

pharmacophore searches), similarity score (for shape searches), molecular weight, and the number of rotatable bonds. The results are given in SDF file format. The database screening interactions are displayed in Figure 5.

3.8. Virtual screening of Pharmacophore

The results of docking were sorted in a decreasing order based on RMSD (for pharmacophore searches), similarity score (for shape searches), molecular weight, and the number of rotatable bonds. The results are given in the SDF file formats. Database screening interactions are displayed in Figure 5. The model with the lowest ACE score and the highest number of clusters was selected for further analysis.

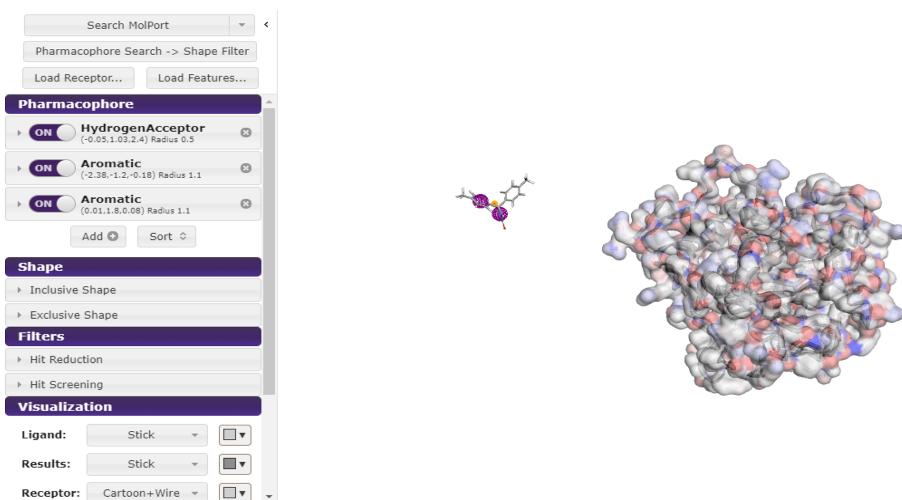
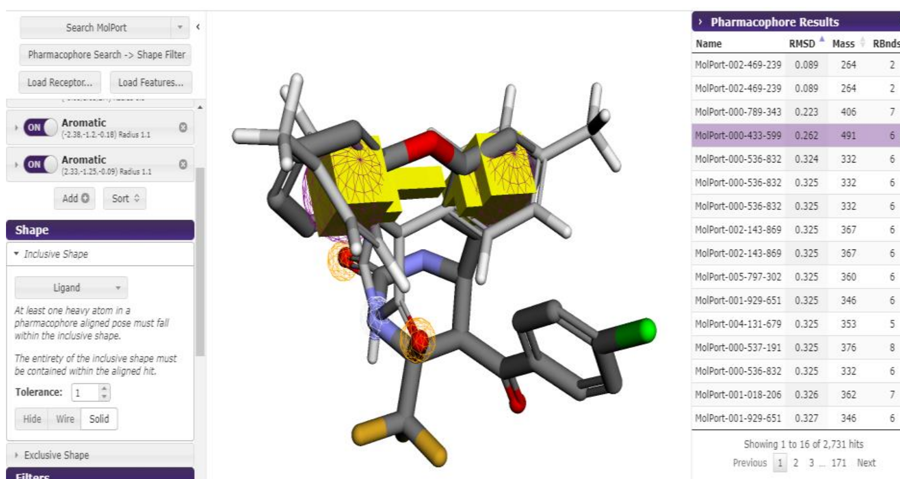


Figure 5 (a). Interaction of the wild protein of COL7A1 with the constructed pharmacophore



Name	RMSD	Mass	RBnds
MolPort-002-469-239	0.089	264	2
MolPort-002-469-239	0.089	264	2
MolPort-000-789-343	0.223	406	7
MolPort-000-433-599	0.262	491	6
MolPort-000-536-832	0.324	332	6
MolPort-000-536-832	0.325	332	6
MolPort-000-536-832	0.325	332	6
MolPort-002-143-869	0.325	367	6
MolPort-002-143-869	0.325	367	6
MolPort-005-797-302	0.325	360	6
MolPort-001-929-651	0.325	346	6
MolPort-004-131-679	0.325	353	5
MolPort-000-537-191	0.325	376	8
MolPort-000-536-832	0.325	332	6
MolPort-001-018-206	0.326	362	7
MolPort-001-929-651	0.327	346	6

Figure 5 (b). Inhibitor 1 designed after data base screening with a possible low RMSD value

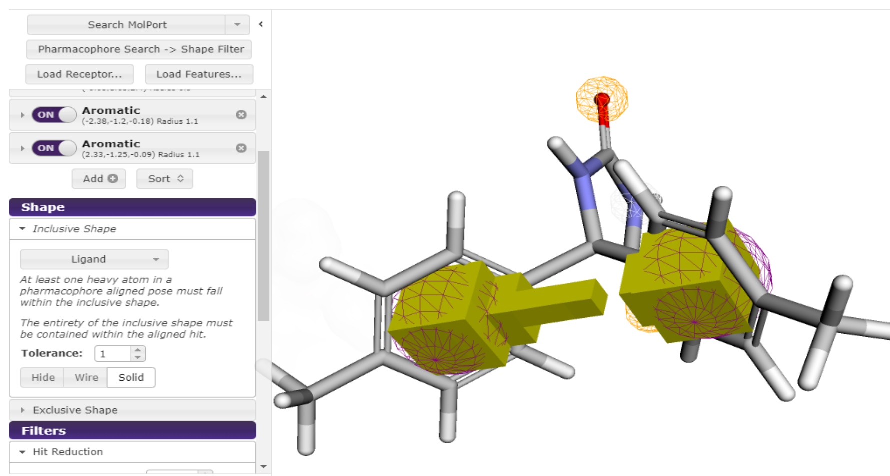


Figure 5 (c). Inhibitor 2 made after database screening of a large number of data bases present *online*

Pharmacophore Results

Name	RMSD	Mass	RBnd
MolPort-028-738-771	0.017	349	3
MolPort-010-962-586	0.017	445	7
MolPort-015-163-726	0.018	367	2
MolPort-008-337-616	0.018	395	2
MolPort-029-883-702	0.018	367	3
MolPort-009-649-776	0.018	329	2
MolPort-008-335-714	0.018	367	2
MolPort-008-340-945	0.018	369	2
MolPort-008-336-561	0.018	409	4
MolPort-008-338-543	0.018	423	4
MolPort-008-335-714	0.018	367	2
MolPort-029-883-730	0.018	405	2
MolPort-000-857-720	0.020	318	6
MolPort-039-158-404	0.020	326	4
MolPort-000-123-851	0.020	328	4
MolPort-002-772-579	0.020	405	4

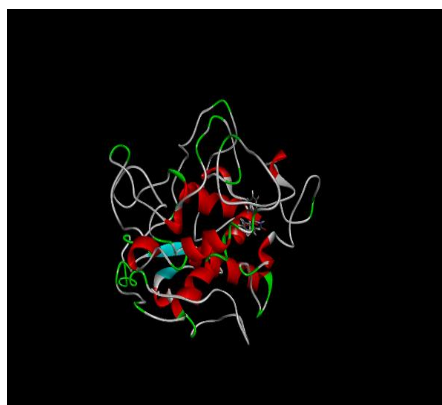
Figure 5 (d). Inhibitor of COL7A1 wild protein with a low RMSD value

3.9. Docking Analysis

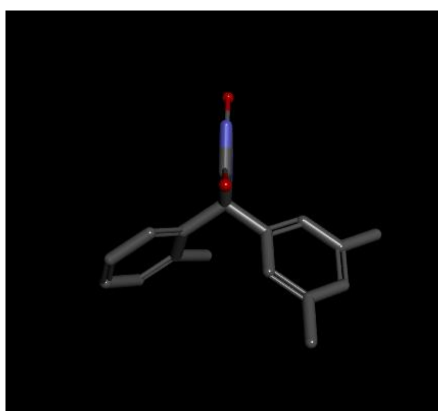
The wild protein of EB obtained from I-Tasser containing the 3D structure of some exons (important from a mutational point of view) of COL7A1 was chosen as the target protein for molecular docking using patch dock and auto dock vina. The docking interaction plot of COL7A1 at its active site with the inhibitors obtained using Pharmit is given below. The docking analysis was executed using an online server PatchDock 4.0 (URL: https://bioinfo3d.cs.tau.ac.il/PatchDock).

(a)

(b)



(c)



(d)

Figure 6. (a) Docking pose of COL7A1 receptor with the designed inhibitor 1. (b): Docking pose of COL7A1 receptor with the designed inhibitor 2. (c) Docking pose of COL7A1 receptor with the designed inhibitor 1. (d) Most potent inhibitor of COL7a1 protein with the best docking energies.

It gives multiple results with 10 ranked results for analysis based on the docking energy score of the mentioned inhibitor (d) with the affinity of -7.3kj/mol. The docking structures are shown in Figure 6.

3.10. Characterization of the Designed Inhibitor

An initial IDSC report provides data for IND filing regarding physical characterization and reformulation. The inhibitor compiles, reviews, and summarizes every single detail required for regulatory documents to help identify barriers to the production of the compound. Physical data includes type recognition, identification of solvents, hygroscopicity, micrometrics, and clarification of the structure. Polymorphic scanning and recognition of types can also be performed. Data on reformulation includes solubility in balance, solubility to pH, coefficient partition, determination of pKa, and rapid physical and chemical stability.

3.11. ADMET Analysis of Inhibitor

The hit compound most correlated with the pharmacophore model obtained after docking and which had the highest inhibitory effect against EB was characterized by ADMETlab. It determines the toxicity of the best hit compound obtained by Pharmit. The characterization involves the naming of a hit compound and determining its properties using online ADMET profiling. ADMETlab finds the likeness of inhibitors in our body as well as the distribution, solubility, and toxicity of the drug designed using computer-aided drug designing techniques. Table 3 and Table 4 represent the ADMET properties of the designed pharmacophore.

Table 3. Characterization of the Inhibitor by Swiss-ADME

Physicochemical Properties	
Formula	C18H18N2O2
Molecular weight	294.35 g/mol
Num. heavy atoms	22
Num. arom. heavy atoms	12
Fraction Csp3	0.22
Num. rotatable bonds	2
Num. H-bond acceptors	2
Num. H-bond donors	2
Molar Refractivity	92.40
TPSA	58.20 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.40
Log $P_{o/w}$ (XLOGP3)	3.56
Log $P_{o/w}$ (WLOGP)	1.82
Log $P_{o/w}$ (MLOGP)	2.74
Log $P_{o/w}$ (SILICOS-IT)	3.91
Consensus Log $P_{o/w}$	2.89
Water Solubility	
Log *S* (ESOL)	-4.18
Solubility	1.95e-02 mg/ml ; 6.62e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-4.47
Solubility	1.00e-02 mg/ml ; 3.41e-05 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-6.77
Solubility	5.03e-05 mg/ml ; 1.71e-07 mol/l
Class	Poorly soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-5.57 cm/s

Table 4. ADMET Properties by ADMETlab

Category	Property	Total	Positive	Negative	Train	Test
Basic physicochemical property	LogS	5220	-	-	4116	1104
	LogD7.4	1031	-	-	773	258
	LogP					
Absorption	Caco-2	1182	-	-	886	296
	Pgp-Inhibitor	2297	1372	925	1723	574
	Pgp-Substrate	1252	643	609	939	313

Category	Property	Total	Positive	Negative	Train	Test
	HIA	970	818	152	728	242
	F (20%)	1013	759	254	760	253
	F (30%)	1013	672	341	760	253
Distribution	PPB	1822	-	-	1368	454
	VD	544	-	-	408	136
	BBB	2237	540	1697	1678	559
Metabolism	CYP1A2-Inhibitor	12145	5713	6432	9145	3000
	CYP1A2-Substrate	396	198	198	297	99
	CYP3A4-Inhibitor	11893	5047	6846	8893	3000
	CYP3A4-Substrate	1020	510	510	765	255
	CYP2C19-Inhibitor	12272	5670	6602	9272	3000
	CYP2C19-Substrate	312	156	156	234	78
	CYP2C9-Inhibitor	11720	3960	7760	8720	3000
	CYP2D6-Inhibitor	12726	2342	10384	9726	3000
	CYP2C9-Substrate	784	278	506	626	156
	CYP2D6-Substrate	816	352	464	611	205
Excretion	Clearance	544	-	-	408	136
	T1/2	544	-	-	408	136
Toxicity	hERG	655	451	204	392	263
	H-HT	2171	1435	736	1628	543
	Ames	7619	4252	3367	5714	1905
	SkinSen	404	274	130	323	81
	LD50 of acute toxicity	7397	-	-	5917	1480
	DILI	475	236	239	380	95

4. Discussion

In this study, the inhibitors of COL7A1 were made using pharmacophore modeling. It is imperative to come up with a drug to treat DEB because no drug or inhibitor is known to be effective against the wild protein of EB. No EB cure and medications specifically designed to manage the symptoms are available. However, awareness about the root cause of EB has improved with ongoing studies [15]. This has contributed to many possible therapies and clinical trials are in progress to determine their benefits for the EB patients [16]. Thus, it was found imperative that the effect of wild protein COL7A1 needs to be suppressed. Therefore, computational biology techniques were used to develop the inhibitors of COL7A1 [15].

In the past, only a few methods were employed to design drugs and to come up

with possible solutions to treat the diseases. These days, computational technology is widely used in the discovery of therapeutic compounds such as anti-HIV compounds, medication for treating snake bites and neurotransmitter inhibitors [17]. Ligand-based approach and structure-based approach to design inhibitors have been used to design the inhibitors of COL7A1 protein. The former is based on the structural activity relationship widely used in the discovery of various therapeutic compounds [18]. The structure-based approach identifies the potential ligand by docking compounds into receptive active sites and then determines the receptor and ligand interaction [19].

Overall, this study focuses on the designing of the inhibitors of COL7A1 using advanced technological and bioinformatic approaches to provide humanity with new drugs that could cure DEB. The wild protein of COL7A1 was used for the designing of the inhibitors of EB [20]. Protein structure was retrieved after downloading the gene of COL7A1 from NCBI and its mutational analysis was done and verified using a mutation verification tool. Mutations were inserted in COL7A1 gene using the BioEdit software and its 3D structure was retrieved using I-Tasser. For the construction of the inhibitors of COL7A1 gene, 32 test sets of pharmacophores were generated using CAAD drug designing tool. Pharmacophores comprise the spatial arrangements of features required for the specific receptor to interact with the COL7A1 gene.

The hit molecule obtained through virtual screening was docked with the active site of the COL7A1 protein obtained through I-Tasser. Before proceeding towards docking, the binding site interacting residues were computed using COACH and the results were further validated using CastP tool. Then, docking was performed and the high score of docking showed the quality of interaction with the receptor. Chemical absorption, delivery, metabolism, excretion, and toxicity (ADMET) played important roles in drug exploration and development. To validate the results of docking, ADMET analysis of the hit compound was carried out to determine its solubility, drug-likeness, and toxicity. Many other physiochemical parameters of the inhibitor were calculated and found to be satisfying for it to act as a potential inhibitor candidate of DEB [21].

Previous studies in patients with recessive dystrophic EB (RDEB) covering the transplantation of healthy bone marrow stem cells indicated that treatment reduces blister formation and greatly improves wound healing [22]. Stem cell therapy consists of the treatment of the defective sections of the body using stem cells. These are regenerative cells that can produce the desired body component. Stem cells are hemopoietic cells originating from the bone marrow, transplanted and labeled with a green florescent pigment to the RDEB mice. Yet, stem cell treatments are very expensive and are not easily accessible to everyone [23].

The physiochemical properties revealed the high inhibitory effect of the hit compound and it was found to be a good inhibitory candidate of the wild protein of COL7A1. PDB structure of the COL7A1 gene was also unavailable in different protein data banks, so it was also designed using various bioinformatics software and validated for its authenticity. Overall, this study focused on the designing of the inhibitors of COL7A1 through the use of advanced technological and bioinformatics approaches to provide humanity with new drugs that could cure DEB [24]. Cell therapy is used currently to treat EB. Cell therapy uses healthy donor transplant cells to treat patients.

There are a few studies available on Dystrophic Epidermolysis Bullosa (DEB) in the current literature. However, there is no treatment approach or drug design available, currently. This study would provide a breakthrough in the field of drug discovery and design and it would also provide a gateway to the future scientists who wish to work on untreatable diseases. If performed *In vitro,* this drug may prove very efficient in the treatment of Dystrophic Epidermolysis Bullosa (DEB).

5. Conclusion

In the current study, an inhibitor of COL7A1 was designed to be used against DEB with the help of computational biology and bioinformatics approaches. In an artificial environment, the inhibitor showed promising results with satisfying solubility and good interaction with the wild protein. *In silico* designed drug candidate can prove to be effective if it is also tested *In vitro* and *In vivo*. This drug was designed to be used against one sub-type of EB. There is still no cure available for other subtypes of EB, so this study may provide a potential treatment option for Dystrophic Epidermolysis Bullosa (DEB). If the designed drug is synthesized *in-vitro*, it may prove as an efficient treatment option.

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Conflict of interest
The authors declare no conflict of interest.

References

[1] Yao YY, Zhang Y, Xie XH, Chen L, Zhu F, Zhou M. Identification of a novel COL17A1 compound heterozygous mutation in a Chinese girl with non-herlitz junctional epidermolysis bullosa. *Curr Med Sci*. 2020;40(4):795-800. https://doi.org/10.1007/s11596-020-2234-9

[2] Firing, C, Bygum A. *Epidermolysis Bullosa.* 2017:V07170568.

[3] Chen F, Huang L, Li C, et al. Next-generation sequencing through multigene panel testing for the diagnosis of hereditary epidermolysis bullosa in Chinese population. *Clin Genetics*. 2020;98(2):179-84. https://doi.org/10.1111/cge.13791

[4] Yang WS, Kang S, Sung J, Kleinman HK. Thymosin β4: potential to treat epidermolysis bullosa and other severe dermal injuries. *Euro J Dermatol*. 2019;29(5):459-67. https://doi.org/10.1684/ejd.2019.3642

[5] Naveed M, Tehreem S, Arshad S, Bukhari SA, Shabbir MA, Essa R, Ali N, Zaib S, Khan A, Al-Harrasi A, Khan I. Design of a novel multiple epitope-based vaccine: An immunoinformatics approach to combat SARS-CoV-2 strains. *J Infect Public Health*. 2021;14(7):938-946. https://doi.org/10.1016/j.jiph.2021.04.010

[6] Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y. The I-TASSER Suite: protein structure and function prediction. *Nat Method*. 2015;12(1):7-8. https://doi.org/10.1038/nmeth.3213

[7] Benson D, Lipman DJ, Ostell J. GenBank. *Nucleic Acids Res*. 1993;21(13):2963-2965.

[8] Gasteiger E, Hoogland C, Gattiker A, Duvaud Se, Wilkins MR, Appel RD. Protein Identification and Analysis Tools on the ExPASy Server. In: Walker JM, ed. *The Proteomics Protocols Handbook*. Totowa, NJ: Humana Press; 2005:571-607. https://doi.org/10.1385/1-59259-890-0:571

[9] Zhang Y. I-TASSER server for protein 3D structure prediction. *BMC Bio Informatics*. 2008;9(1):1-8. https://doi.org/10.1186/1471-2105-9-40

[10] Ko J, Park H, Heo L, Seok C. GalaxyWEB server for protein structure prediction and refinement. *Nucleic Acids Res*. 2012;40(W1):294-297. https://doi.org/10.1093/nar/ gks493

[11] Wang W, Xia M, Chen J, et al. Data set for phylogenetic tree and RAMPAGE Ramachandran plot analysis of SODs in Gossypium raimondii and G. arboreum. *Data Brief*. 2016;9:345-348. https://doi.org/ 10.1016/j.dib.2016.05.025

[12] Schneidman-Duhovny D, Dror O, Inbar Y, Nussinov R, Wolfson HJ. PharmaGist: a webserver for ligand-based pharmacophore detection. *Nucleic Acids Res*. 2008;36(suppl_2):W223-228. https://doi.org/10.1093/nar/gkn187

[13] Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Res*. 2005 Jul 1;33(suppl_2):W363-367. https://doi.org/10.1093/nar/ gki481

[14] Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*. 2017;7(1):1-13. https://doi.org/ 10.1038/srep42717

[15] Koga H, Prost-Squarcioni C, Iwata H, Jonkman MF, Ludwig RJ, Bieber K. Epidermolysis bullosa acquisita: the 2019 update. *Front Med*. 2019;5(362):1-28. https://doi.org/ 10.3389/fmed.2018.00362

[16] Prodinger C, Reichelt J, Bauer JW, Laimer M. Epidermolysis bullosa: advances in research and treatment. *Exp Dermatol*. 2019;28(10):1176-1189. https://doi.org/10.1111/exd.13979

[17] Ludwig RJ. Clinical presentation, pathogenesis, diagnosis, and treatment of epidermolysis bullosa acquisita. *Int Sch Res Notices*. 2013;2013:1-25. http://dx.doi.org/10.1155/2013/812029

[18] Khan KM, Ambreen N, Taha M, et al. Structure-based design, synthesis and biological evaluation of β-glucuronidase inhibitors. *J Comput Aided Mol Des*. 2014 May;28(5):577-585. https://doi.org/10.1007/s10822-014-9745-z

[19] Afzal MS, Khan A, Qureshi UU, et al. Community-based assessment of knowledge, attitude, practices and risk factors regarding COVID-19 among Pakistanis residents during a recent outbreak: a cross-sectional survey. *J Comm Health*. 2021;46(3):476-486. https://doi.org/10.1007/s10900-020-00875-z

[20] Zahra A, Saleem MA, Javed H, Khan MA, Naveed M, Shakoori AR. Single Nucleotide Polymorphism Induces a Positive Selection Pressure at Gag-Pol Sites in Human Immunodeficiency Virus Favoring Drug Resistance Mutations. *Pak J Zool*. 2021;2021:1-17. https://dx.doi.org/10.17582/journal.pjz/20200525130507

[21] Koga H, Recke A, Vidarsson G, et al. PDE4 inhibition as potential treatment of epidermolysis bullosa acquisita. *J Investig Dermatol*. 2016 Nov;136(11):2211-2220. https://doi.org/10.1016/j.jid.2016.06.619

[22] Kern JS, Loeckermann S, Fritsch A, et al. Mechanisms of fibroblast cell therapy for dystrophic epidermolysis bullosa: high stability of collagen VII favors long-term skin integrity. *Mol Therapy*. 2009;17(9):1605-1615. https://doi.org/10.1038/ mt.2009.144

[23] Wong T, Gammon L, Liu L, et al. Potential of fibroblast cell therapy for recessive dystrophic epidermolysis bullosa. *J Investig Dermatol*. 2008;128(9):2179-2189. https://doi.org/10.1038/jid.2008.78

[24] Van den Akker PC, Jonkman MF, Rengaw T, et al.. The international dystrophic epidermolysis bullosa patient registry: an online database of dystrophic epidermolysis bullosa patients and their COL7A1 mutations. *Human Mutation*. 2011;32(10):1100-1107. https://doi.org/10.1002/umu.21551

[25] Naveed M, Mumtaz AS, Saleem MA. Role of Pyrroloquinoline Quinone in Biocontrol Together with Induced Systemic Resistance: A Novel Resource Trialed for Rice Disease Control. *Intl J Agric Biol*. 2020;2020:1-10.