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Screening of potential GCMS derived antimigraine compound from the leaves of *Abrus precatorius Linn* to target "calcitonin gene related peptide" receptor using *in silico* analysis

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ABSTRACT

Calcitonin receptor-like receptor (CRLR) is a human protein, that produces a calcitonin gene-related peptide receptor (CGRP) when associates with human receptor activity-modifying protein-1 (HRAMP1). CGRP is believed to be involved in triggering of migraine. Many strategies were employed to design antimigraine drug using various CGRP antagonist/ligands but most of them have failed due to their inability to reach the target "CGRP receptor" as they get metabolized before conferring their pharmacological action and exhibit toxic effect to the liver. The present study, evaluated the binding of 13 phyto-chemical compounds of "*Abrus precatorius*" identified through GCMS analysis against the protein CGRP using "Discovery Studio software". The molecular docking study and ADME/T properties prediction were performed with the compounds using C-DOCKER module. The results showed that five lead compounds of *A. precatorius* may act as good inhibitors for migraine headache and the compounds can be re-designed and synthesized for better antimigraine activity.

1. Introduction

For long times the medicinal plants, herbs and spices have been well known as the treatment of human as well as animal diseases. Owing to the lack of awareness and deforestation the rare and precious medicinal plants were lost. Since the medicinal plants are associated with lesser adverse effects they are considered as an important for the therapy in developing and developed countries [1]. Last few decades some of the countries are promoting the research on herbal medicine for their economic growth and development.

Abrus precatorius Linn. (*Fabaceae*) commonly known as Indian liquorice, which is a climbing shrub found in subtropical regions of India [2]. The leaves are glabrous with long internodes. It has slender branches with cylindrical wrinkled stem with a smooth textured brown bark [3]. The leaves of *A. precatorius* possess medicinal properties to treat herpes zoster, wounds, rheumatoid arthritis, catarrhal, fever, and cough. The leaves of *A. precatorius* are also been used in Nigeria for the treatment of myriad of diseases including malaria, typhoid, respiratory tract infections and hepatitis [4]. The leaves of the plant have sweet taste that lasts long on the tongue upon ingestion. Interestingly, if the leaves are taken orally as medicine it does not contain as much of the deadly component abrin, a potent toxin as found in the seed of the same plant [5]. Hence, the leaves of *A. precatorius* have been used as a food and medicine. It is reported that the leaves are commonly chewed or sucked to obtain its sweet taste [6] and boiled with food such as cereal pulp and vegetables as a sweetener. In addition to this, the fresh leaves have been reportedly pressed on the gum for sore mouth and in many countries the preparations of leaves of *A. precatorius* are being used for skin cancer [7].

Migraine is a unilateral throbbing headache associated with nausea and vomiting. The exact cause of migraine remains unknown, but the most widespread theory is that it is a disorder of the serotonergic system [8]. Serotonin is a type of neurotransmitter, which passes messages between nerve cells and control mood, pain sensation, sexual behavior, sleep as well as dilation and constriction of the blood vessels. Migraine is also characterized by over excitability of certain active protein which leads to inflammatory pain in specific area of the brain. The migraine attack is three times more common in women than men. Goadsby and co-workers

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reported that the migraine gets triggered by brain dysfunction and leads to activation and sensitization of the trigeminovascular system, particularly trigeminal nociceptive afferents innervating "meninges" and leads to headache [10]. The serotonin level in the brain involved in the constriction and followed by dilation of the blood vessels which triggers the migraine. Triptans are the activate serotonin receptors which stops the migraine attack [9].

Calcitonin gene-related peptide (CGRP) is a neuropeptide reported to play a key role in the onset of migraine [11]. Previous studies reported that the sensitivity of neuronal CGRP receptors is strongly enhanced by the *in vitro* and *in vivo* expression of human receptor activity-modifying protein-1 (hRAMPI), an obligatory subunit of the CGRP receptor [12]. CGRP mRNA levels and promoter activities were also increased endogenously due to an activation of CGRP receptors [13]. The CGRP receptor antagonists were successfully alleviates the symptoms of a migraine attack [14].

CGRP receptor is a heterodimeric protein with Calcitonin receptor like receptor (CALCRL) and Receptor Activity Modifying Protein-1 (RAMP-1) as its subunits. Human RAMP1 is a small single-transmembrane protein which is required for CGRP binding to CLR (Calcitonin-like receptor) [15–17]. The activation of this receptor by the endogenous natural ligand leads to various physiological disorders like migraine, Crohn's disease, irritable bowel syndrome, systemic inflammatory response syndrome and so on [18,19]. Whereas, RAMP is an activity modulator associated with different GPCRs like Calcitonin receptor or Calcitonin receptor like receptor to incarnate various dimeric receptors [20,21]. There are different types of RAMPs namely, RAMP-1, RAMP-2, and RAMP-3 [22]. Considering the central role of CGRP receptor in migraine, blocking the physiological effects of CGRP receptor appears to be a logical therapeutic strategy [23].

The present study focused on the screening of potential phytochemicals from the petroleum ether extracts of the leaves *Abrus precatorius* using GCMS analysis. The compounds identified from *Abrus precatorius* were used to screen its potential antimigraine activity using *in-silico* analysis. The docking studies were carried out with the protein CGRP receptor and the PDB ID is 3N7S. The potential molecules were tested for Lipinski rule and ADME/T properties prediction analysis.

2. Materials and methods

2.1. Collection of plant material

The leaves of *Abrus precatorius* were collected in the early morning from the nearby village of and packed in linen bag. The leaves were brought to the laboratory.

2.2. Preparation of sample

The collected leaves were washed with tap water and dried under shade at room temperature for 10 days. About 400 g of the dried leaves were ground using electrical mixer for about 2 min to get a coarse powder.

2.3. Extraction of leaves

The powdered leaves of *Abrus precatorius* (200 gm) were extracted with 500 ml of petroleum ether at 60–80°C for 48 h using soxhlet extractor. The extract was concentrated by condensation using steam distillation until to get a semi solid mass.

2.4. GCMS analysis

The chemical composition of the petroleum ether extract of *Abrus precatorius* was investigated using Gas Chromatography Mass Spectrometry/Mass Spectrometry Electron Ionization (GC–MS/EI) mode. The GC–MS/MS (Bruker Scion 436-GC) united with a triple quadruple mass spectrophotometer fused with silica capillary column BR-5MS (5% Biphenyl / 95% Dimethyl polysiloxane) with 30 m long, internal diameter of 0.25 mm and the thickness of 0.25 μm. Helium gas (99.999%) was used as a carrier gas at a flow rate of 1 ml/min. The injection volume of the gas was 2 μl (split ratio of 10:1). The temperature program set for the column oven at 80 °C hold for 2 min followed by 160 °C at the rate of 20 °C/min, with no hold. Up to 280 °C at a rate of 5 °C/min with no hold, up to 300 °C at the rate of 20 °C/min with 10 min hold. The injector temperature was kept at 280 °C and total GC running time was 41 min [24,25].

The mass spectrometer was operated in the positive electron ionization (EI) mode with ionization energy of 70 eV. The solvent delay was 0–3.0 min A scan interval of 0.5 s and fragments from *m/z* 50 to 500 Da was programmed. The inlet temperature was set at a source temperature of 250 °C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

2.5. Identification of compounds

The software "MS work station 8" was installed into the PC to handle the mass spectra and chromatograms. The NIST Version 2.0 library database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

The GC–MS/MS analysis of the petroleum ether extract of *Abrus precatorius* leaves was performed at Food Safety and Quality Testing Laboratory, Institute of Crop Processing Technology, Tanjore, Tamilnadu, India.

2.6. Preparation of protein

The 3D structural information of the macromolecules was determined by X-ray crystallography and NMR studies using the website (http://www.rcsb.org/pdb) [26]. The crystal structure of Calcitonin Gene-Related Peptide (CGRP) PDB ID of 3N7S [27] with the resolution of 2.1 Å (X-ray diffraction) obtained from the protein data bank. The crystallographic water molecules were removed from the protein followed by the addition of missing hydrogen. Additionally, the crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. CHARMm (Chemistry at HARvard Macromolecular Mechanics), a force-field was used for energy minimization of the protein.

2.7. Identification of active site

The binding or active sites of the receptor proteins were predicted based on 'receptor cavity method' and the inhibitory property of the amino acid residues present in the binding sites using Accelry's Discovery Studio Ver. 2017 (Fig. 1).

Fig. 1. Leaves and flowers of *Abrus precatorius Linn.*

2.8. Preparation of ligands

The identified chemical compounds namely, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; n-Hexadecanoic acid; Phytol; 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-; Octadecanoic acid; Hexanedioic acid; mono(2-ethylhexyl) ester; Heneicosane 11-(1-ethylpropyl)-; Squalene; Heptadecane 9-octyl-; 17-Pentatriacontene; Ergosterol; Cholestane-3,5-diol, 5-acetate, (3β,5α)- and Stigmasterol were derived from *Abrus precatorius* leaves and the structures were obtained from PubChem online server [27]. The structures were constructed using the Chem-Draw [28] tool and saved in MOL format. Each ligand molecule were added to the Discovery Studio software and the hydrogen bonds were also added. The energy minimization was performed using the CHARMm force field with smart minimizer of about 30,000 steps. The three dimentional optimizations were performed for all the ligands and then saved in SDF format. The 2D diagram of ligands are shown in Fig. 2.

2.9. Docking analysis

Docking is the virtual method of screening compounds using the database and predicting the strongest binders based on various scoring functions [29]. The docking analysis was performed using Discovery studio Ver. 2017 [30]. The compounds identified from the petroleum ether leaf extract of *Abrus precatorius* by GCMS analysis were docked to see the interaction with the amino acids of the protein hRAMPI. The active site (binding site) of the protein was first identified and defined based on the ligands already present in the PDB file. In addition to these, the active site was marked using the definition of a sphere. The ligand binding energies of the protein can be determined by the ligand binding affinity and calculated using Dock score or LigScore and docking energy. The other parameters were set as default while using C-DOCKER for molecular docking [31].

2.10. Lipinski rule and the analysis of ADME/T properties

The *in silico* analysis helps in early preclinical assessment and thereby avoiding expensive late stage preclinical and clinical failures. The best known physical property filters is Lipinski's "rule of five", which focuses on bioavailability. The rule states that the compounds have molecular mass less than 500 daltons, not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors and an octanol-water partition coefficient "log P" not greater than 5 [32]. The pharmacokinetic properties of the 13 compounds were predicted using the tool of Discovery Studio Ver. 2017.

3. Results and discussion

The petroleum ether leaf extract of *A. precatorius* was analyzed using GCMS and the compounds were identified using

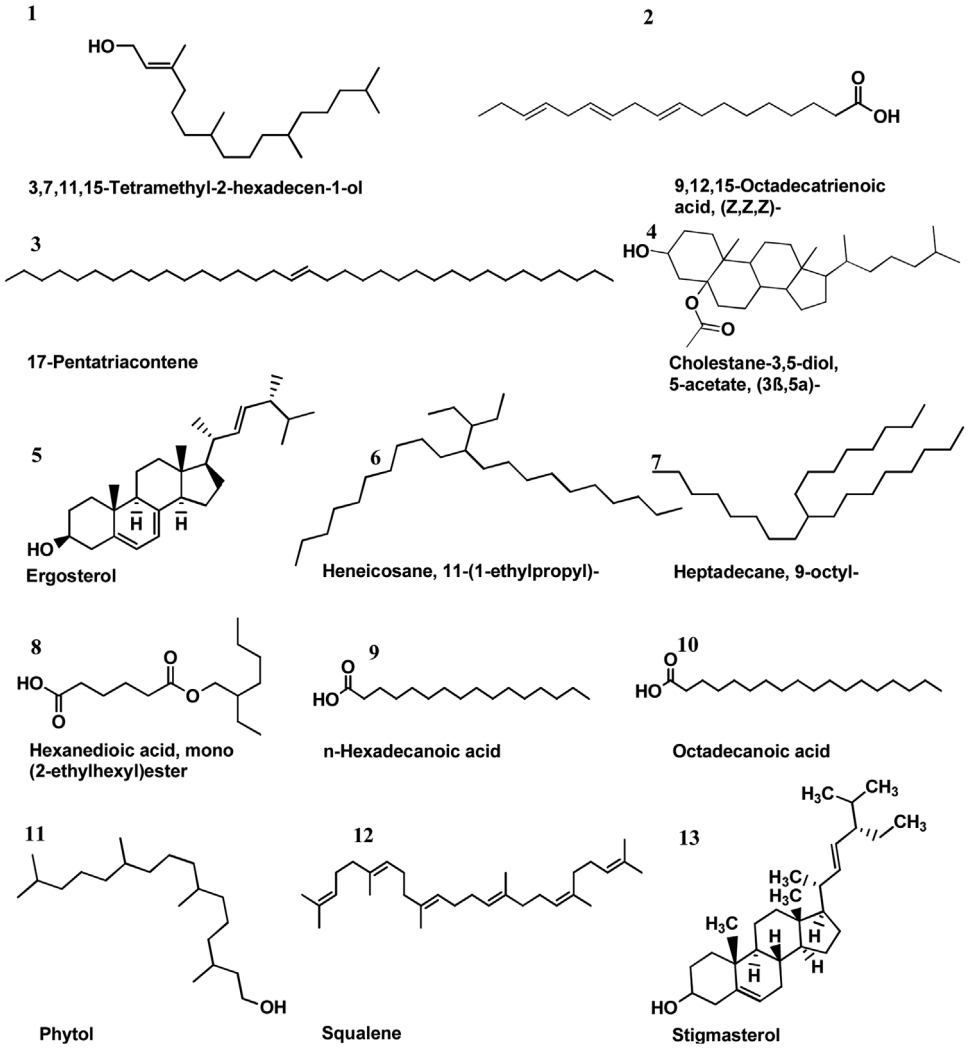

Fig. 2. The 2D structure of 13 compounds identified in the petroleum ether extract of *Abrus precatorius* using GCMS analysis.

Table 1
Compounds identified using GCMS analysis from the petroleum ether leaf extract of *A. precatorius*.

No.	RT	Name of the compound	Molecular Formula	Molecular Weight	% Peak Area
1.	12.54	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.44
2.	14.23	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.25
3.	16.22	Phytol	$C_{20}H_{40}O$	296	2.31
4.	16.79	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	12.83
5.	17.08	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.93
6.	20.28	Hexanedioic acid, mono(2-ethylhexyl)ester	$C_{14}H_{26}O_4$	258	0.49
7.	24.82	Heneicosane, 11-(1-ethylpropyl)-	$C_{26}H_{54}$	366	0.66
8.	26.36	Squalene	$C_{30}H_{50}$	410	3.34
9.	27.56	Heptadecane, 9-octyl-	$C_{25}H_{52}$	352	1.27
10.	31.20	17-Pentatriacontene	$C_{35}H_{70}$	490	6.16
11.	33.05	Ergosterol	$C_{28}H_{44}O$	396	1.47
12.	33.66	Cholestane-3,5-diol, 5-acetate, (3β,5α)-	$C_{29}H_{50}O_3$	446	1.09
13.	34.44	Stigmasterol	$C_{29}H_{48}O$	412	9.33

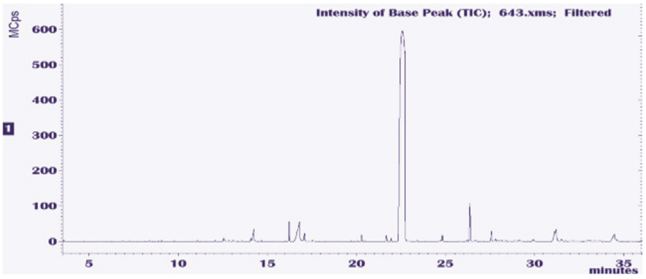

Fig. 3. The GCMS chromatogram of the petroleum ether leaf extract of *Abrus precatorius*.

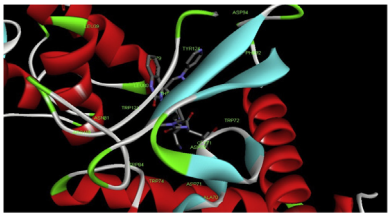

Fig. 4. Amino acids present in the active site of the Calcitonin Gene-Related Peptide (CGRP) protein.

Helium at 280^{0}C to 300^{0}C. Based on the GCMS chromatogram (Fig. 3) from the GCMS analysis and the retention time (RT) peaks, 13 compounds were identified. The RT and the corresponding compounds identified were namely, (R.T = 12.54) 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, (R.T = 14.23) n-Hexadecanoic acid, (R.T = 16.22) Phytol, (R.T = 16.79) 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)-, (RT = 17.08) Octadecanoic acid, (RT = 20.28) Hexanedioic acid, mono (2-ethylhexyl) ester, (RT = 24.82) Heneicosane, 11-(1-ethylpropyl)-, (RT = 26.36) Squalene, (RT = 27.56) Heptadecane, 9-octyl-, (RT = 31.20) 17-Pentatriacontene, (RT = 33.05) Ergosterol, (RT = 33.66) Cholestane-3,5-diol, 5-acetate, (3β,5α)- and (RT = 34.44) Stigmasterol. The phytochemicals identified from the petroleum ether extract of *A. precatorius* using GC–MS analysis is given in Table 1.

3.1. Protein preparation and the identification of active site

The 3D structure of Calcitonin Gene-Related Peptide protein (CGRP) (ID: 3N7S) was obtained from the protein data bank. The minimized energy of the protein 3N7S and the amino acids present in the active site is shown in Fig. 4. The amino acid residues present in the active site is responsible for the inhibitory activity of the target protein is shown in Table 2 [30].

Table 2
Amino acid residues present in the active site of target protein "CGRP".

Target protein	Active site	Amino acid residues
Calcitonin Gene-Related Peptide protein ID: **3N7S**	Site 1	ASN31, LEU39, ALA70, ASP71, TRP72, TRP74, LYS79, LEU80, TRP84, ASP94, ARG119, TRP121, THR122, TYR124.

3.2. Molecular docking

The 13 phyto-compounds identified from *A. precatorius* were docked with the Calcitonin Gene-Related Peptide protein and all the compounds showed their interactions with the amino acids present in the active site of the receptor. The interaction scores of the phytochemicals targeting the protein 3N7S is shown in Table 3. The ligand or molecules with the least binding energy are considered as the compounds of highest binding affinity. The phytochemicals like Octadecanoic acid, Hexanedioic acid, mono (2-ethylhexyl) ester, n-Hexadecanoic acid, Heptadecane, 9-octyl- and Phytol showed the CHARMm values such as -26.21, -27.63, -25.32, -2.64 and 8.68 kcal/mol^{-1} respectively. This binding affinity indicates a focused interaction between the above compounds with the targets as compared to others. The parameters for finding the best inhibitors such as C-Docker energy and C-Docker interaction energy were also evaluated. C-Docker energy is the combined energy produced by the sum of internal ligand strain energy and receptor-ligand interaction energy. C-Docker interaction energy is the interaction energy between the protein and ligand. The values of these two parameters indicate the strength of interaction between the proteins and ligands.

Besides the least CHARMm energy as well as the compounds with least atomic energy difference between C-Docker energy and C-Docker interaction energy were also analyzed. The C-Docker energy values and the interaction values for the 5 compounds are, 48.80, 44.38, 44.16, 33.35, and 31.70. The C-Docker interaction energy values for the first 5 potential compounds are 49.07, 42.36, 45.16, 43.83, and 43.67. Using the value of the C-Docker and C-Docker interaction energy the 5 seven compounds namely, Octadecanoic acid, Hexanedioic acid, Mono(2-ethylhexyl) ester, n-Hexadecanoic acid, Heptadecane, 9-octyl- and Phytol are considered as the potential phyto-compounds based on their better interaction. The comparative analysis of receptor ligand interactions showed the potential compounds to target Calcitonin Gene-Related Peptide protein to treat migraine headache.

The 5 lead compounds showed specific interaction with the following amino acids, which includes LYS79, TRP121, ARG119, ASP70, LEU80, TRP72, TRP74, TRP84, LEU39, LEU79, PRO85, and PHE92. The interaction of amino acids of the protein with 5 compounds showed hydrogen bond, Pi-Pi, Pi-alkyl Pi-sigma inter-

Table 3
Docking results of the 13 phytochemicals with antimigraine target identified from GCMS analysis and their score calculated using C-Docker module of Discovery Studio version 2017.

Target protein	Ligand	CDocker energy	CDocker interaction energy	CHARMm Energy (kcal/mol^{-1})	Interacting amino acid residues
CGRP	Octadecanoic acid	48.80	49.07	−26.21	TRP74, LYS79 & LEU80
	Hexanedioic acid, mono(2-ethylhexyl) ester	44.38	42.36	−27.63	TRP72, TRP121, TRP84 & PRO85
	n-Hexadecanoic acid	44.16	45.16	−25.32	TRP72, LEU80, PHE92 & ARG119
	Heptadecane, 9-octyl-	33.35	43.83	−2.64	TRP74
	Phytol	31.70	43.67	8.68	LEU39, ASP70, LYS79 &TRP121,
	Heneicosane, 11-(1-ethylpropyl)-	29.09	46.81	7.75	TRP84 & PRO85
	17-Pentatriacontene	23.27	49.45	4.95	LEU39
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	21.63	50.54	21.16	LEU39, LYS79, LEU80, TRP84& CYS127
	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	−7.41	39.99	25.40	TRP74, LEU80, TYR124 & THR125
	Cholestane-3,5-diol, 5-acetate, (3β,5α)-	−21.6	44.72	132.45	LEU35, LEU39 &LEU80
	Stigmasterol	−84.14	24	99.35	LEU35, LYS79, LEU80 & PRO85
	Ergosterol	−91.43	22.08	103.177	LEU80 & PRO85
	Squalene	−92.39	42.93	174.11	LEU35, LEU39 & PRO85

Table 4
Values of the five screened compounds based on the Lipinski rule.

S. No	Compound	Molecular Weight (<500)	H-Bond Donor (<5)	H-Bond Acceptor (<10)	Log P O/W (<5)	Rule of 5 (0)
1	Octadecanoic acid	284.481	1	2	6.07	1
2	Hexanedioic acid, mono(2-ethylhexyl)ester	258.357	1	4	3.538	0
3	n-Hexadecanoic acid	256.428	1	2	5.291	1
4	Heptadecane, 9-octyl-	352.686	0	0	14.228	1
5	Phytol	298.551	1	1.7	6.415	1

Table 5
Predicted ADME/T values of the five lead compounds identified using Discovery Studio Ver.2017.

S.No	Ligand	ADME/T solubility level (2-4)	BbB level (2-4)	Hepato-toxicity (False)	Absorption level (0-1)	Alogp98 (<4)	CYP2D6 (false)
1	Octadecanoic acid	2	2	False	0	1.655	False
2	Hexanedioic acid, mono(2-ethylhexyl)ester	3	2	False	0	1.78	False
3	n-Hexadecanoic acid	3	2	False	1	1.473	False
4	Heptadecane, 9-octyl-	3	2	False	0	2.062	False
5	Phytol	4	2	false	0	0.972	False

actions. The 3D and 2D interaction images of the five lead compounds are shown in Fig. 5.

3.3. Lipinski rule and ADME/T properties

Based on the values of the C-Docker and C-Docker interaction energy first 5 compounds were chosen for further ADME/T and Lipinski analysis. The studies were carried out based on the absorption, distribution, metabolism, excretion, and toxicity (ADME/T) properties of the compounds. These study was performed using the Discovery studio inbuilt ADME/T protocol. The parameters such as aqueous solubility, blood brain barrier (BBB) level, hepatotoxicity, absorption level, AlogP and CYPD26 were studied. The results of the study provided the pharmacokinetic properties of the compounds. The Lipinski rule and the ADME/T value of the 5 compounds are shown in Tables 4 and 5. The results of the Lipinski rule showed few violations except in rule of five and LogP (o/w). The compound Hexanedioic acid, mono (2-ethylhexyl) ester showed no violations of the Lipinski rule and it is expected that the compound is orally active. The compound Octadecanoic acid, n-Hexadecanoic acid, Heptadecane, 9-octyl- and Phytol showed two violations in the Lipinski's rule and are expected to be orally inactive.

The compounds namely, Octadecanoic acid, Hexanedioic acid, mono (2-ethylhexyl) ester, n-Hexadecanoic acid, Heptadecane, 9-octyl- and Phytol were passed all the parameters and they exhibited good interaction score along with ADME/T properties. Hence, they are considered as the best compound and desirable to be a drug candidate to treat migraine attack.

4. Conclusion

The *in silico* molecular docking and pharmacokinetic analysis with the petroleum ether extract of the leaves of *Abrus precatorius* revealed that the five lead phyto-compounds present in the extract showed a favorable interaction with Calcitonin Gene-Related Peptide protein. The scores of C-Docker and C-Docker interaction energy from the Discovery Studio software showed the better affinity towards CGRP due to their hydrophobic, pi-pi, pi-alkyl, and pi-sigma interaction. In addition to these, five screened compounds and their ADME/toxicity prediction as well as the Lipinski rules properties indicated that the compounds with high dock score were within the acceptable range of various pharmacological parameters with few violations. These may have better characteristic for health effects and can inhibit the activation of CGRP receptor signaling. The present study provided the strong evidence that the lead compounds present in *A. precatorius* leaves may act as an inhibitor to Calcitonin Gene-Related Peptide receptor. Hence, these compounds may have the potential to prevent or treat migraine

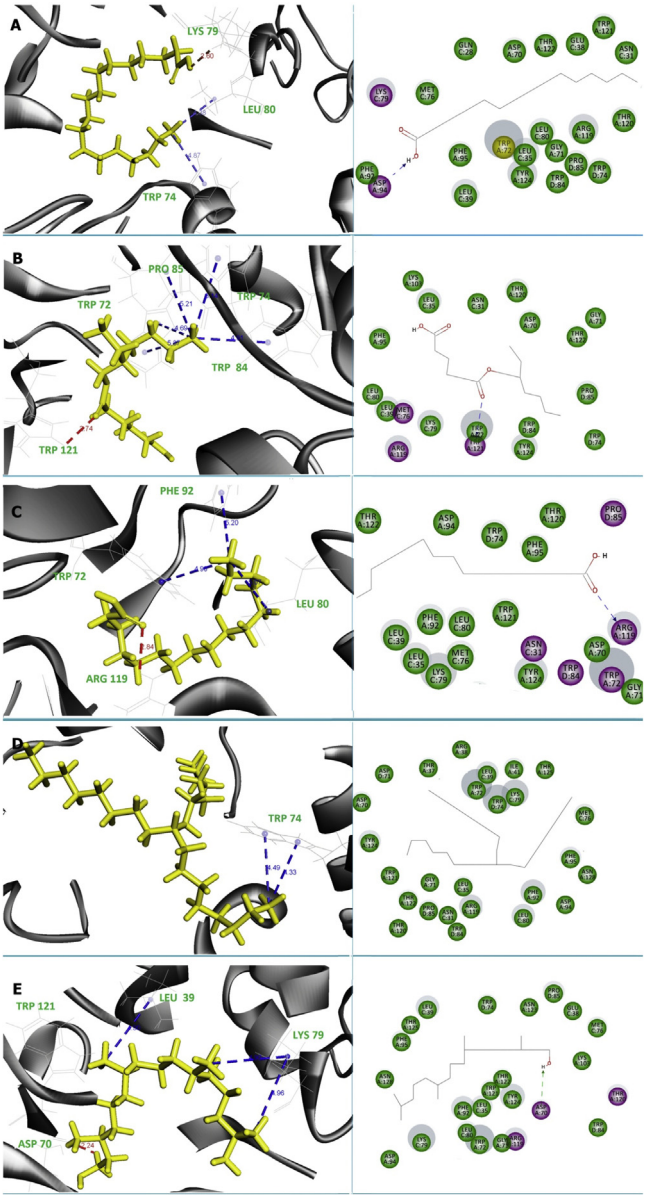

Fig. 5. 3D and 2D interaction images of five screened compounds (1–5) identified with anti-migraine properties to target Calcitonin Gene-Related Peptide protein ID: 3N7S.

headache. These compounds can be screened further for their use as an anti-migraine drug.

Conflict of interest

The authors confirm that the contents of this article have no conflict of interest.

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References

[1] H.A. Padh, Report of seminar on herbal drugs, Curr. Sci. 81 (1) (2001) 15–16.
[2] A. Rajani, K. Hemamalini, S.K. Arifa Begum, et al., Anthelmintic activity of ethanolic seed extract of *Abrus precatorius Linn*, Pharm. Innov. J. 1 (11) (2013) 17–19.
[3] H. Hara, L.J.H. Williams, An Enumeration of Flowering Plants of Nepal, vol. I, Trustees of British Museum (Natural History), London, 1979.
[4] A.S. Saganuwan, P.A. Onyeyili, Biochemical effects of aqueous leaf extracts of *Abrus precatorius* (Jecquirity bean) in Swiss albino mice, Herbapolonica. 56 (3) (2010) 63–80.
[5] L. Reedman, R.D. Shih, O. Hung, Survival after an intentional ingestion of crushed *abrus* seeds, West. J. Emerg. Med. 9 (3) (2008) 157–159.
[6] E.J. Kennelly, R. Suttisri, A.D. Kinghorn, et al., Abruoside E, a further sweet tasting cycloartane from the leaves of *Abrus precatorius*, Phytochemistry 41 (5) (1996) 1381–1383.
[7] A.A. Adedapo, O.A. Omoloye, O.G. Ohore, Studies on the toxicity of an aqueous extract of the leaves of *Abrus precatorius* in rats, Onderstepoort J. Vet. Res. 74 (2007) 31–36.
[8] C.F. Gasparini, H.G. Sutherland, L.R. Griffiths, Studies on the pathophysiology and genetic basis of migraine, Curr. Genom. 14 (5) (2013) 300–315.
[9] P.J. Goadsby, R.B. Lipton, M.D. Ferrari, Migraine-current understanding, and treatment, N. Engl. J. Med. 346 (2002) 257–270.
[10] S.K. Ashish, V. Parthasarthy, N. Sayyed, et al., Determination of antimigraine property of leaves extracts of *Abrus precatorius* by serotonergic receptor agonist activity, J. Pharm. Res. 4 (4) (2011) 1000–1003.
[11] U. Arulmani, A. Maassenvandenbrink, C.M. Villalon, et al., Calcitonin gene-related peptide and its role in migraine pathophysiology, Eur. J. Pharmacol. 500 (2004) 315–330.
[12] Z. Zhang, C.S. Winborn, B. Marquez de Prado, et al., Sensitization of Calcitonin gene-related peptide receptors by receptor activity-modifying protein-1 in the trigeminal ganglion, J. Neurosci. 27 (10) (2006) 2693–2703.
[13] L.E. Anderson, V.S. Seybold, Calcitonin gene-related peptide regulates gene transcription in primary afferent neurons, J. Neurochem. 91 (6) (2004) 1417–1429.
[14] J. Olesen, H.C. Diener, I.W. Husstedt, et al., Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine, N. Engl. J. Med. 350 (11) (2004) 1104–1110.
[15] E. Ter Haar, C.M. Koth, N. Abdul-Manan, et al., Crystal structure of the ectodomain complex of the CGRP receptor, a class-B GPCR, reveals the site of drug antagonism, Structure. 18 (9) (2010) 1083–1093.
[16] L.M. McLatchie, N.J. Fraser, M.J. Main, et al., RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor, Nature 393 (1998) 333–339.
[17] S. Kunso, M. Kukimoto-Niino, R. Akasaka, et al., Crystal structure of the human receptor activity-modifying protein 1 extracellular domain, Protein Sci. 17 (11) (2008) 1907–1914.
[18] D. Vecchia, D. Pietrobon, Migraine: a disorder of brain excitatory-inhibitory balance, Trends Neurosci. Educ. 35 (8) (2012) 507–520.
[19] L. Delafoy, A. Gelot, D. Ardid, et al., Interactive involvement of brain derived neurotrophic factor, nerve growth factor, and calcitonin gene related peptide in colonic hypersensitivity in the rat, Gut 55 (7) (2005) 940–945.
[20] M.F. Steven, K.C. Roger, Isolation and characterization of human calcitonin-gene-related-peptide receptor, Eur. J. Biochem. 170 (1–2) (1988) 373–379.
[21] G. Skofitsch, D.M. Jacobowitz, Autoradiographic distribution of 125I Calcitonin gene-related peptide binding sites in the rat central nervous system, Peptides 6 (5) (1985) 975–986.
[22] S.L. Armour, S. Foord, T. Kenakin, et al., Pharmacological characterization of receptor-activity-modifying proteins (RAMPs) and the human Calcitonin receptor, J. Pharmacol. Toxicol. Methods 42 (4) (1999) 217–224.
[23] S.W. Shirley, Health, and wellness, Wall Street J. (January (17)) (2012).
[24] K. Srinivasan, S. Kumaravel, Unraveling the potential phytochemical compounds of gymnema sylvestre through GC-MS study, Int. J. Pharm. Pharm. Sci. 8 (1) (2016) 450–453.
[25] P. Balachandran, Ajay Kumar T.V, Parthasarathy V, Screening of potential anticancer compounds from *Sargassum wightii* to target breast cancer specific HER2 receptor using *in silico* analysis, Nat. Prod. J. 6 (2) (2016) 1–8.
[26] H.M. Berman, G.J. Kleywegt, H. Nakamura, et al., The Protein Data Bank archive as an open data resource, J. Comput. Aided Mol. Des. 28 (10) (2014) 1009–1014.
[27] W.D. Inlenfeldt, E.E. Bolton, S.H. Bryant, The PubChem chemical structure sketcher, J. Cheminform. 1 (20) (2009) 1–9.
[28] R.E. Buntrock, ChemOffice ultra 7.0, J. Chem. Inf. Comput. Sci. 42 (6) (2002) 1505–1506.
[29] T. Usha, S.K. Middha, A.K. Goyal, et al., Molecular docking studies of anti-cancerous candidates in Hippophae rhamnoides and Hippophae salicifolia, J. Biomed. Res. 28 (5) (2014) 406–415.
[30] Discovery Studio Client Software, Version 4.5, 2017, U.S http://www.accelrys.com.
[31] G. Wu, D.H. Robertson, C.L. Brooks, et al., Detailed analysis of grid based molecular docking: a case study of CDOCKER-A CHARMm based MD docking algorithm, J. Comput. Chem. 24 (13) (2003) 1549–1562.
[32] C.A. Lipinski, F. Lombardo, B.W. Dominy, et al., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (1–3) (2001) 3–26.