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Research Article

MOLECULAR MECHANISM OF ANALGESIC ACTION OF SOLANOGLYCOSYDANE – AN IN SILICO STUDY

M. DUTTA CHOUDHURY[1, 2], MONJUR AHMED LASKAR*[1, 2], SHUVASISH CHOUDHURY[3] AND PANKAJ CHETIA[1, 2]

[1]Bioinformatics Centre, Assam University, Silchar – 788011, Assam, India,[2] Department of Life Science and Bioinformatics, Assam University, Silchar – 788011, Assam, India,[3] Central Instrumentation Laboratory, Assam University, Silchar – 788011, Assam, India,
Email: monjur@bioinfoaus.ac.in

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ABSTRACT

There are few natural products analgesics which target TRPV1. Solanoglycosydane a glucosidal steroid isolated from the tender fruits of the plant *Solanum torvum* Swartz reported to have analgesic action. But molecular mechanism of action of this product in inducing analgesic action was not explained. In the present work we wanted to see if the analgesic action of Solanoglycosydane is mediated through TRPV1 using computational tool. The three dimensional structure of TRPV1 was downloaded from Protein Data Bank (RCSB PDB). The molecule was analyzed to find its probable active site using online server, Q-Site Finder. This program depicts the amino acids of the probable ligand binding sites of the enzyme. The very first site was selected as it was the energetically most favorable. The three dimensional structure of Solanoglycosydane was generated using another online server, CORINA. This structure of Solanoglycosydane was also saved in PDB format and is converted to sdf format using OpenBabel. The ADME/Tox properties of the compound were also studied using Mobyle ADME/Tox server. To study the binding efficacy of Solanoglycosydane to TRPV1, FlexX was used. The ADME/Tox screenings of Solanoglycosydane have not shown any negative results, which indicate the potentiality of the molecule to become drugs. The compound showed greater binding affinity towards TRPV1. The study suggested that Solanoglycosydane induces analgesic action by inhibiting TRPV1.

Keywords: Analgesics, ADME/Tox, FlexX, Solanoglycosydane, TRPV1

INTRODUCTION

Natural products are being utilized since the time immemorial for different purposes including the treatment of pain. Initially different plants like opium etc. were used to cure pain in the raw form. But the effort to isolate the pure and active form of natural products started in the 19th century. With the development of modern tools to isolate and screen the drug efficacy, many natural products have reached the market as effective analgesics. Aspirin (Acetylsalicylic acid) the popular analgesic compound was isolated from *Salix alba*. Similarly morphine, codeine, thebaine, menthol etc. have been widely popular as analgesics under different brand names [1]. Very recently, computational tools have been used to screen the activity and toxicity profile of drug molecules.

Extracts from *Diclea grandiflora*, a vine found in North eastern Brazil have been reported to have analgesic effects [2]. Several non-nitrogenous compounds were isolated from these extracts and a minor component, dioflorin was discovered to be a potent analgesic in a mouse model of tail-flick [3]. The analgesic activity was reversed by naloxone indicating a potential mode of interaction with opioid receptors.

Cocaine is mostly known and studied for its ability to block the dopamine transporter due to its ability to create a euphoric state [4]. However, its utilization as a local anesthetic is known by its interactions through sodium channel blockage.

Epibatidine was isolated from the skin of the Ecuadorian dart-frog, *Epipedobates tricolor*, has been reported to be a potent analgesic compound that could be antagonized by mecamylamine, a nicotinic receptor antagonist, but not by opioid antagonists.[5]

Resiniferatoxin isolated from the succulent plant *Euphorbia resinifera*, is another natural product having analgesic activity [6].

Scutigeral isolated from the non-pungent mushroom Albatrellus ovinus, has been shown to stimulate rat dorsal root ganglion neurons by activation of vanilloid receptors [7].

Thapsigargin, an active compound isolated from the plant *Thapsia garganica*, generally used in traditional European and Arabian medicines for rheumatic pain. Thapsigargin inhibited TRPV1 mediated [45Ca2+] -uptake and blocked [3H]-RTX binding sites in rTRPV1-CHO cells [8].

Yohimbine, an alkaloid obtained from the bark of the tree *Pausinystalia yohimbe* and the root of *Rauwolfia.* Yohimbine inhibits Na+ channels and TRPV1 channels in a dose dependent way [9].

Two acylpolyamine toxins, AG489 and AG505 were isolated from the North American funnel web spider, *Agelenopsis aperta,* which inhibit TRPV1 channels from the extracellular side of the membrane. The toxin AG489 inhibits TRPV1 in a voltage-dependent way[10].

In this work, we tried to predict the molecular mechanism of action of analgesic activity Solanoglycosydane a glucosidal steroid, isolated from the tender fruits of the plant *Solanum torvum* Swartz by Paul et al [11]. Analgesic activity of the compound was established by them using animal models. However mechanism of action of the compound in inducing analgesic action was not studied.

Generally some target proteins or enzymes or receptors are responsible for the therapeutic activity of drug molecules. Here we studied the probable action of solanoglycosydane on the transient receptor potential vanilloid, subfamily V, member 1 (TRPV1), also known as the capsaicin receptor is a protein that, in humans, is encoded by the TRPV1 gene. This protein is a member of the TRPV group of transient receptor potential family of ion channels [12, 13, 14]

TRPV1 is distributed in the peripheral and central terminals of the sensory neurons and plays a role in initiating action potentials at the nerve terminals and modulating neurotransmitter release at the first sensory synapse [15, 16]. TRPV1 is considered as a target for analgesics through evaluation of different antagonists [17, 18, 19]

MATERIAL AND METHODS

Preparation of Ligand Structures

Chemsketch, chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com) was used to construct the structure of the ligands. Using draw mode of Chemsketch, the ligands were drawn and the SMILE notations of the compounds were generated. The three dimensional structures of the compounds in PDB formats were generated using another online server called CORINA using the SMILE notation and again converted to SDF format using OpenBabel [20].

Preparation of Protein Structure

The crystal structure of the drug targets TRPV1 (PDB ID: 2NYJ) has been obtained from RCSB Protein Data Bank (http://www.pdb.org).

Active site identification

The structure of the drug target was obtained from Protein Data Bank. The PDB file was loaded into Q-Site Finder [21, 22] to identify the active site amino acids.

ADME/Tox Screening

The ADME/Tox parameters of the compounds were studied using online server Mobyle@RPBS maintained by the University of Paris. The compounds were input in the server in SMILES format using the following parameters:

Molecular weight : min 200.0 max 600.0
Hydrogen donors : min 0.0 max 6.0
Hydrogen acceptors : min 0.0 max 12.0
Flexible bonds : min 0.0 max 15.0
Rigid bonds : min 0.0 max 50.0
Ring number : min 0.0 max 7.0
Ring size : min 0.0 max 12.0
Atom number : min #carbons: 5.0 min #non carbons 2.0
Ratio carbon/hetero : min 0.1 max 1.0
Charge number : min 0.0 max 3.0
Total charge : min -2.0 max 2.0
logP : min -2.0 max 6.0
Polar Surface Area : min 0.0 max 150.0

Protein – Ligand interaction using FlexX

The PDB file of the target was loaded in the BioSolveIT FlexX [23]. The active site amino acids were defined in the target molecule during the target preparation step of FlexX. A sphere of 9Å radius was defined as active site. The SDF file of all the compounds was loaded in FlexX as docking library. The Protein Ligand clash was set to 2.9 Å and Intra Ligand clash was set to 0.6 in the docking. The docking was performed to study the binding efficacy of the compounds and the drug target. The docked ligand-target complexes were analyzed carefully to identify the interactions. The docking score was noted down and docking poses were saved for reference.

RESULTS

The active site characterisation of the enzyme using Q-site finder showed that GLU 311, TYR 351, LYS 303, THR 306, SER 307, ASN 310 are the key amino acids forming active site.

Various drug relevant properties of the compound were studied using OSIRIS Property Explorer. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red. Where as a green colour indicates drug confirm behaviour.

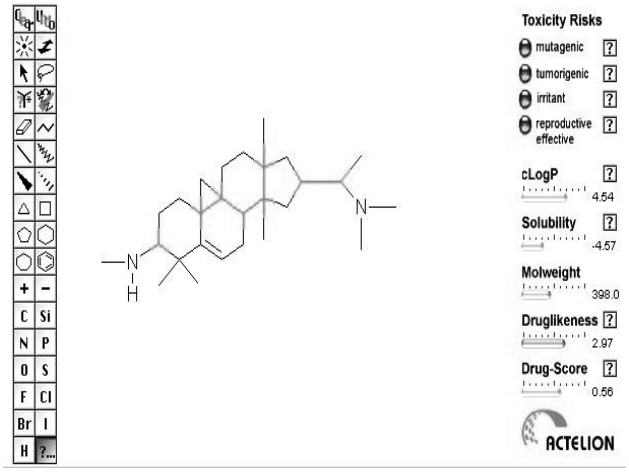

Fig 1: Drug relevant properties of the compound

For any molecule to become a drug, it should not have any toxic or allergenic effects. The ADME/Tox screenings of Solanoglycosydane have not shown any negative results, which indicate the potentiality of the molecule to become drugs. The results of the ADME/Tox screening are described in Table.1

Table 1 :Results of ADME/Tox Screening

Parameters	MW	Drs	Ars	FB	RB	#R	RL	C	nC	C/nC	#Chrg	Chrg	LogP	PSA
Parameter standards	200-400	0-6	0-12	0-15	0-50	0-7	0-12	5-2	>2	0.1-1.0	0-3	(-2)-2	(-2)-6	0-150
Solanoglycosydane	398.3	1	2	4	22	5	6	27	2	0.074074	0	0	5.56	15.27

MW : Molecular weight, Drs : Donors, Ars : Acceptors, FB : flexible bonds, RB : Rigid Bonds, #R : Ring Number, RL : Ring Length, C : carbons, nC : non carbons, C/nC : ratio non carbons/carbons, #Chrg : number of charges, Chrg: Total Charge, LogP : logP (octanol / water), PSA: Polar surface area.

Interaction energies between ligand and receptor play the most crucial role in drug designing. In this work, TRPV1 was selected as drug target and the interactions of Solanoglycosydane were studied using FlexX and following results were obtained (Table.2).

Table 2: Docking result of TRPV1 with Solanoglycosydane

Compound	Total score	Bond Property		
		Bonds	Energy(Kcal/mol)	Length(Å)
Solanoglycosydane	-8.4030	OE2 GLU311 – H57	-7.36	2.01
		OE1 GLU311 – H57	-3.16	2.05
		OH TYR 351 – H76	-4.70	1.72

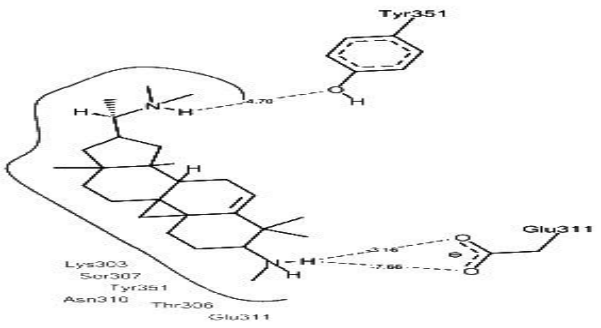

Fig 2 : Docking of Solanoglycosydane with TRPV1 residues

DISCUSSION

The vanilloid receptor TRPV1 is recognized as a molecular integrator of painful stimuli ranging from noxious heat to endovanilloids in inflammation. Pharmacological blockade of TRPV1 represents a new strategy in pain relief. TRPV1 is considered to be a potential target for developing drugs to treat different modalities of pain and it is distributed in the peripheral and central terminals of the sensory neurons and plays a role in initiating action potentials at the nerve terminals and modulating neurotransmitter release at the first sensory synapse. Docking of Solanoglycosydane with analgesic target TRPV1 shows a good result. Solanoglycosydane binds with the residues of TRPV1 with the estimated free energy of binding -8.4030kcal/mol. From the docking results it can be predicted that Solanoglycosydane may be used as an Analgesic Drug and the

analgesic activity of Solanoglycosydane is mediated through TRPV1. On the one hand the study confirms the report of Paul et al. (2009) for analgesic activity of the compound and on the other it predicts the possible mechanism of action of the compound.

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