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In Silico Prediction of Novel Drug Molecule for Migraine Using Blind Docking

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Abstract: This study deals with a comprehensive pathway of lead molecule design for migraine focusing on the emerging in silico trends and techniques which include generation of candidate molecules, checking for their toxicity and human body likeliness, docking them with the target and ranking them based on their binding affinities. Protein sequence of Human receptor activity-modifying protein-1 (hRAMPI) was taken and prediction of missing side chain was performed using SQWRL. Energy minimization was done in Chimera using AMBER force field, followed by blind docking of the available drugs with Autodock software. Ligandscout was used to analyze the pharmacophore and its derivatives were derived using ProDRG. The derivatives were analyzed for drug-likeliness using Lipinski filters and ADME/Tox filter. Protein-ligand interactions for all the derivatives were determined using Autodock and Hex. Sumatriptan was found to be the best ligand as it had the lowest binding energy. Its 15 derivatives were drawn with minimized energy. It was found that compound number 13, 9, 10 and 12 had lowest binding energy and all had drug likeliness. In all the four compounds, ASP90A, TRP84A, ALA70A and TYR66A amino acids were found to participate in ligand-protein interaction. By the comparative analysis of the binding energies of all the complexes thus formed, three of the best ligands were chosen and analyzed for active amino acid groups mainly involved in ligand-protein interaction using Ligand Scout 2.0. The amino acid groups ALA70A and ASP90A were found to be involved in favorable binding interaction.

Key words: Migraine · hRAMPI · Sumatriptan · Blind docking

INTRODUCTION

Migraine is basically a disorder of neurological origin causing severe pain typically on one side of the head but sometimes on both sides [1]. Most of the sufferers have a family history of Migraine and women are affected more than men. Calcitonin gene-related peptide (CGRP) is a neuropeptide found to play a key role in the development of migraine [2-4]. Previous study provides the evidence that the responsiveness of neuronal CGRP receptors is strongly enhanced *in vitro* and *in vivo* by expression of human receptor activity-modifying protein-1 (hRAMPI), an obligatory subunit of the CGRP receptor [5]. CGRP mRNA levels and promoter activities were also found to increase endogenously due to activation of CGRP receptors [5, 6]. CGRP receptors have expression in a wide array of tissues and also involved in biologically significant and pathophysiological events [7, 8]. Also, the CGRP receptor antagonists were successful in improving the symptoms of a migraine attack [9-13].

Migraine can be classified as common migraine, classic migraine, ophthalmoplegic migraine, Hemiplegic migraine, basilar type migraine, abdominal migraine and acephalgic migraine [14]. The common symptoms of migraine include intense pulsating or throbbing headache. It may also include nausea, vomiting, diarrhea and increased sensitivity to smell, sound or light [15]. Visual disturbances or 'aura' (flashing lights, wavy lines, distorted vision, blind spots) [16], difficulty in concentrating, speaking, tingling sensation in the limbs, tension in the neck and shoulder region and problems with coordination are other symptoms. Certain drugs alleviate the symptoms of migraine by vascular or neuronal effects [17, 18] but there are no guidelines to determine the effective therapy. In 2006, FDA warned about the serotonin agonist, as they lead to symptoms like dilated pupil seizures and blood pressure problems. The commonly available drugs are listed in (Table 1).

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Table 1: Classification of drugs used in therapy against migraine attacks.

Drug type	Examples
Non-steroidal anti-inflammatory drug (NSAIDS)	Naproxen, Paracetamol
Serotonin agonists	Sumatriptan
Ergot alkaloids	Ergotaminetartratetablets
Analgesics combined with Antiemetic	Metoclopramide, Aspirin
Herbal treatment	Feverfew

The purpose of this study was to compare the available drugs and determine the pharmacophore of the best compound by blind docking approach. Five different drugs previously available for migraine (aspirin, ibuprofen, naproxen, paracetamol and Sumatriptan) were taken for initial study. After drawing the ligands and energy minimizing, the ligands were docked with the protein molecule using AUTODOCK software. After docking the drug having the minimum binding energy value was taken and the binding affinities their derivatives were rationalized by blind docking technique.

MATERIALS AND METHODS

Target Identification: Human receptor activity-modifying protein-1 (hRAMPI) protein is sufficient for functionality of *CGRP* and is identified as a drug target for migraine. By inhibiting the activity of *hRAMPI* the migraine attacks can be substantially reduced. The structure of human receptor activity-modifying protein-1 (hRAMPI) is taken from RCSB Protein data bank whose PDB code is 2yx8.

Target Validation: Receptor structure plays a central role in the target based drug design. The crystal structure of 2yx8 was obtained from the PDB site [19] and identification of secondary structure was done through the STRIDE WEB INTERFACE (http://webclu.bio.wzw.tum.de/stride) [20]. It generates protein secondary structure assignment from atomic co-ordinates based on the combined use of hydrogen bond energy and statistically derived backbone tensional angle information to identify the number of secondary structure helix, sheet and coil. SCWRL 3.0 software [21] was used to check for any missing side chain (SCWRL3.0 is based on graph theory to solve the side chain prediction problem.) The missing residues were fixed using Deep View (The Swiss PDB Viewer available at (http://us.expasy.org.spdbv/) [22].

Energy minimization of Target Receptor: Before energy calculations can be performed it is necessary to correct structural inconsistencies, add hydrogen and associated atoms with force field parameters. Minimization routines are provided by MMTK, which is included with Chimera [23]. The Kollman charges were added to each atom of the remained promoter. The Amber ff99 force field is used for standard residues and Amber Antechamber module (also included with Chimera) is used to assign parameters to nonstandard residues.

Preparation of Ligands: The compounds were drawn and energy minimization was done in PRODRG server (http://davapc1.bioch) using the GROMOS 96.1 force field [24]. Since ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogen atoms were merged.

Docking: The widely distributed molecular docking software Autodock 4.0 was used in this study [25, 26]. This program addresses automatically the flexible docking of the ligands into a known protein structure. The flexibility of the target is also taken into account. But for this study, we had considered only the flexible docking of ligands into a known protein structure. The binding site was unknown in this case, hence called as blind docking. As Autodock searches for the best way to fit a Ligand molecule into a receptor, which results in a docking log file that contains a detailed record of the Docking. For blind docking study it is necessary to set up the docking to search the full surface of receptor [27]. To achieve this, size of the grid maps were increased.

In-Silico Docking: The aim of this part is the use of an automated docking suite called 'Autodock'. The GUI for Autodock is AutoDockTools (ADT), which was used to perform the entire docking task. More information about Autodock is available on the Autodock suite homepage available at Scripps University server, (http:// www.scripps.edu/ mb/ olson/ doc/ autodock/) [28]. After preparing the protein and ligand, further modification to the protein and ligand like fixing the torsion residues were made and the files are saved in Autodock directory. The grid box was settled on the protein, Autodock was run and the obtained Docking results were saved. The conformation with the lowest docking energy was ranked best by Autodock. Then the results were arranged according to cluster rank, lowest docked energy and the number of configurations in the cluster.

Table 2: Docking parameters.

Parameter type	Value
Maximum number of GA runs	100
Population size	150
Maximum number of evaluation	250000
Rate of Gene mutation	0.02
Rate of Crossover	0.8

Steps of Autodock: Grid Generation: The Grid box was centered on the human receptor activity-modifying protein-1 (hRAMPI) the spacing between the Grid points was 0.375 angstroms and the number of grid points in each x, y and z dimension was set to 70.

Docking: The GA-LS (Lamarckian genetic algorithm) was chosen to search for the best conformation [26]. During the docking process, parameters were set for each compound as given in (Table 2). The parameters were set using the software Autodock Tools [29]. The Calculations of Autogrid and Autodock were performed on Linux Operating system followed by evaluation of modified ligands by a flexible docking procedure and screening with ADME/Tox filters.

At the end of the docking, the lowest energy conformation of the ligands is determined. This conformation is a combination of translation, quaternion and Torsional angles and is characterized by intermolecular energy, internal energy and Torsional energy. The first two of these combined give the 'Docking energy' while the first and third give 'Binding energy'. The overall lowest binding energy output was used as the criterion for ranking. After ranking the 3 top conformers, the Ligands were carefully checked for good steric complementarity. Furthermore the main features shared by these 3 top conformers were studied with help of Ligand Scout 2.0 [30] and on basis of the important pharmacophores of three top conformers 16 derivatives were designed by PRODRG using the GROMOS 96.1 force field [24]. All the 16 derivatives were passed through ADME/Tox Filter and Lipinski filter. The derivatives were then docked to the receptor using Autodock tools and 4 best ligands were taken into consideration. The important pharmacophores and critical amino acids which are common in all four ligand protein complex were predicted using Lignd Scout 2.0.

RESULTS AND DISCUSSION

Stride Evaluation: Secondary structure of protein molecule was evaluated with STRIDE, which provided the physical features of hRAMPI*, such* as helix, coil as well as extended stands and the like.

Secondary structure of *hRAMP1* has only H alpha helix and isolated Beta Bridge and generally the protein consists of three long chains of alpha helix.

Missing Side Chain Pridiction By Scwl 3.0 Software: Human receptor activity-modifying protein-1 (hRAMPI) was taken from RCSB Protein data bank. The resolution of the structure was 2.40 Armstrong which is accepted for docking study. Sometimes the side chain of the protein deposited in PDB is missing hence it is important to check for missing side chains using SCWL 3.0 SOFTWARE. The missing residues were also fixed using this software.

Molecular Docking Study for Pharmacophore Identification Preparation of Ligand and Protein: Minimization of protein molecule was done through Chimera software and for Sumatriptan minimization was done in PRODRG server. Rigid roots were automatically defined rather manually for Sumatriptan. Docking-grid was generated over the whole protein molecule to find out the most appropriate active site for binding purpose. The protein-ligand complex after docking is extracted in the pdb file format. Visualization of Pharmacophores was generated with help Ligandscout 2.0.

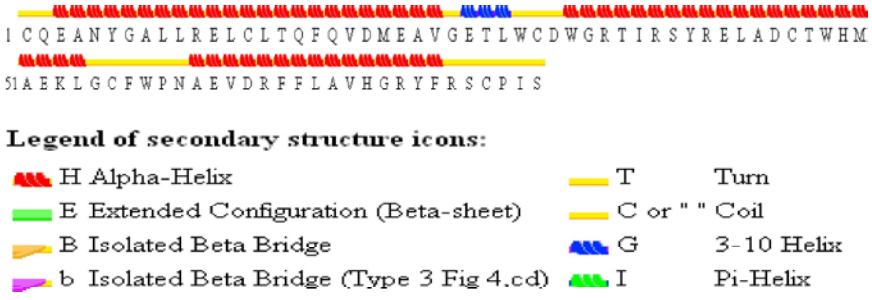


Fig. 1: Secondary structure of *receptor* activity-modifying protein-1 (hRAMPI).

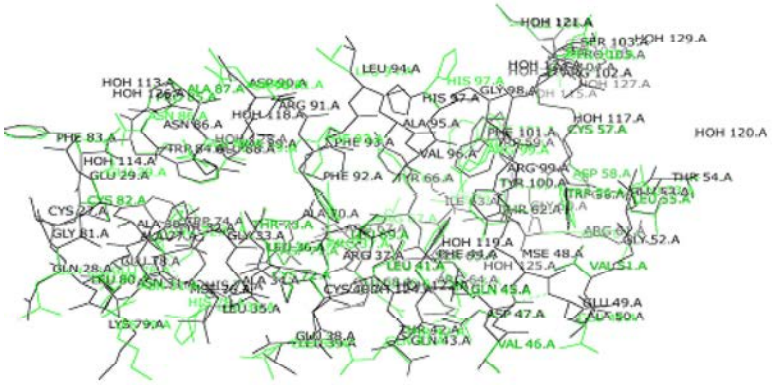


Fig. 2: White color-original pdb-2yx8, pink color-predicted protein with all side chain conformation.

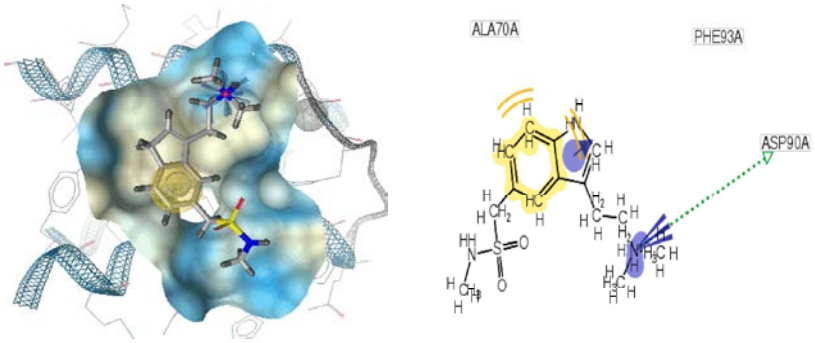


Fig 3: (a) Showing cavity surface of the receptor *activity-modifying protein-1* complex with Sumatriptan molecule and. Fig3 (b) showing 2d representation of protein and ligand interaction.

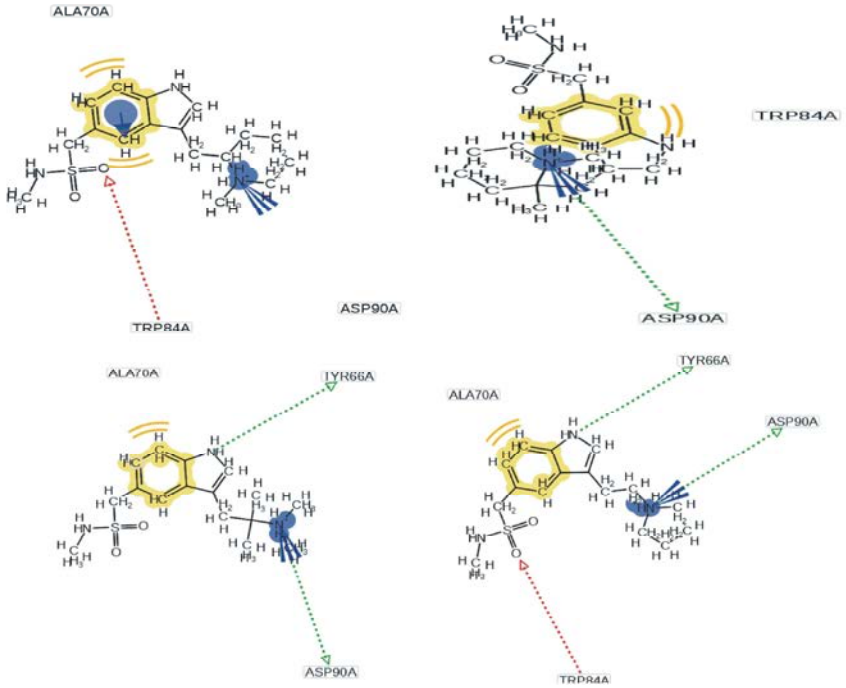
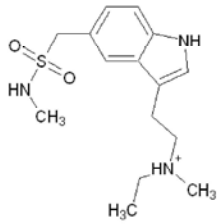
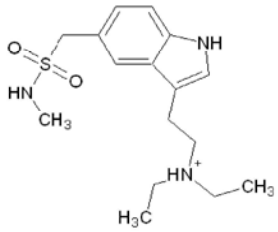


Fig. 4: Left top figure showing the interaction between hRAMPI receptor and compound-9, Right top figure showing interaction between hRAMPI and compound-13, Left bottom showing the interaction between hRAMPI and compound-10, Right bottom showing the interaction between compound-12 and hRAMPI receptor



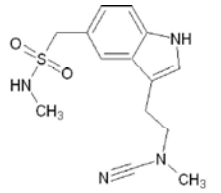
N-ethyl-*N*-methyl-2-{5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl}ethanaminium

■ COMPOUND-**1**



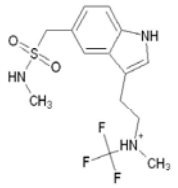
N,*N*-diethyl-2-{5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl}ethanaminium

■ COMPOUND-**2**



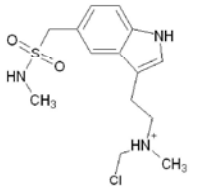
1-(3-{2-[cyano(methyl)amino]ethyl}-1*H*-indol-5-yl)-*N*-methylmethanesulfonamide

■ COMPOUND-**3**



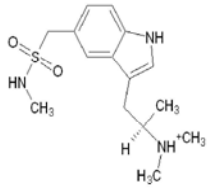
N-methyl-2-{5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl}-*N*-(trifluoromethyl)ethanaminium

■ COMPOUND-**4**



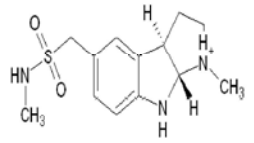
N-(chloromethyl)-*N*-methyl-2-{5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl}ethanaminium

■ COMPOUND-**5**



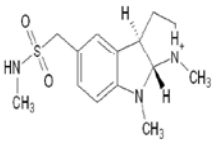
(2*R*)-*N*,*N*-dimethyl-1-{5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl}propan-2-aminium

■ COMPOUND-**6**



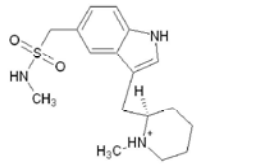
(3a*R*,8a*S*)-1-methyl-5-[(methylsulfamoyl)methyl]-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-1-ium

■ COMPOUND-**7**



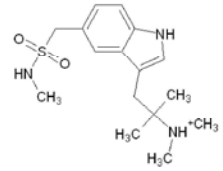
(3a*R*,8a*S*)-1,8-dimethyl-5-[(methylsulfamoyl)methyl]-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-1-ium

■ COMPOUND-**8**



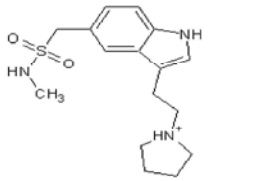
(2*R*)-1-methyl-2-({5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl}methyl)piperidinium

■ COMPOUND-**9**

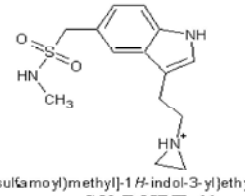


N,*N*,2-trimethyl-1-{5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl}propan-2-amipium

■ COMPOUND-**10**

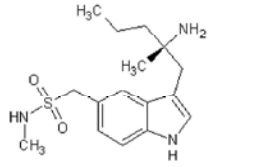


1-(2-{5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl}ethyl)pyrrolidinium



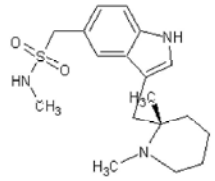
1-(2-{5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl}ethyl)aziridinium

■ COMPOUND-**11**

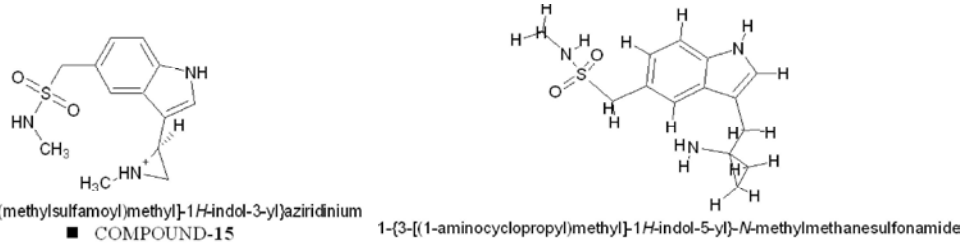


1-{3-[(2*S*)-2-amino-2-methylpentyl]-1*H*-indol-5-yl}-*N*-methylmethanesulfonamide

■ COMPOUND-**14**



• COMPOUND-13



Supplementary-1 (ligand structure with IUPAC name)

Based on the pharmacophores generated by the protein-ligand complex structure, 16 derivatives were designed in PRODRG server with GROMOS 96.1 force field for minimization.

Docking Study of 16 Derivatives: The docking results were ranked according to the ascent of the docking energies of the 100 conformers for each of the ligands, ranking the energy results according to the Binding Energy which included the Intermolecular Energy and the Torsion terms. It was found that most of the ligands interacted quite well with the receptor in the pocket. A comparison of the results between the top 4 ranked conformers suggests that some large sized ligands suffer from more loss of Torsional freedom upon binding. The top three compounds were selected based on the scoring function, which predicts the ranking of different ligands in approximate order of ligand size, order of affinity and allows selectivity [31]. It was found that compound no 13, 9, 10 and 12 had lowest binding energy. The compounds were also checked for their conformations from the output dlg file of a docking run and were sorted out according to their RMSD values. It was found that a cluster of 2 conformations in compound 13, a cluster of 8 conformations in Compound 9, a cluster of 19 conformations in Compound 10 and a cluster of 35 conformations in compound 12 were all observed with a RMSD tolerance set to 2.0. The recurrence of the identical conformations of one ligand means that it fits well to the pockets and is likely a good inhibitor candidate [33].

Validation of Docking: HEX docking score was used to estimate the binding affinity of the ligand receptor complex. The four top conformations which show lowest binding energy in Autodock (Table 3) were valueated using HEX (Table 4). The results show that the ligands are binding to the same active site and binding energy is also consistent with the Autodock result [31, 32].

Table 3: Comparision of experimenal binding affinities and docking scores using autodock 4.0

Compounds	Lowest Binding Energy	Calculated Pki conc	Rank
Compound 1	-5.58	80.62µM	RANK14
Compound 2	-5.75	61.3µM	RANK7
Compound 3	-5.31	127.47µM	RANK16
Compound 4	-5.57	82.31µM	RANK15
Compound 5	-5.7	66.87µM	RANK10
Compound 6	-5.82	54.41µM	RANK5
Compound 7	-5.74	62.01µM	RANK8
Compound 8	-5.75	60.65µM	RANK6
Compound 9	-6.51	16.88µM	RANK2
Compound 10	-6.16	30.62µM	RANK3
Compound 11	-5.62	76.47µM	RANK13
Compound 12	-6.07	35.82µM	RANK4
Compound 13	-6.6	14.45µM	RANK1
Compound 14	-5.68	68.79µM	RANK11
Compound 15	-5.66	71.24µM	RANK12
Compound 16	-5.73	62.58µM	RANK9

Table 4: Evaluation of hex docking result for the top 4 conformers

COMPOUND	HEX SCORE
Compound-13	189.63
Compound-9	188.73
Compound-10	184.24
Compound-12	180.24

Study of Interaction: In all the four ligand-protein complexes the ASP90A, TRP84A and in some cases ALA70A, TYR66A amino acids participate in ligand-protein interaction. ASP90A acts as hydrogen bond acceptor, TRP84A act as hydrogen bond donor and ALA70A is involved in hydrophobic interaction. In compound-9 piperidinium nitrogen as positive ionizable residue interacts with ASP90A which acts as hydrogen bond donor; phenyl reside in indol ring acts as hydrophobic moiety and oxygen atom of sulfamoly group involved with TRP84A acts as hydrogen acceptor. In compound-10 amininium atom acts as positive ionizable residue which interacts with ASP90A amino acid, Nitrogen atom in indol ring acts as hydrogen donor and TYR66A as acceptor, phenyl ring of indol ring acts as a hydrophobic group interacting with ALA70A. In compound-12 oxygen atom of sulfamoly group

interacts with TRP84A, nitrogen atom in indol ring acts as hydrogen donor for TYR66A and pyrrrolidinium nitrogen donates hydrogen to ASP90A.

CONCLUSIONS

Sumatriptan derivatives which have been designed on the basis of pharmacophores of Sumatriptan bind to the same active site with lower binding energy than original compound. The amino acid group ASP90A and TRP84A play an important role in protein-ligand binding. This study provides strong evidence that ASP90A and TRP84A are the crucial residues for binding of Sumatriptan derivatives to hRAMPI receptor. These findings advance our knowledge on treatment of migraine. Compound 13 is the best derivative and can be further used for treatment of migraine.

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