



## PACE4 - A Novel Therapeutic Drug Target for Osteoarthritis

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### Abstract:

Osteoarthritis (OA) is a degenerative joint disease which occurs mainly due to aging and mechanical stress. So far only temporary methods like giving pain alleviating drugs are employed for the treatment of OA but a permanent solution to cure the disease has not yet been established. The need of the hour is to find this permanent solution and completely undo the disease. The current work is on Computer Aided Drug Designing against Osteoarthritis. PACE4, a pro protein convertase involved in aggrecanase activation in cartilage is a major protein responsible for the occurrence of this disease. Therefore targeting PACE4 can be an effective therapy. The sequence, structure and functional analysis were performed using various insilico tools. The target site on the receptor protein was selected based on the analysis of mutational sites and the presence of the binding site among the selected mutational site. The potential chemical compounds which could be used for the treatment were screened for drug likeliness, toxicity and ambiguity. The binding affinity between the receptor protein and final screened chemical ligands were analyzed by performing Docking studies. Based on Binding affinity it was shown that the chemical compound named Calpain Inhibitor I was found to be the best drug ligand acting against the targeted PACE4 protein. Thus our studies support that Calpain Inhibitor I can be used as a potent drug candidate for the treatment of Osteoarthritis.

### Introduction:

Osteoarthritis is a chronic degenerative disorder of multifactorial etiology characterized by loss of articular cartilage, hypertrophy of bone at the margins, subchondral sclerosis and range of biochemical and morphological alterations of the synovial membrane and joint capsule<sup>[1]</sup>. OA is best

considered as a disease of the whole “joint organ”. Medical conditions that can lead to OA involve bleeding disorders that cause bleeding in the joint such as hemophilia, disorders that block the blood supply near a joint and lead to a vascular necrosis and other types of arthritis, such as chronic gout, pseudo gout, or rheumatoid arthritis<sup>[2][3]</sup>. Proteolytic degradation of the major cartilage macromolecules, aggrecan and type II collagen, is a key pathological event in OA<sup>[4]</sup>. The particular chondrocytes are able to maintain the phenotypic characteristics of the tissue such as resistance to mineralization and vascular invasion. However, when challenged in the onset and progression of osteoarthritis not only are these cells activated and increase matrix molecule synthesis, but they also produce pro inflammatory cytokines and tissue-destructive enzymes. The catabolic processes gradually outmatch anabolic efforts, resulting in progressive degradation of the cartilage<sup>[5][6]</sup>. ADAMTS-4 and ADAMTS-5, the primary aggrecanases capable of cartilage aggrecan cleavage, are synthesized as latent enzymes and require prodomain removal for activity. The N-termini of the mature proteases suggest that activation involves a pro protein convertase, but the specific family member responsible for aggrecanase activation in cartilage in situ has not been identified. PACE4 was identified as a pro protein convertase responsible for activation of aggrecanases in osteoarthritic and cytokine-stimulated cartilage. Posttranslational activation of ADAMTS-4 and ADAMTS-5 in the extracellular milieu of cartilage results in aggrecan degradation<sup>[7]</sup>. These findings suggest that PACE4 represents a novel target for the development of OA therapeutics.

### Materials and Methods:

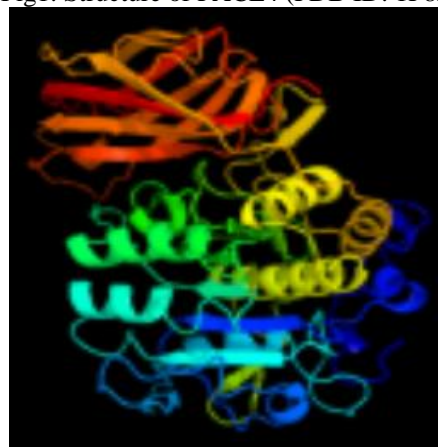
PACE4 protein Sequence was retrieved from NCBI [8]. The sequence was used for further various Insilico analyses. Primary, Secondary and Tertiary Structure analysis were performed using the tools of EXPASY Server. Disordered regions in the sequence were scrutinized using the tools DisEMBL and RONN [9]. The functional regions of the protein were analyzed using the tool SMART [10]. In the preparation of ligands, the chemicals having the therapeutic property against Osteoarthritis were selected from the chemical database Pubchem. The chemicals shortlisted from Pubchem were screened for druglikeliness, Toxicity and Ambiguity. A drug library was generated for all the screened ligands. The protein and Ligand structures were retrieved, following which their structure optimization and energy minimization was performed using the software Argus lab. The Docking analysis was carried out and the ligand binding affinity with the receptor was studied. The comparative analysis was undertaken and based on the best binding affinity the best drug candidate was predicted. This can be used in the treatment of osteoarthritis after a considerable clinical study.

### Results and Discussion:

#### PACE4 Sequence and Structural Analysis:

The sequence of PACE4 consisting of 959 amino acids was retrieved. Through primary structure analysis using Protparam [11] the physicochemical properties of the protein was determined. The protein under consideration was found to be unstable, hydrophilic, negatively charged, aliphatic, a molecular weight of 86247.0 Daltons and a theoretical PI of 5.5. The secondary structure analysis using SOPMA [12] showed the presence of 25.22% alpha helices, 18.22% of extended strands, 5.99% of beta turns and the majority forming random coils with a 50.57%. From tertiary structure analysis, the protein structure of PACE4 was predicted. The PDB ID 1P8J was selected as the ideal representative for the structure of PACE4.

Fig1: Structure of PACE4 (PDB ID: 1P8J)



#### Functional region and Mutational site analysis:

The functional domain was shown through SMART, where the amino acid length and varied functions were noted. The domain Peptidase\_S8 ranging from 179 to 479 has the property of acting on aggrecans and thus, targeting this region will effectively halt the effect caused by this protein on cartilage degradation. From the domain analysis the most disordered or mutation prone regions within the domain were analyzed. The probable mutational sites found were 229-255, 306-319, 377-385, 416-417, and 419-420.

#### Preparation of Ligands:

According to the 'Lipinski Rule of Five', the chemicals found to have a molecular weight of less than 500 daltons, logP not greater than 5, not more than 5 hydrogen bond donors and less than 10 hydrogen bond acceptors were selected for further screening. Based on this rule, 13 chemicals from PubChem were selected. The lead molecules obtained after screening through Lipinski rule were MDL201053, Calpain Inhibitor, Atrophine23622704, Atrophine5318386, Atrophine64692, Atrophine25817, Atrophine17184, Atrophine6321299, Urinastatin, Gabapentin, Acetaminophen, (6-(4-(2-piperidin-1-ylethoxy)phenyl))-3-pyridin-4-ylpyrazolo(1,5-

a)pyrimidine and Capsaicin Precursor. The compounds were run through Mobyly ADME and their toxicity was determined. (6-(4-(2-piperidin-1-ylethoxy) phenyl))-3-pyridin-4-ylpyrazolo (1, 5-a) pyrimidine had more than 3 benzene rings and hence was not taken into further consideration. Twelve out of thirteen compounds were accepted. The ambiguity studies for all the compounds were performed and the biophysical properties along with their allowed hydrogens were recorded. Calpain Inhibitor I was found to be the most favourable ligand as it had the least ambiguity.

**Docking Analysis:**

From the Primary (Drug likeliness), Secondary (Toxicity) and Tertiary (Ambiguity) Screening process, 10 chemicals were selected. The PACE4 receptor protein and the Screened chemical compounds were optimized using Argus lab. The optimized structures were processed further for Docking. Docking results were tabulated in the below Table 1. From the overall analysis, Calpain Inhibitor I was found to have the best binding affinity with the receptor owing to its lowest docking energy of -265.64 Kcal/mol.

**Table1: List of Screened Drug Candidates**

Serial Name	Chemical Name	Chemical ID	Optimization Energy (K cal/mol)	Docking Energy (K cal/mol)
MDL 201053	Benzyloxycarbonyl-phe-ala-fluormethylketone	122019	39.60	-255.87
Urinastatin	Urinary trypsin inhibitor	105102	49.74	-185.65
Calpain inhibitor I	N-acetylleucylleucylnorleucinal	4332	39.32	-265.64
Acetaminophen	4-Acetamidophenol	1983	20.83	-161.65
Gabapentin	1-(Aminomethyl)cyclohexanecarboxylic acid	3446	36.25	-170.76
Capsaicin Precursor	4-hydroxy-3-methoxyphenyl)methylazanium	6951328	16.82	-159.75
Atrophine25817	Hyoscyamine (D)-, Daturine	25817	66.30	-216.31
Atrophine64692	L-Hyoscyamine, Cystospaz, l-Atropine	64692	60.58	-219.75
Atrophine23622704	Hyoscyamine sulfate	23622704	63	-251.11
Atrophine5318386	Hyoscyamine	5318386	53.91	-239.10

**Conclusion**

In light of Pace4 receptor protein being an aggrecanase stimulator, it serves as a therapeutic target in Osteoarthritis. When Pace4 is docked, it will cease to act as an aggrecanase stimulator which in turn will prevent the aggrecanases from acting on aggrecan, a major cartilage macromolecule. This process can avert proteolytic` degradation to a major extent and thereby block the progression of Osteoarthritis. Out of the ten compounds that were docked with Pace4 receptor in Hex, Calpain Inhibitor

I showed the least docking energy and thus can be considered as the most acceptable drug candidate having the best binding affinity with the Pace4 receptor. The study can be promising if followed by clinical study.

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